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Review Article

Ethnobotanical, Phytochemical and Pharmacological Aspects of *Daphne mucronata* (Thymeleaceae)

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Abstract

Daphne mucronata is a shrub well known as a medicinal plant in different regions of Asia. Ethnobotanical, phytochemical and pharmacological studies have revealed strong anti-cancer potential of the plant. Literature reports the evaluation of the initial bioactivity profile and extraction of the plant followed by different chromatographic techniques to obtain fractions. As an outcome, isolation and identification of coumarins, flavonoids, triterpenoids, lignin cumarinolignans, glucosides, daphnecin, aquillochin, daphnine and umbelliferone from the plant have been reported. Of these compounds, a diterpene, named gnidilatimonoein, has shown promising anticancer potency in in vitro tests on various cancer cell lines. This review article is an effort to summarize literature published in recent years on the bioactivity of Daphne mucronata.

Keywords: *Thymelaeaceae, Daphne mucronata, Diterpene, Gnidilatimonoein, Anti-cancer, Bioactivity*

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INTRODUCTION

Ethnobotany is the principal approach to investigate natural resource management of native society. It is a science of human synergy with plants and ecosystem [1]. To relieve discomfort, disease or pain, people generally use those plants which are repeatedly emphasized by informants as a possible cure for the particular illness compared to other plants which are less known.

Thymelaeaceae family constitutes of 50 genera and 900 species that are mostly impound Asia, Africa and Australia. It is typified in Pakistan by 5 genera and 7 species [2]. *Daphne mucronata* is a wild shrub of the Thymelaeaceae family and is distributed in Northern areas of Pakistan as well as in several regions of Iran and is considered as an important medicinal plant in these areas; it is well known for its ethno pharmacological importance and has been employed in conventional medication for the treatment of various diseases [1-8]. The common names of the plant are presented in Table 1.

Generally, its flowering period is from April to May, life cycle is perennial and habitat is woodland [3,8,9]. *Daphne mucronata* is used as a remedy for skeleto-muscular problems whereas its fruit and leaves mash dressing is used against rheumatism [3,5,9]. Its liniment is effective in treating infectious wounds, the plant material is ground and mixed with water to

prepare the paste; moreover, a pain relieving effect has been observed when weary muscles are either exposed directly to the smoke of branches or steam of its water extract; its decoction and cooked leaves are employed for curing women infertility, gynecological problems, infections, menstruation disorders and constipation [7].

Table 1: Common names of *Daphne mucronata* in different languages

Name	Language	Reference
Toye	Elam	[8]
Alef	Kurdish	
Lovomekeen	Chitrali	[4]
Khoshak		
Kheshk	Persian	[7]
Khesht-e-garmsiri		
Daphne	English	[8]
Laighonai	Pashto	[1,3,9]

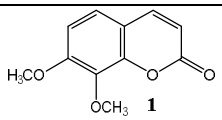
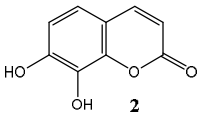
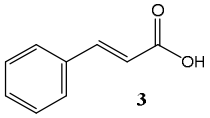
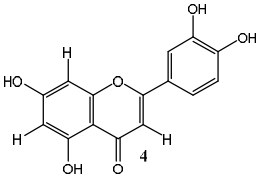
The fruit of *Daphne mucronata* is edible and its fruit poultice is used for treating pimples and freckles on face. Although its leaves are poisonous for animals, but they are bearable by goats, therefore, the laniment of leaves is applied to cure abscesses in goats. Bark of the plant is used to cure disease of bones and for washing hair [4,6]. Plant extract is considered good for curing skin related diseases and allergies [1,6]. Its wood is finely ground to make powder (surmeh) which is either applied directly with the help of a wooden stick or mixed with rose water, and is also used as an eyeliner to clean eyes and relieve eye pain [8]. The non-medicinal uses of *Daphne mucronata* include boiling of dried branches in water to produce yellow color which is used for leather dyeing; flowers are ornamental; wood is used as fuel and to make gunpowder charcoal [5,7].

