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Antidiabetic activity of *Abies Alba* seeds using *In vitro* starch iodine method

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Abstract
Abies alba has long tradition of use in natural based medicine system of Greece for the treatment of cancer and diabetes. Few scientific studies revealed anticancer and antibacterial potential of the plant but traditional claims for antidiabetic activity have not been validated. Thus, it was considered worthwhile to investigate *A. alba* seeds for antidiabetic activity. Seeds of plant were extracted utilizing solvents in expanding request of polarity *viz*., petroleum ether, chloroform, methanol, and water. The chloroform, methanol and water 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. The extract showed greatest action was additionally fractionated successively using solvents in order of increasing polarity *viz.*, ethyl acetate and 1-butanol for further purification and subjected to *in vitro* antidiabetic activity using starch iodine method via inhibition of α-amylase enzyme. The ethyl acetate showed huge antidiabetic action as compared to other tested samples. Our preliminary phytochemical investigations showed presence of flavonoids and phenolic compounds in the bioactive ethyl acetate fraction. 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 seeds. These antidiabetic compounds will be isolated from bioactive ethyl acetate fraction using chromatographic techniques in future investigations.

Keywords: *Abies alba*, diabetic, flavonoids, phenol, pinaceae

Introduction
The conifer *Abies alba* is a conifer mainly called as European Silver Fir and belonging to family Pinaceae. It is mainly distributed in the native areas of Europe situated at higher attitude and many other regions such as Bosnia, Herzegovina, Montenegro, Serbia, south Italy, Bulgaria, Albania and northern Greece. The present survey of literature has been collected from various scientific databases such as Sci Finder, PubMed, Scirus, Google Scholar, Open J Gate, Science Direct, Scopus, King's American Dispensatory, Rain tree Nutrition Incorporation, AGRICOLA, etc. *Abies alba* is a large evergreen coniferous tree growing to 40-50 metres tall and with a trunk diameter of up to 1.5 metres (Farjon, 2010) [4]. Traditionally, the plant in the treatment of cancer and diabetes in various parts of Greece (Karkabounas *et al*., 2000; Lunder *et al*., 2019) [12]. A total number of 31 phytoconstituents such as sesquiterpenoids, diterpenoids, triterpenoids, phenolic and steroids have been isolated from plant till date (Khan *et al*., 1988 [9]; Khan & Pentegova, 1988 [10]; Ribo & Mitja, 1974 [14]; Yang *et al*., 2008) [17]. The plant essential oil possess antibacterial activity (Bagci & Digrak, 1991) [1]. The water extract of plant has been reported anticancer potential against L-1210 cells (Karkabounas *et al*., 2000) [8]. The two species of *Abies* named *A. alba* and *A. nigra* has been investigated clinically for the treatment of skin and gastroesophageal reflux diseases, respectively. The volatile oil extracted from *A. alba*, *A. grandis* and *A. balsamea* has been reported to be safe at higher doses and did not show any type of toxicity on body organs (Dweck, 2009) [3].
Traditionally, the plant in the treatment of cancer and diabetes in various parts of Greece (Karkabounas *et al*., 2000 [8]; Lunder *et al*., 2019) [12]. A survey of literature revealed that no antidiabetic related work has been carried out on this potential plant seeds till date. Therefore, it was envisaged to undertake detailed *in vitro* antidiabetic activity on *A. alba* seeds to validate traditional claims of the plant using well developed method.

Materials and methods
Collection and identification of plant material
The dried *Abies alba* seeds were purchased from online market in the month of November 2020. The identification / authentication of the plant material was confirmed on the basis of literature reported and various photographic pictures available online.

Preparation of various extracts/fractions and phytochemical screening
The various extracts and fractions from bioactive crude extract from plant seeds under present studies were prepared using standard extract protocols such as Soxhlet and reflux methodology as per scientific records available online (Kumar *et al*., 2014) [11]. Crude extracts of *A. webbiana* were screened for detection of different classes of phytoconstituents using specific standard reagents (Farnsworth, 1966) [5].

