

# Airways and vascular smooth muscles relaxant activities of *Gaultheria trichophylla*

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**Abstract:** The aim of this experimental work was to explore the potential pharmacological activities of *Gaultheria trichophylla* Royle in hyperactive respiratory and vascular conditions. *Gaultheria trichophylla* was extracted with solvents, phytochemical detection tests were performed, and rabbit trachea and aorta strips were used to evaluate its effects on airways and vascular smooth muscles. Qualitative phytochemical tests showed the presence of flavonoids, alkaloids, anthraquinones, saponins, terpenoids, and condensed tannins. The methanol extract caused inhibition (EC<sub>50</sub> values of 3.12 mg/mL) of carbachol (1 μM) and partial relaxation of K<sup>+</sup>(80 mM) caused contractions in tracheal strips. The chloroform extract was comparatively more potent against carbachol than K<sup>+</sup> induced contraction with EC<sub>50</sub> values of 0.64 and 2.26 mg/mL, respectively. However, the n-hexane extract showed more potency against K<sup>+</sup> than cabachol induced contractions, as in case with verapamil, with EC<sub>50</sub> values of 0.61 and 6.58 mg/mL, respectively. In isolated prepared trachea, the extracts displaced the carbachol concentration response curves and maximum response was suppressed. In rabbit aorta preparations, methanol and n-hexane extracts partially relaxed phenylephrine (1 μM) and K<sup>+</sup> induced vasoconstrictions. However, the chloroform extract inhibited phenylephrine induced contractions and exhibited a vasoconstrictor effect at lower concentrations and a relaxant effect at higher concentrations against K<sup>+</sup> precontractions. The data indicates that, in addition to others, the extracts of *G. trichophylla* possess verapamil like Ca<sup>++</sup> channel blocking components which explain the possible role of this plant in respiratory and vascular conditions.

**Keywords:** *Gaultheria trichophylla*, rabbit trachea, rabbit aorta, Ca<sup>++</sup> antagonist.

## INTRODUCTION

*Gaultheria trichophylla* belongs to family Ericaceae which consists of 90 genera and over 1700 species. It is native to Himalayas and commonly known as Himalayan snowberry (Kao, 1987). The flowers range in color from red to pink; fruits are blue-colored berries; and small green leaves are approximately 3-7 mm in length. This plant is an inhabitant of cold and lofty situations of the mountains and like many plants of such situations it is furnished with setae (Royle, 1839; www.efloras.org). Other species of the genus *Gaultheria* like *G.yunnanensis*, *G. fragrantissima* and *G. procumbens* are used in traditional medicine to treat arthritis in China, India, America and Canada (Simon *et al*; 1984). Research work on other species of this plant indicates anti-inflammatory, antibacterial, and anti-arthritis activities. Gaultherin a natural salicylate isolated from *G. yunnanensis* possess analgesic and anti-inflammatory activity. The phytochemical investigation of *Gaultheria* reported to contain methyl salicylate, diterpenoids, acids, dilactone, alkaloids and glycosides (Liu *et al*; 2005).

To our knowledge, there is no report in the literature on pharmacological and or biological activities of *Gaultheria trichophylla*. The present work was therefore conducted to investigate the potential usefulness of *Gaultheria trichophylla* in respiratory and vascular ailments using *in vitro* pharmacological protocols.

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## MATERIAL AND METHOD

### *Plant material*

*Gaultheria trichophylla* herb was collected by the author from Kaghan valley, District Mansehra, KPK, Pakistan (November, 2013). After authentication by taxonomist, Professor Dr. Qazi Najum us Saqib, voucher specimen (CTPHM-GT01, 13) was deposited in the herbarium of the Department of Pharmacy, COMSATS, CIIT, Abbottabad. The whole plant was washed with water and dried in shade at room temperature and was grinded to coarse powder. The powder drug was stored in a container to keep safe from air and light before extraction.

### *Extract preparation*

200g of powder material was extracted with each of methanol, chloroform and n-hexane using soxhlet extractor for 20 hours each. It was filtered through a Whatman Grade-I filter paper. The filtrate was reduced on a vacuum rotary evaporator at 40°C. Extractive yields (percent) of the methanol (Gt. Meth), chloroform (Gt. Chlor) and for n-hexane (Gt. Hex) were 21.85%, 16.35% and 7% respectively.