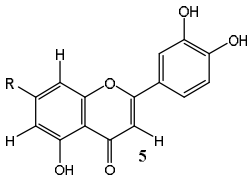
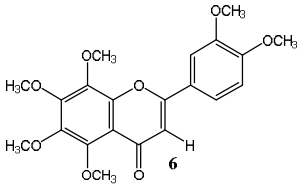
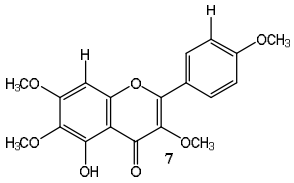
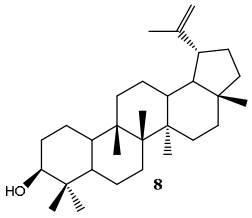
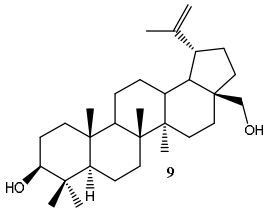
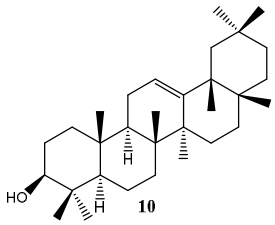
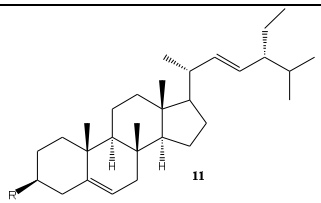
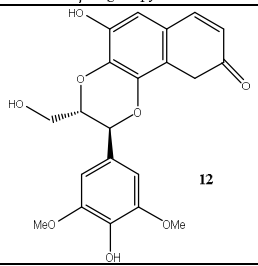
Isolation, extraction and phytochemical analysis

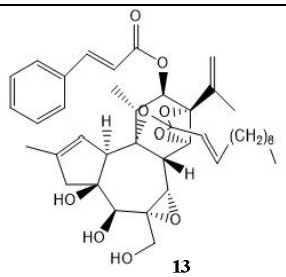
Methanol extract of shade dried *Daphne mucronata* was dried and dissolved in water and extracted with regular solvents including n-hexane, chloroform, n-butanol, water and ethyl acetate by Rasool *et al* [2]. The column and flash chromatography of ethyl acetate fraction eluted with chloroform/methanol mixture (with increasing methanol concentration) was reported. This resulted in six fractions. Further column chromatography of these fractions resulted in the isolation of thirteen compounds which are presented in Table 2 [2,10-12].

Daphne comprises diverse range of biologically active natural products including coumarins [13,14], flavonoids [13,15,16], triterpenoids [13], lignin [17] and cumarinolignans [2,18]. Glucosides, daphnecin, coumarin, aquillochin, daphnine and umbelliferone are among the important constituents of *Daphne mucronata* (Table 2) [1,11].

Table 2: Classes and structures of compounds isolated from *Daphne mucronata* [2,10-12]

Sr. No.	Class	Structure	Name
1	Coumarins	 1	7,8-Dimethoxycoumarin
		 2	7,8-Dihydroxycoumarin
2	Carboxylic acids	 3	Cinnamic acid
3	Flavanoids	 4	5,7,3',4'-Tetrahydroxyflavone

		 R = 7-*O*-*β*-D-glucopyraniside **5**	5,3',4'-Trihydroxyflavone 7-O-β-D-Glucopyronaside
		 **6**	5,6,7,8,3',4'-Hexamethoxyflavone
		 **7**	5-Hydroxy-3,6,7,4'-Tetrahydroxyflavone
4	Triterpenoids	 **8**	Lupeol
		 **9**	Betulin
		 **10**	B-Amyrin
5	Sterols	 **11** R = 7-*O*-*β*-D-glucopyraniside	Stigmasterol 3-O-β-D-Glucopyranoside
6	Coumarinolignin	 **12**	Daphnecin

7	Diterpene		Gnidilatimonoein

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Pharmacological aspects

Thymelaeaceae family is often documented as pharmacologic agent such as anti-leukemia [19], anti-tumor [20], abortifacient [21], anti-gout [22], anti-inflammatory [23], anti-microbial [24], nucleic acid inhibitor [25], against breast adenocarcinoma [26] and cell adhesion inhibitor in folk medicines. To evaluate the pharmacological behavior of *Daphne mucronata*, it has been reported that the compounds 1 to 11 (table 2) isolated from plant extract were subjected to 1, 1-diphenyl-2-picryl-hydrazil (DPPH) assay. The reported IC_{50} values presented in Table 3 show the anti-oxidant potential of these compounds [2].

Table 3: The IC_{50} values of compounds isolated from *Daphne mucronata* against standard in DPPH assay

Compounds	IC_{50} (µM/mL)± SEM.*
1	314.125 ± 1.047
2	Inactive
3	341.023± 0.349
4	257.853 ± 0.258
5	296.854 ± 1.258
6	356.012 ± 1.023
7	324.156 ± 2.035
8	370.015±2.125
9	Inactive
10	Inactive
11	Inactive
Standard	BHA ± 44.0

**SEM = standard error mean taken from triplicate experiments*

Literature reveals that the bioactive components from genus *Daphne* reside in roots, stem or leaves of the plant [21,27-31]. The ethanolic extract of leaves, stem and roots of *Daphne mucronata* have been reported to possess anti-microbial effects against *Escherichia coli* and *Pseudomonas aeruginosa* whereas the ethanolic extract of root alone has been stated to show activity against *Staphylococcus aureus* and *Bacillus subtilis*, however, the toxicity was highest in the leaves extract (LD_{50}: 403µg/mL) and lowest in the root extract (LD_{50}: > 1000 µg/mL); this association of root extract with low and leaf extract with high toxicity was verified by brine shrimp test. However, no part of the plant has been stated to show anti-fungal activity [32]. Several species of *Daphne* possess anti-leukemia activity and have been used for this purpose since long [13,21,33,34].