Antidiabetic activity
The *in vitro* antidiabetic activity of various extracts and fractions was investigated using starch iodine test via α-amylase enzyme inhibition (Sivaraj *et al*., 2020) [16]. 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 (Scheffer, 1980) [15].

Results and discussion

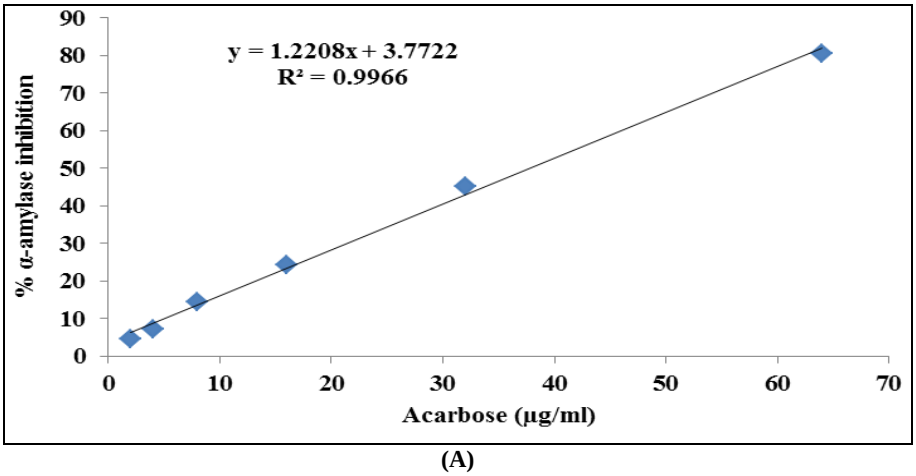
A. alba seeds were extracted exhaustively and successively using solvents in order of increasing polarity. The percentage yields (% w/w) of various extracts of plant seeds *viz.*, petroleum ether, chloroform, methanol and water was found to be 1.89, 3.40, 6.10 and 5.08 %w/w respectively. All the crude extracts of plant seeds were screened for the presence of different classes of phytoconstituents using standard specific chemical reagents. The petroleum ether extract showed presence of fixed oils, steroids; chloroform extract showed presence of alkaloids, triterpenoids; methanol extract showed presence of tannins, saponins, flavonoids and water extract showed presence of carbohydrates, proteins, saponins.

It is clearly evident from results of preliminary phytochemical screening that petroleum ether extract did not show any presence of any main class of phytoconstituents. Therefore, chloroform, methanol and water 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} = 142.02 µg/ml) followed by chloroform extract (IC_{50} = 248.20 µg/ml), water extract (IC_{50} = 352.25 µg/ml). These results are statistically compared with Acarbose standard antidiabetic drug (IC_{50} = 37.86 µg/ml). These observations suggested that the most polar water extract was found to be devoid of antidiabetic activity whereas chloroform extract exhibited mild antidiabetic activity. The most bioactive methanol extract was fractionated successively using solvents in order of increasing polarity *viz.*, ethyl acetate and 1-butanol for further purification. All fractions as well as remaining bioactive extract were subjected to screen *in vitro* antidiabetic activity using starch iodine method via inhibition of α-amylase enzyme.

Table 1: The results of antidiabetic activity of various extracts of plant seeds using starch iodine method.

Treatment	Concentration (µg/ml)	% α-amylase inhibition (Mean ± S.D.)	IC_{50} values (µg/ml)
Acarbose	2	4.58 ± 1.04	37.86
	4	7.35 ± 1.11	
	8	14.50 ± 1.58	
	16	24.28 ± 1.08	
	32	45.31 ± 1.69	
	64	80.44 ± 1.40	
Chloroform extract	50	7.51 ± 1.02	248.20*
	100	18.90 ± 1.66	
	150	27.36 ± 1.98	
	200	40.11 ± 1.32	
	250	48.91 ± 1.47	
	300	62.44 ± 2.90	
Methanol extract	50	30.21 ± 1.80	142.02*
	100	42.80 ± 1.95	
	150	52.40 ± 1.08	
	200	61.01 ± 3.44	
	250	70.69 ± 2.35	
	300	83.44 ± 3.87	
Water extract	50	2.90 ± 1.11	352.25*
	100	10.08 ± 1.25	
	150	18.64 ± 1.87	
	200	25.45 ± 1.09	
	250	32.89 ± 1.36	
	300	42.88 ± 1.70	

n= 3; **P<0.05* vs standard; Statistical Comparison by one way ANOVA further using by Tukey's test.