### *Preliminary phytochemical analysis*

Preliminary analysis of the crude extracts of *G. trichophylla* was carried out for the detection of various classes of phytochemicals, like saponins, alkaloids, coumarins, terpenes, sterols, flavonoids and tannins (Gilani *et al*; 2005).

**Chemical and drugs**

Analytical grade chemicals were used in all the experiments. Acetylcholine chloride, carbochol, dimethylsulfoxide (DMSO), ethylene diamine tetraacetic acid, glucose, potassium chloride, phenylephrine, magnesium chloride, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, and sodium dihydrogen phosphate were purchased from Sigma (St. Louis, MO, USA). The salt calcium chloride was used of Merck (Darmstadt, Germany).

hexane extracts of *G. trichophylla* on isolated rabbit trachea (All values are expresses as the mean  $\pm$  SEM; n=3).

**Preparation of samples**

The stock solution of methanol and chloroform extract (300 mg/mL) was prepared in dimethylsulfoxide (1% w/v solution) which was then made dilution of 30 and 3 mg/mL. The fresh physiological salt solutions were prepared in distilled water prior to start of experimental work.

**Animals**

Adult rabbits of either gender were used each weighing 1.0 to 1.5 kg. Animals were maintained in optimum conditions in well aerated animal house at the institute and provided with fresh green fodder, standard diet and water *ad libitum*. The experiments were performed according to the rulings of National Research Council (1996) about the Laboratory Animals Resources, Commission on Life Sciences, and approved by the Ethical Committee of Department of Pharmacy, COMSATs CIIT, Abbottabad.

**Isolated rabbit tracheal preparations**

The dissected trachea was cut into rings each containing two cartilages. A longitudinal cut was made to open up the ring on ventral side opposite to the smooth muscle layer, resulting into a tracheal strip with of smooth muscle located centrally. The prepared strip was suspended in a 10 mL tissue bath filled with Kreb's physiological salt solution at 37°C and supplied with carbogen. The Kreb's solution was composed of (mM):

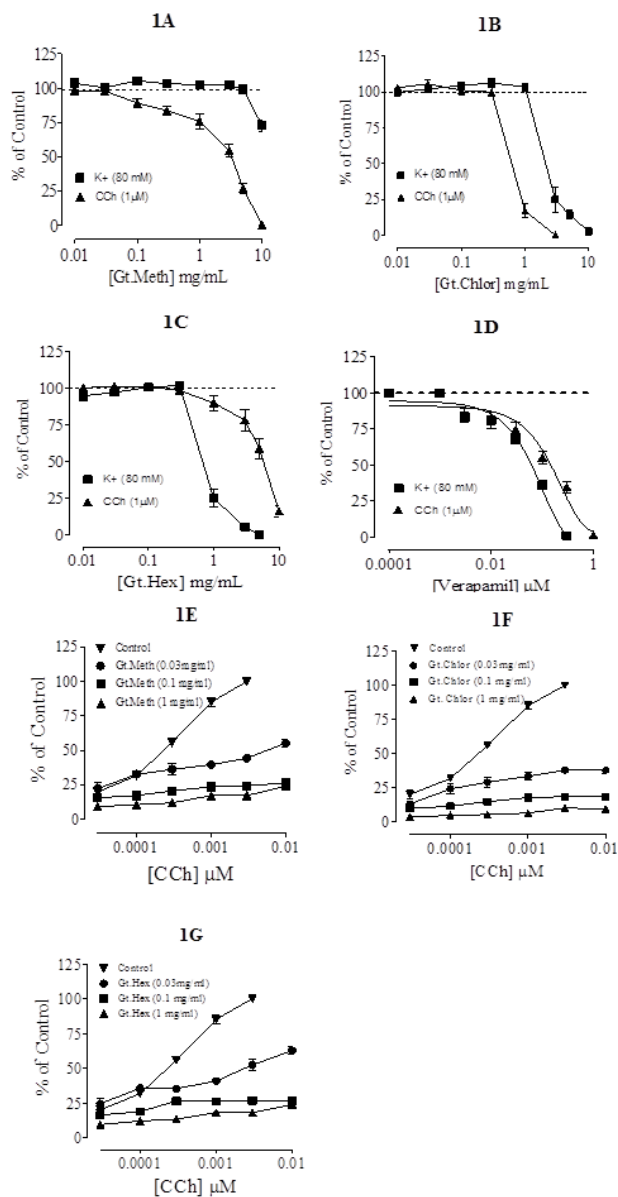
KCl4.7, NaCl 118.2, NaHCO<sub>3</sub> 25.0, CaCl<sub>2</sub> 2.5, KH<sub>2</sub>PO<sub>4</sub> 1.3, MgSO<sub>4</sub> 1.2 and glucose 11.7 (pH7.4).

