International Journal of Pharmaceutical Science and Research
www.pharmacyjournal.net
ISSN: 2455-4685
Received: 13-03-2024, Accepted: 03-04-2024, Published: 27-04-2024
Volume 9, Issue 2, 2024, Page No. 19-23

In vitro Hyaluronidase inhibitory investigations of *Abies Webbiana* Roots

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Abstract
A. webbiana has a long tradition of utilization in the treatment of heart diseases, cough, inflammation, tumour, amoebiasis, hypochlorhydria, vomiting, antispasmodic, aphrodisiac, diuretic and mouth disorders yet has not been systematically assessed to approve its conventional cases. Hence, the current examinations were attempted so as to assess *A. webbiana* aerial parts efficiently for hyaluronidase inhibitory activity. The chloroform and methanol extracts of plant aerial parts was prepared in a Soxhlet mechanical assembly with chloroform and methanol, respectively, subsequent to defatting with petroleum ether. Preliminary phytochemical screening of various crude extracts suggested that petroleum ether extract showing presence of fixed oil; chloroform extract showing presence of steroids, alkaloids, triterpenoids; methanol extract showing presence of tannins, flavonoids and water extract showing presence of carbohydrates, proteins. The methanol extract of plant aerial parts exhibited strong hyaluronidase inhibitory activity followed by respective chloroform extract and water extract, as compared to standard hyaluronidase inhibitory drug tannic acid. The water and chloroform extracts almost devoid of tested pharmacological activity. The available literature also reveals that a large number of flavonoid and phenolic compounds have been reported to exhibit hyaluronidase inhibitory activity. The contents of total phenols were found to be almost three times in comparison to total flavonoids content in methanol extract. In agreement to these reports, it is suggested from our results that hyaluronidase inhibitory activity of *A. webbiana* aerial parts is attributed to these bioactive groups of phytoconstituents. Finally, it can be suggested that it is better to isolate main constituents responsible for hyaluronidase inhibitory activity in future studies using column chromatography.

Keywords: Abies webbiana, hyaluronidase, phenols, flavonoids

Introduction
Abies webbiana Lindl. (Family: Pinaceae) Syn. *Abies spectabilis* (D. Don Spach.), commonly known as Talispatra in Hindi and Bengali, Talispatram in Sanskrit, Badar in Kashmir and The West-Himalyan high level Fir in English, is a large, tall, evergreen tree, up to 50 m in height (Pullaiah, 2002 [18]; Khare, 2007) [11]. The plant is widely distributed in Himalayan region from Kashmir to Assam states in India and other parts of country such as Sikkim at an altitude of 1,600-4,000 m (Chatterjee and Pakrasi, 1991 [3]; Khare, 2004) [10]. It is also found in Afghanistan (Hindu Kush range), Tibet (China), Nepal, in Karakoram range and Bhutan at an altitude of 2,500-4,000 m (Ganguly and Kar, 1999) [6]. *A. webbiana* has been reported to contain alkaloids (Ghosh *et al*., 2010) [7]; phytosterols (Rao *et al.*, 2012) [19]; glucosides (Chatterjee *et al*., 1984) [2]; resins (Pullaiah, 2002) [18]. The volatile oil obtained from leaves contain α-*pinene*, *l*-limonene, Δ-carene, dipentene, *l*-bornyl acetate and *l*-cardinene as major constituents (Khare, 2007) [11]. The plant has been reported to exhibit various pharmacological activities such as: antitussive activity (Nayak *et al*., 2003) [17], antibacterial activity (Vishnoi *et al*., 2007a) [24], antifungal activity (Vishnoi *et al*., 2007a) [24], sedative activity, anti-inflammatory activity (Nayak *et al*., 2004) [16], antipyretic activity (Vishnoi *et al*., 2007b) [23], antispasmodic activity, antiplatelet activity, bronchodilator activity (Yasin *et al*., 2014) [25], oral contraceptive (Kamat and Hiremath, 2012) [9], antioxidant activity (Tote *et al*., 2009) [22], anti-allergic and mast cell stabilizer (Kumar *et al*., 2008) and anti-tumour activity (Ghosh *et al*., 2001 [8]; Nayak *et al*., 2004) [16].
Traditionally, *A. webbiana* has been used for the treatment of various diseases mainly heart diseases, cough, inflammation, tumour, amoebiasis, hypochlorhydria, vomiting, antispasmodic, aphrodisiac, diuretic and mouth disorders (Pullaiah, 2002 [18]; Chopda and Mahajan, 2009) [4]. Despite a long tradition of use, this traditionally used and medicinally promising plant has not been properly investigated for validation of its traditional claims especially in the treatment of hypersensitive, dengue and edema via inhibition of hyaluronidase enzyme. Therefore, it was envisaged to undertake detailed hyaluronidase inhibitory profile on *A. webbiana* roots to validate traditional claims of the plant.