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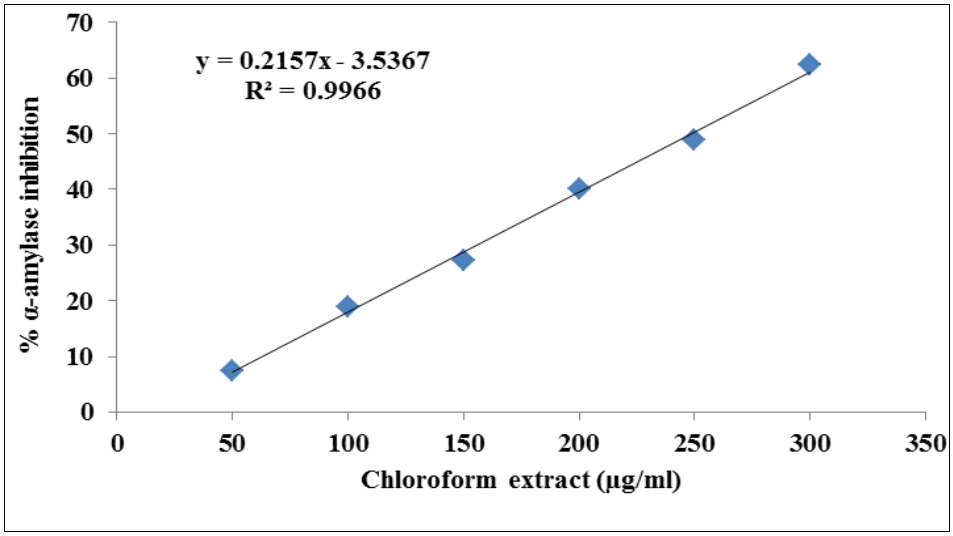

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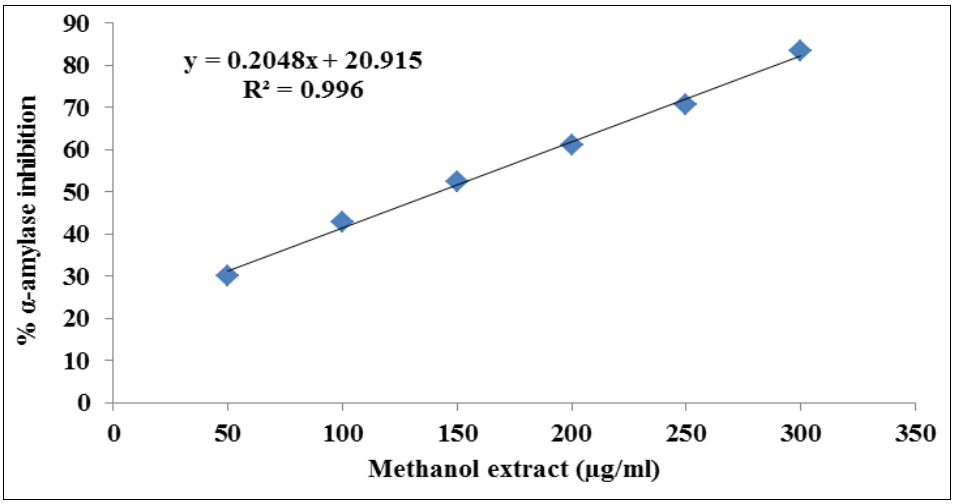