The tracheal preparation was equilibrated for about 60 min before recording isometric contractions via force displacement transducers connected to a Power lab Data Acquisition System (AD Instruments, Sydney, Australia) (Gilani *et al*; 2010).

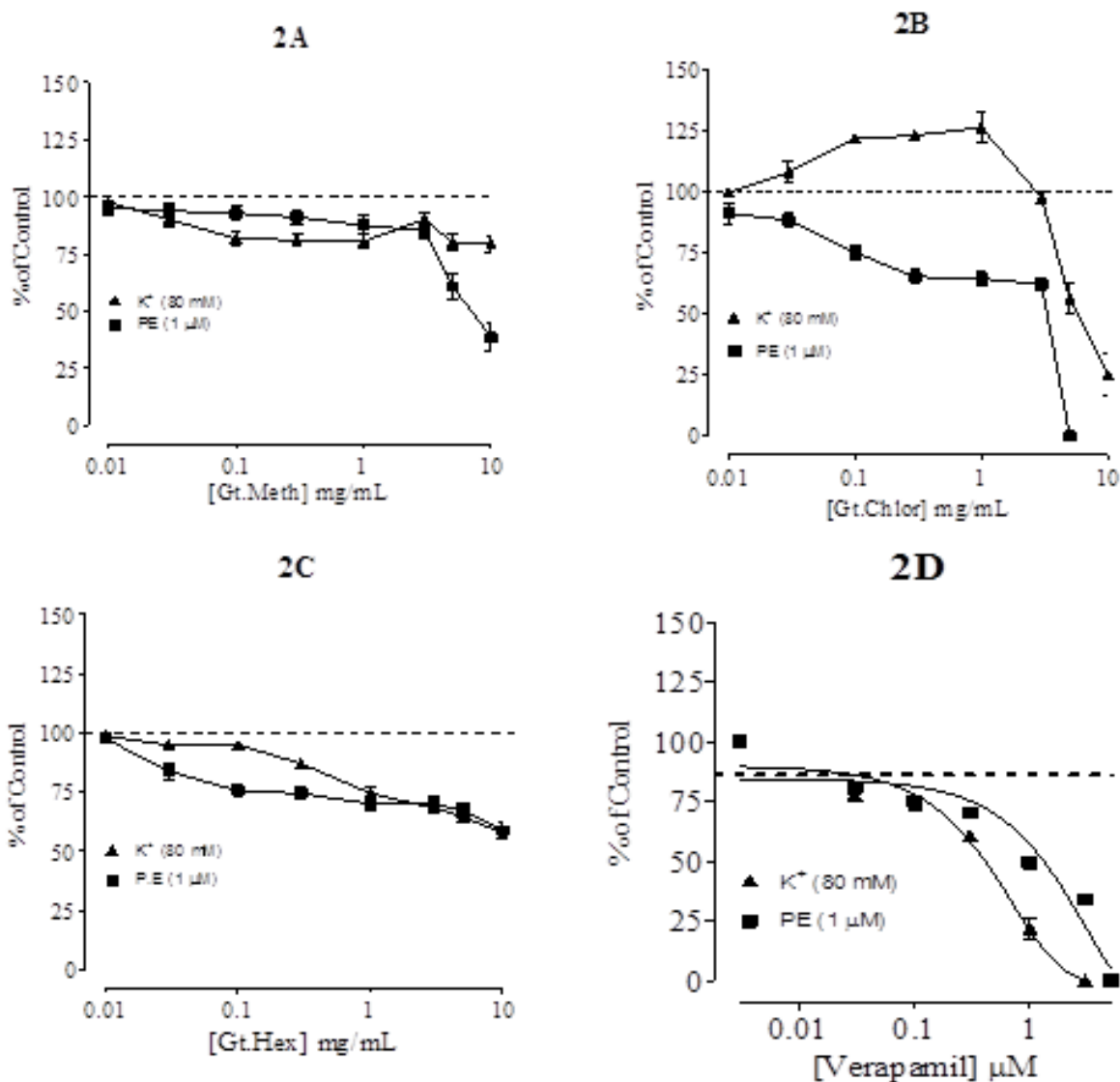
K<sup>+</sup> (80mM) and Carbachol (1 $\mu$ M) were applied to produce sustained contractions in prepared isolated trachea strips, on which the possible relaxant activity of the extracts was studied incumulative fashion. The carbochol cumulative concentration response curves for were constructed through increase in concentration of agonist in tissue bath in a cumulative fashion. The tissues were bathed to re-establish the base-line tension, and concentration response curve for carbachol was prepared in the presence of different concentrations of the extracts (Gilani *et al*; 2010).