Materials and methods
Collection and identification of plant material
Abies webbiana aerial parts were procured from authentic source D.G. Ayurvedic Sangrah, Andheri, Mumbai, India in November, 2020.

Preparation of various extracts and phytochemical screening
The various extracts from plant material 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) [13]. Crude extracts of *A. webbiana* were screened for detection of different classes of phytoconstituents using specific standard reagents (Farnsworth, 1966) [5].

Hyaluronidase inhibition assay
The various extracts were subjected to Hyaluronidase inhibition assay as per standard procedures available online (Tatemoto *et al*., 2006) [21]. The results have been 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 Tukey's test (Scheffer, 1980) [20].

Estimation of total phenols and flavonoids content

The estimation of total phenols (Folin Ciocalteu's assay) and flavonoids (aluminium chloride assay) content in plant aerial parts was done by using the following standard procedure (Kumar *et al*., 2014) [13].

Results and discussion

Preliminary Phytochemical screening

The aerial parts of plant were successively extracted in a Soxhlet apparatus using solvents in increasing order of polarity viz., petroleum ether, chloroform, methanol and water. The percentage yields (w/w) of various crude extracts of plant aerial parts are presented in table 1. The percentage yield of each extract was calculated as: weight of extract obtained / weight of plant material taken × 100. All extracts of plant aerial parts were dissolved in their respective solvents and screened for different classes of phytoconstituents using specific standard reagents and results are shown in table 2. It is evident from table 2 that petroleum ether extract showing presence of fixed oil; chloroform extract showing presence of steroids, alkaloids, triterpenoids; methanol extract showing presence of tannins, flavonoids and water extract showing presence of carbohydrates, proteins.

Table 1: The percentage yield of various extracts of plant aerial parts.

Extract	Colour of extract	Consistency	Percentage yield (% w/w)
Petroleum ether	Dark green	Semisolid	1.38
Chloroform	Dark brown	Semisolid	1.79
Methanol	Dark reddish brown	Semisolid	5.26
Water	Dark reddish brown	Semisolid	8.49

Table 2: The phytochemical screening of various extracts of plant aerial parts.

Class of phytoconstituents	Petroleum ether extract	Chloroform extract	Methanol extract	Water Extract
Alkaloids	-	+	-	-
Carbohydrates	-	-	-	+
Anthraquinone glycosides	-	-	-	-
Cyanogenetic glycosides	-	-	-	-
Cardiac Glycosides	-	-	-	-
Steroids / Triterpenoids	-	+/+	-/-	-/-
Saponins	-	-	-	-
Coumarin	-	-	-	-
Flavonoids	-	-	+	-
Tannins	-	-	+	-
Proteins	-	-	-	+
Fixed oils	+	-	-	-