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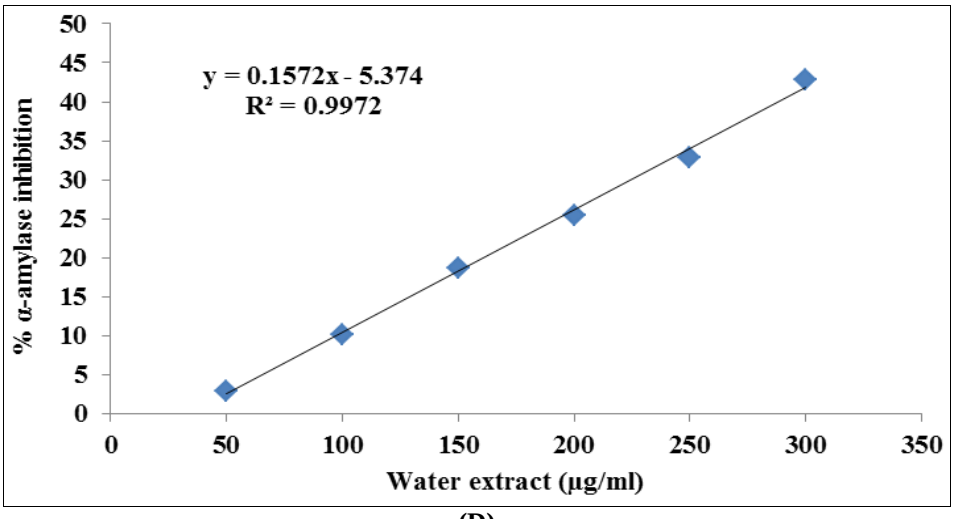

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Fig 1: Graphical representation of antidiabetic activity of various extracts of plant seeds using starch iodine method.

The percentage yields (% w/w) of various fractions obtained from bioactive methanol extract *viz.*, *n*-hexane, ethyl acetate, 1-butanol and remaining bioactive extract was found to be 4.80, 25.12, 16.78 and 54.69 respectively, in relation to methanol extract. Preliminary phytochemical screening of various fractions prepared from bioactive methanol extract of plant seeds was performed and *n*-Hexane fraction did not show any presence of any main class of phytoconstituents. The ethyl acetate fraction showed presence of flavonoids, tannins; 1-butanol fraction showed presence of saponins and remaining bioactive extract showed presence of saponins.

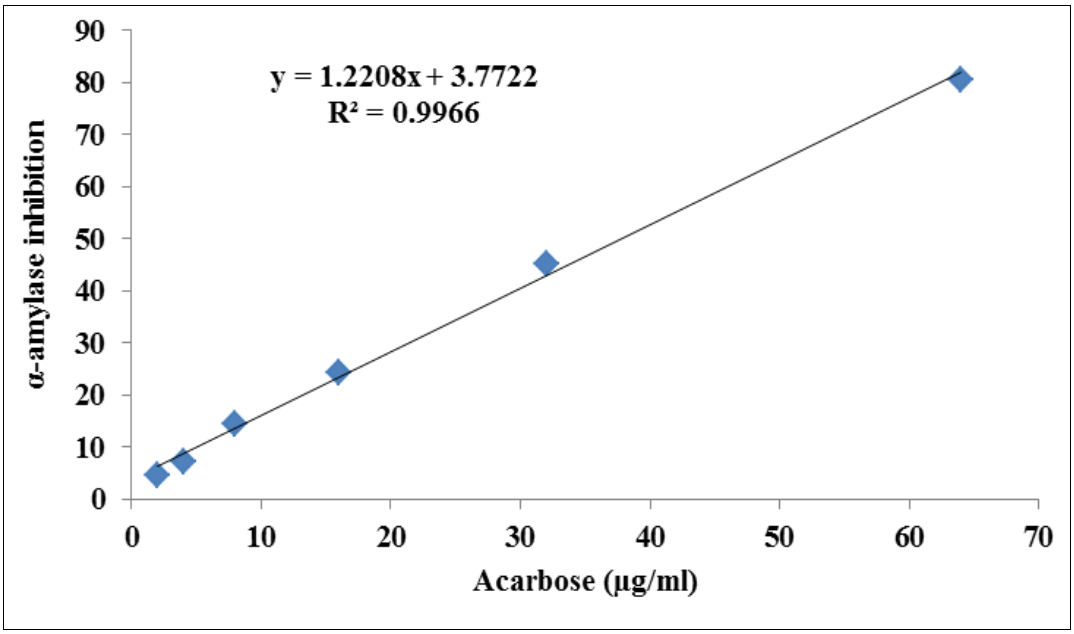
Therefore, ethyl acetate fraction, 1-butanol fraction and remaining bioactive extract obtained from bioactive