Moreover, ethanolic extract of *Daphne mucronata* holds breast anti-cancer potential; during *in vivo* studies, this anti-cancer behavior was evaluated by first introducing breast tumor in rats with 5 mg administration of 9, 10-Dimethyl-1, 2-benzanthracene (DMBA) two times per week for one month, subsequently, the rat was orally administrated with 50 % ethanolic plant extract for 20 days and a significant reduction in diameter of breast tumor has been reported [26] whereas complete tumor elimination was reported with long term dosage of the ethanolic plant extract [35]. The ethanolic extract of *Daphne mucronata* leaves has cytotoxicity and anti-tumor activity against K562 and CCRF-CEM human leukemia cell lines. The reported LC_{50} values of *Daphne mucronata* extract based on shrimp test are 0.062 mg/L for plant leaves and 0.3 mg/L for plant stem [36]. It has been reported that *Daphne mucronata* extract shows a decrease in cell viability on leukemia cell lines by a factor of four whereas the plant extract concentration required a half reduction in cell number as expressed in Table 4.

Taxol is a natural antineoplastic agent which has the tendency to down regulate tumor necrosis factor alpha (TNF-α) receptor and stimulate its release in isolated human monocytes. *Daphne mucronata* extract has the tendency to increase the release of TNF-α and down regulate the TNF-α receptor by a mechanism identical to toxic on cultured human monocytes [26,35]. TNF-α determination in co-culture cell media revealed that the concentration of TNF-α increased in a dose dependent manner under the effect of plant extract (Table 5).

Table 4: The independent effect of *Daphne mucronata* extract as well as when co cultured with human monocytes on cell viability percentage in K562 and CCRF-CEM cell lines [36]

Diluted extract volume (µL)	Cell viability in K562, (%)		Cell Viability in CCRF/CEM, (%)	
	Without human monocytes	*With human monocytes*	*Without human monocytes*	*With human monocytes*
0	100	100	100	100
10	95	80	81	97
20	93	60	61	95
40	90	40	50	93
80	80	39	40	83
160	53	--	--	58
320	30	--	--	33

Table 5: Effect of *Daphne mucronata* extract on TNF-*α* release [36]

TNF-*α* (Pg/mL)	Diluted extract volume (µL)
25	0
100	10
155	20
250	40
160	80

Daphne mucronata contains a very promising antineoplastic diterpene known as Gnidilatimonoein. Literature reports the cytotoxic activity of gnidilatimonoein against various cancer cell lines like K562, HL-60, CCRF-CEM and MOLT-4 leukemia cell lines, prostate cancer cell line LNaP-FGC-10 and a mouse BALB/C fibrosarcoma cell line WEHI-164 [37]. Yazdanparast and Sadeghi have reported that gnidilatimonoein inhibits the cell growth in K562 cell lines through G1 phase by 15 % as compared to the untreated cells and the population in G2 and S phases of treated cells also decreased by 8.3 % and 5.4 % respectively [37]. Gnidilatimonoein effects the DNA synthesis mainly and to a lesser extent RNA synthesis in treated cells. It also reduces the activity of inosine-5'-monophosphate dehydrogenase (IMPDH) by 44 % in the treated cells which depicts that purine biosynthetic pathway is troubled by gnidilatimonoein. However, the exact mode of action of gnidilatimonoein on IMPDH activity is still unknown [37].

Daphne mucronata extract and its purified diterpene has a tendency to transform cell surface glycoprotein's of WEHI-164 cancerous cells in a single none toxic dose which inhibits later on their adhesion to substrate such as fibronectin coated wells. It has been reported that morphology of treated cells changed significantly and they became spherical shaped [38]. Gnidilatimonoein possesses strong anti-proliferative and anti-tumor activities. It can induce monocytic differentiation and apoptosis in HL-60, KG1, NB4 and U937 leukemia cell line in a dose- and time dependent manner [12,39]. However, the low concentrations of drug has been proven by the scientists as an effective generator of differentiation in leukemia cell toward monocyte linages; apoptosis finally eliminates the differentiated cells [12]. The IC_{50} of the diterpene was found to be 1.3 µM [39].

CONCLUSION

Daphne is an important genus of Thymelaeaceae family, and it exhibits pharmacological activities of significance. *Daphne mucronata* is a member of this genus and is used as folk medicine in Iran and northern areas of Pakistan. The extract of *Daphne mucronata* has a reducing effect on rat breast adenocarcinoma and enhances TNF-α release from monocytes. The mechanism of action of the plant extract in the body is similar to the mechanism of action of taxol. [36,40-42]. Gnidilatimonoein is a potential candidate for the development new anti-leukemia drugs.

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