+: present, -: absent

Hyaluronidase inhibitory activity

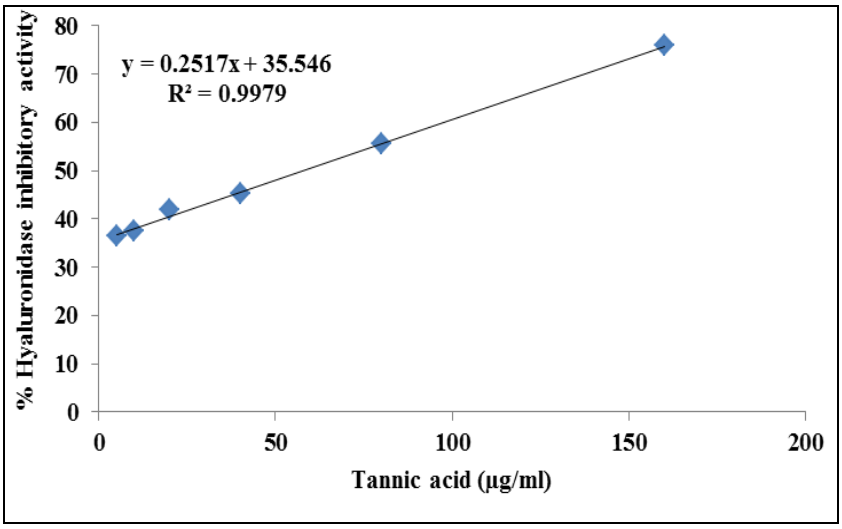
The chloroform, extract, methanol extract and water extract of plant aerial parts were screened for hyaluronidase inhibitory potential using *in vitro* standardized spectrophotometric method. The methanol extract (IC_{50} = 180.21 µg/ml) of plant aerial parts exhibited strong hyaluronidase inhibitory activity followed by respective chloroform extract (IC_{50} = 547.22 µg/ml) and water extract (IC_{50} = 763.09 µg/ml), as compared to standard hyaluronidase inhibitory drug tannic acid (IC_{50} = 57.43 µg/ml). The water and chloroform extracts almost devoid of tested pharmacological activity. The results of the hyaluronidase inhibitory activity are depicted in table 3 and figure 5.

Table 3: The results of hyaluronidase inhibitory activity of standard drug and various extracts of plant aerial parts.

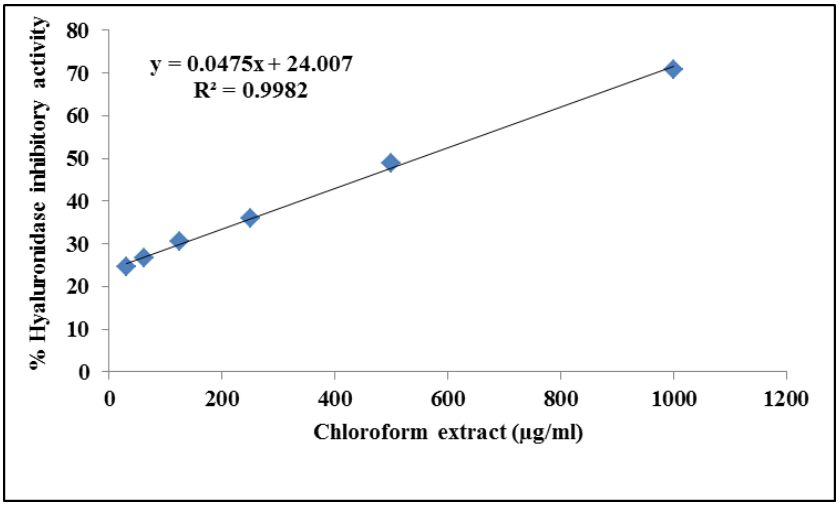
Treatment	Concentration (µg/ml)	% hyaluronidase inhibitory activity (Mean ± S.D.)	IC_{50} values (µg/ml)
Tannic acid	5	37.99 ± 2.04	57.43
	10	39.10 ± 3.78	
	20	42.58 ± 2.69	
	40	46.88 ± 2.78	
	80	54.58 ± 3.58	
	160	70.25 ± 2.88	
Chloroform extract	31.25	24.58 ± 1.55	547.22*
	62.5	26.80 ± 2.48	
	125	30.47 ± 1.98	
	250	35.93 ± 3.12	
	500	48.90 ± 2.47	
	1000	70.92 ± 2.69	
Methanol extract	31.25	40.14 ± 2.40	180.21*
	62.5	43.88 ± 3.25	
	125	46.25 ± 3.02	
	250	54.20 ± 3.64	
	500	70.35 ± 2.88	
	1000	96.47 ± 3.45	