methanol extract were subjected to screen *in vitro* antidiabetic activity using starch iodine method via inhibition of α-amylase enzyme. The antidiabetic activity of various fractions obtained from bioactive methanol extract was assessed in terms of percentage inhibition of α-amylase enzyme. The results of various fractions as 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 ethyl acetate fraction exhibited maximum antidiabetic activity in term of percentage inhibition of α-amylase enzyme (IC_{50} = 73.70 µg/ml) followed by 1-butanol fraction (IC_{50} = 267.97 µg/ml), remaining bioactive extract (IC_{50} = 393.99 µg/ml). These results are statistically compared with Acarbose standard antidiabetic drug (IC_{50} = 37.86 µg/ml). These observations suggested that the remaining bioactive extract was found to be devoid of antidiabetic activity whereas 1-butanol exhibited mild antidiabetic activity.

Table 2: The results of antidiabetic activity of various fractions obtained from bioactive methanol extract of plant seeds using starch iodine method.

Treatment	Concentration (µg/ml)	% α-amylase inhibition (Mean ± S.D.)	IC_{50} values (µg/ml)
Acarbose	2	4.58 ± 1.04	37.86
	4	7.35 ± 1.11	
	8	14.50 ± 1.58	
	16	24.28 ± 1.08	
	32	45.31 ± 1.69	
	64	80.44 ± 1.40	
Ethyl acetate fraction	50	45.58 ± 2.01	73.70*
	100	53.61 ± 2.11	
	150	65.45 ± 1.25	
	200	75.69 ± 1.56	
	250	85.50 ± 1.90	
	300	96.33 ± 3.47	
1-Butanol fraction	50	7.56 ± 1.25	267.97*
	100	17.32 ± 1.96	
	150	26.79 ± 1.65	
	200	35.99 ± 1.77	
	250	45.79 ± 1.32	
	300	57.25 ± 2.78	
Remaining bioactive extract	50	1.08 ± 0.25	393.99*
	100	6.89 ± 1.01	
	150	14.58 ± 1.08	
	200	22.40 ± 1.11	
	250	28.56 ± 1.31	
	300	36.97 ± 1.45	

n= 3; *$P<0.05$ vs standard; Statistical Comparison by one way ANOVA further using by Tukey's test.



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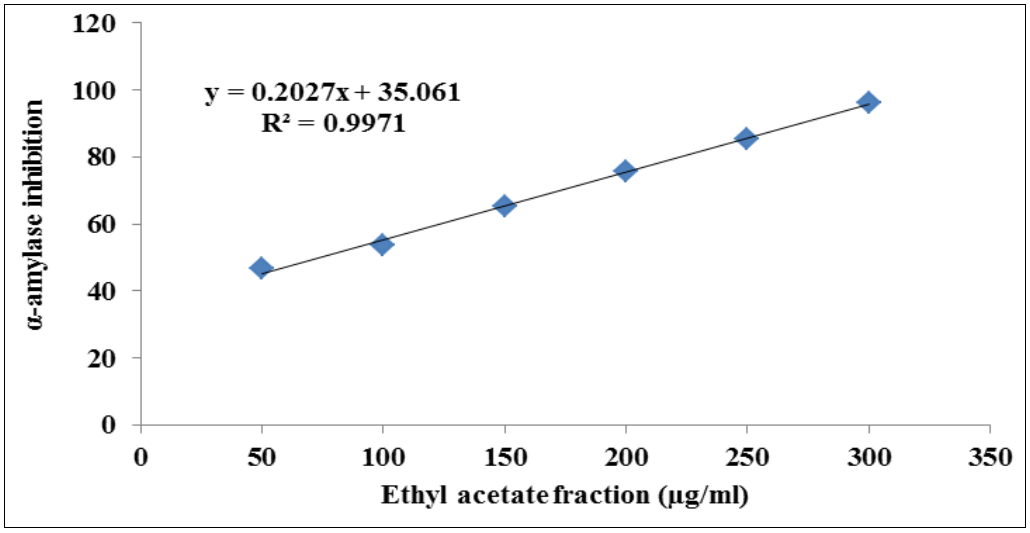