Water extract	31.25	15.27 ± 1.01	763.09*
	62.5	18.07 ± 1.58	
	125	21.89 ± 1.99	
	250	26.47 ± 1.64	
	500	38.58 ± 2.47	
	1000	60.44 ± 3.69	

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



(A)



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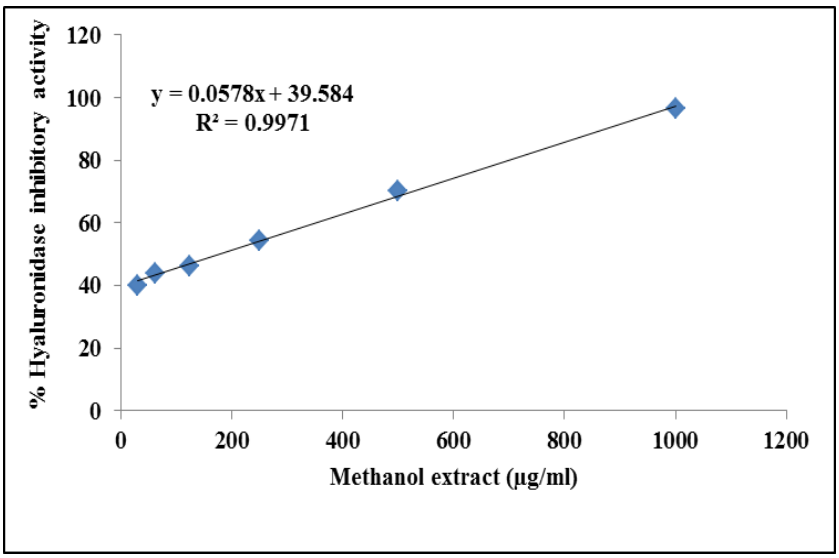

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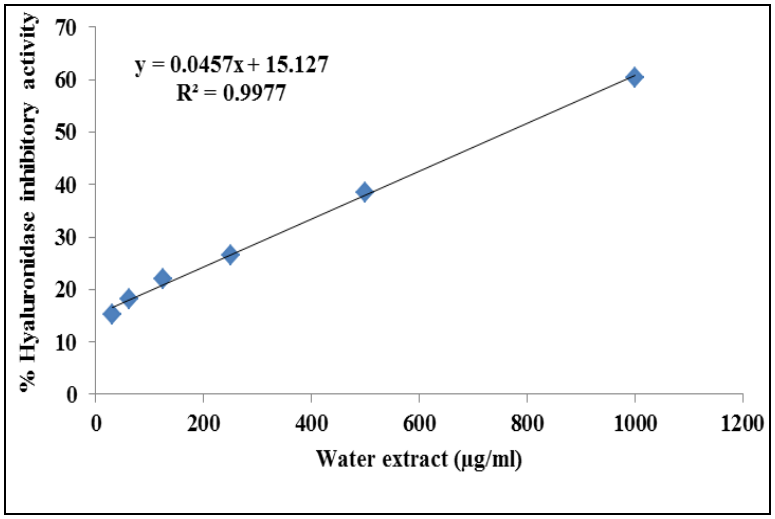


(D)

Fig 5: Graphical representation of percentage hyaluronidase inhibitory activity against concentration of standard drug and various extracts.

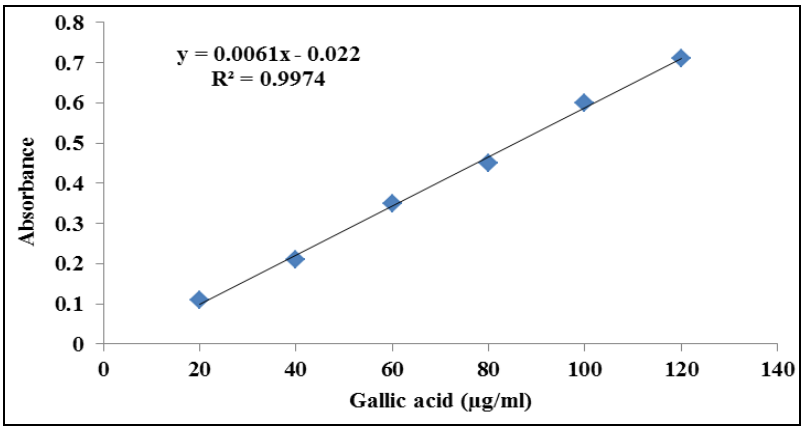
Total phenol and flavonoid contents of plant aerial parts

The methanol extract of plant aerial parts showing presence of phenolic and flavonoid compounds on the basis of preliminary phytochemical screening. The available literature reveals that these are most bioactive group of phytoconstituents exhibited hyaluronidase inhibitory activity. Therefore, quantitative studies were performed to estimate contents of phenolic and flavonoid compounds in methanol extract. Estimation of total phenols and flavonoids was done from the respective regression equations of standard plots of gallic acid (Linearity – 20 to 120 µg/ml; r^2 = 0.9974) and quercetin (linearity – 30 to 180 µg/ml; r^2 = 0.9985) respectively. The contents of total phenols were found to be almost three times in comparison to total flavonoids content. The results of estimation of total phenols and flavonoids content are presented in table 4 and figure 6.

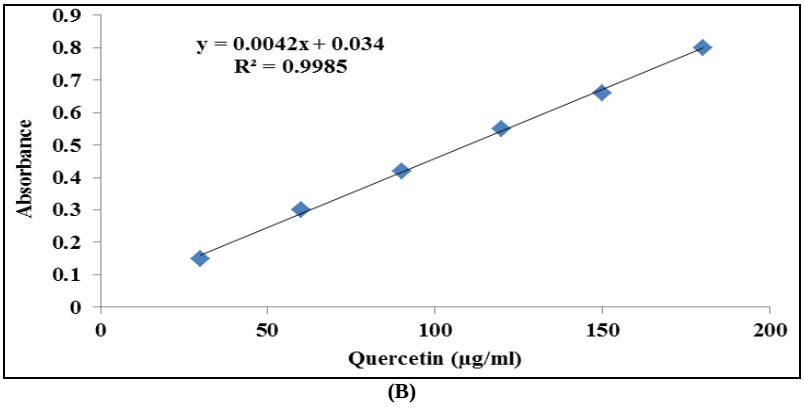
Table 4: Total phenol and flavonoid contents in methanol extract of plant aerial parts.

Test sample	Total phenols content (% w/w) Mean^n ± S.D.	Total flavonoids content (% w/w) Mean^n ± S.D.
Methanol extract	9.11 ± 0.33	3.69 ± 0.19

n=3



(A)



(B)

Fig 6: Standard plots of (A) gallic acid vs absorbance; (B) quercetin vs absorbance.

Preliminary phytochemical studies showed presence of flavonoids and phenolic compounds in bioactive methanol extarct of plant aerial parts. The available literature also reveals that a large number of flavonoid – luteolin, apigenin (Kuppusamy *et al*., 1990) [14], kaempferol, quercetin, myricetin, rutin (Lee *et al*., 2010) [15] and phenolic compounds – protocatechuic acid, chlorogenic acid, methyl chlorogenate, catechin, epicatechin (Ao *et al*., 2010) [1] have been reported to exhibit hyaluronidase inhibitory activity. In agreement to these reports, it is suggested from our results that hyaluronidase inhibitory activity of *A. webbiana* aerial parts is attributed to these bioactive groups of phytoconstituents.

Conclusion

The bioactive methanol extract have shown presence of phenolic and flavonoid compounds, which have been reported to possess strong hyaluronidase inhibitory activity. Thus, it was envisaged to estimate content of total phenol and flavonoid in bioactive methanol extract. The contents of total phenols were found to be almost three times in comparison to total flavonoids content. These observations suggest that it is better to isolate main constituents responsible for hyaluronidase inhibitory activity in future studies using column chromatography.

Acknowledgement

The reaserch facilities provided by Akal College of Pharmacy & Technical Education, Mastuana Sahib, Sangrur are duly acknowledged.

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