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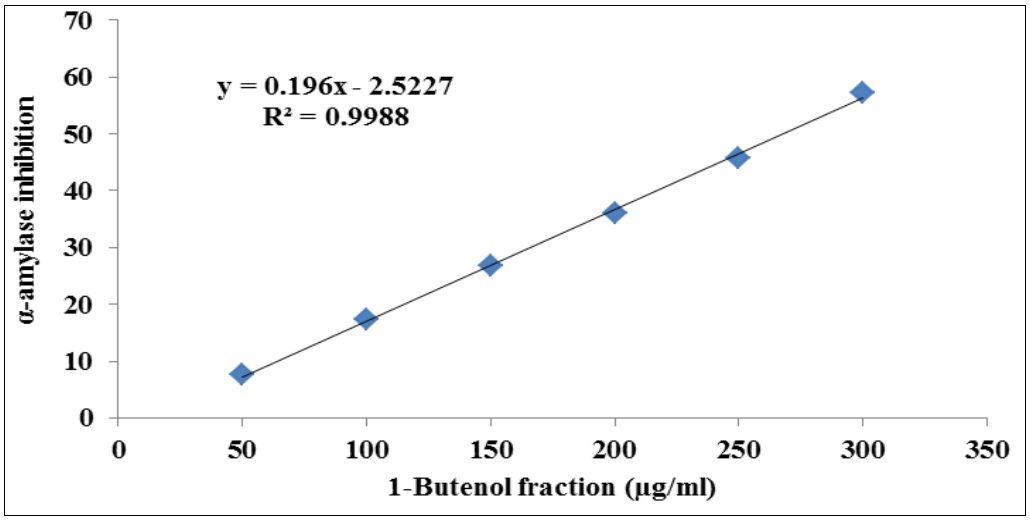

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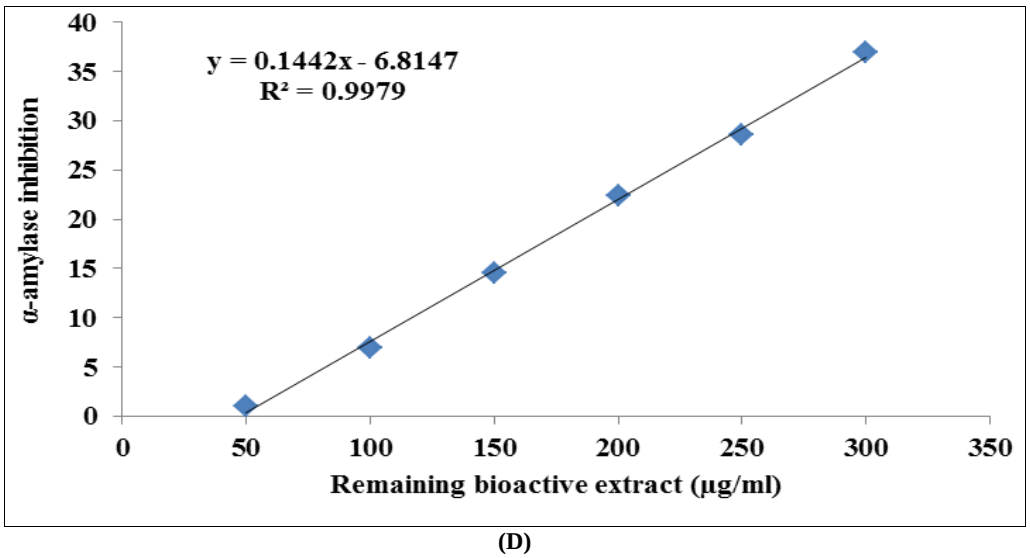

(D)

Fig 2: Graphical representation of antidiabetic activity of various fractions obtained from bioactive methanol extract of plant seeds using starch iodine method.

Our preliminary phytochemical investigations showed presence of flavonoids and phenolic compounds in the bioactive ethyl acetate fraction. Further, exhaustive finding of literature suggested that a number of flavonoids and phenolic compounds are scientifically reported for antidiabetic activity such as flavonoids – luteolin (Josline *et al*., 2013) [7], quercetin, rutin (Jadhav and Puchchakayala, 2012) [6], naringenin (Ortiz-Andrade *et al*., 2008) [13] and phenolic compounds – pinitol (Bates *et al*., 2000) [2] have been reported as antidiabetic agents.

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 seeds. These compounds will be isolated from plant seeds using sophisticated chromatographic methodologies.

Acknowledgement

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References

1. Bagci E, Digrak M. Antimicrobial activity of essential oils of some Abies species from Turkey. Flavour Fragr J,1991:11:251-6.
2. Bates SH, Jones RB, Bailey C. Insulin-like effect of pinitol. Br J Pharmacol,1997:130(8):1944-8.
3. Dweck AC. Toxicology of essential oils reviewed. Personal Care,2009:74:65-77.
4. Farjon A. A Handbook of the World's Conifers. Volume 2. Netherlands: Brill Publishers, 2010, 59-113.
5. Farnsworth NR. Biological and chemical screening of plants. J Pharm Sci,1966:55:225-276.
6. Jadhav R, Puchchakayala G. Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, ellagic acid, quercetin, rutin on streptozotocin-nicotinamide induced type 2 diabetic rats. Int J Pharm Pharm Sci,2012:4:251-256.
7. Josline YS, Michael HN, Eskande EF. Anti-diabetic properties of flavonoid compounds isolated from Hyphaene thebaica epicarp on alloxan induced diabetic rats. Pharmacognosy Res,2013:5:22-29.
8. Karkabounas S, Assimakopoulos D, Malamas M, Skaltsonis AL, Leonce S, Zelovitis J, *et al*. Antiproliferative and anticarcinogenic effects of an aqueous preparation of Abies alba and Viscum album se Abies, on a L-1210 malignant cell line and tumor-bearing wistar rats. Anticancer Res,2000:20:4391-6.
9. Khan VA, Pentegova VA. Volatile components of the oleo resin of Abies alba. Chem Nat Compd,1988:24:162-6.
10. Khan VA, Tkachev AV, Pentegova VA. New sesquiterpenoids from the oleoresin of Abies alba. Chem Nat Compd,1988:24:606-11.
11. Kumar D, Jamwal A, Madaan R, Kumar S. Estimation of total phenols and flavonoids in selected Indian traditional plants. J Pharm Technol Res Manag,2014:2:329-338.
12. Lunder M, Roskar I, Hosek J, Strukeli B. Silver Fir (Abies alba) extracts inhibit enzymes involved in blood glucose management and protect against oxidative stress in high glucose environment. Plant Foods Hum Nutr,2019:74(1):47-53.
13. Ortiz-Andrade RR, Sanchez-Salgado JC, Navarrete-Vazquez G, Webster SP, Binnie M, García-Jimenez S, *et al*. Antidiabetic and toxicological evaluations of naringenin in normoglycaemic and NIDDM rat models and its implications on extra-pancreatic glucose regulation. Diabetes Obes Metab,2008:10:1097-1104.
14. Ribo JM, Mitja MR, Ramentol J. Diterpenoids in Abies alba. Phytochemistry,1974:13:1614.
15. Scheffer WC. Statistics for the Biological Sciences. 32nd ed. Philippines: Addison-Wesley Publishing Company, 1980.
16. Sivaraj C, Saraswathi K, Jayashree M, Srija RC, Arumugam P. Antidiabetic, antioxidant, antibacterial activities and GC-MS analysis of Withania coagulans Dunal- (Rishyagandha). Int J Phytopharmacol,2020:11(1):20-31.
17. Yang XW, Li SM, Shen YH, Zhang WD. Phytochemical and biological studies of Abies species. Chem Biodivers,2008:5:56-81.