**Isolated rabbit aorta preparations**

The rabbit chest was opened and dissected out ((Gilani *et al*; 2010). The aorta was kept in Kreb's solution. The aorta rings were prepared by cutting 2-3 mm wide pieces. Aorta was mounted in 10mL tissue bath in Kreb's solution



**Fig. 1:** Concentration dependent inhibitory effect of *G. trichophylla* (1A) methanol (1B) chloroform (1C) n-hexane extracts (1D) verapamil on carbochol (CCh) and high K<sup>+</sup> induced contractions in isolated rabbit tracheal preparation (1E, 1F and 1G) Concentration response curve of carbochol in the absence and presence of increasing concentration of methanol, chloroform and



**Fig. 2:** Concentration dependent inhibitory effect of *G.trichophylla* (2A) methanol (2B) chloroform (2C) n-hexane extracts (2D) verapamil on phenylephrine (PE) and high  $K^+$  induced contractions in isolated rabbit aorta preparation (All values are expressed as the mean  $\pm$  SEM; n= 3).

and tuned at 37°C. A preload of about 2 g was applied to each tissue preparation and tissues were equilibrated for about 1 hr before the test materials were introduced. The vasorelaxant activity of the extracts was investigated through cumulative addition to tissue bath on phenylephrine (1  $\mu$ M) or  $K^+$  (80 mM) induced contractions. The changes in isometric tension of the aortic ring were recorded.

### STATISTICAL ANALYSIS

The results for activities are presented as the  $\pm$  SEM.  $EC_{50}$  values with 95% confidence interval were calculated using the Graph Pad Prism program version for Windows, (San Diego, USA). The nonlinear regression sigmoidal

response curve was applied to analyze the dose-response curves.

### RESULTS

When tested in isolated trachea, methanol extract of *G. trichophylla* (Gt. Meth) caused inhibition of carbachol (1  $\mu$ M)-induced contractions (3.12 mg/mL (3.11-3.64; n=3) while partially inhibited  $K^+$  (80 mM) pre-contractions (fig. 1A).

Among the other extracts tested, the chloroform (Gt.Chlor) and hexane (Gt.Hex) extracts also inhibited carbachol induced contractions with  $EC_{50}$  values of 0.64 (0.63-0.88., n=3) and 6.58 mg/mL (6.343-7.12; n=3)

respectively, (fig. 1B & C). When experimented against high  $K^+$  pre-contractions, both chloroform and n-hexane extracts caused complete inhibition with  $EC_{50}$  values of 2.26 (2.19-2.83, n=3) and 0.61 mg/mL (0.354-0.911, n=3) respectively.

The methanol, chloroform and n-hexane extracts at low bath concentration of 0.03, 0.01 and 1 mg/mL displaced the carbachol concentration response curves to the right and maximum response was suppressed. The order of suppression was chloroform > methanol > n-hexane (fig. 1 E, F & G).

The extracts of *G. trichophylla* were parallel tested in vascular smooth muscle preparations from rabbit. The methanol extract (Gt. Meth) when tested on phenylephrine (1  $\mu$ M)-induced contractions in isolated aorta strips of rabbit, it showed a relaxant effect with  $EC_{50}$  values 5.12 mg/mL (4.372-5.707; n=3) and was found without any significance inhibitory effect on  $K^+$  (80 mM) induced contractions (fig. 2A). The n-hexane extract was also less effective against the pre-contractions induced with PE and high  $K^+$  (fig. 2C). However, the chloroform extract (Gt. Chlor) caused inhibition of both phenylephrine and  $K^+$  (80 mM) pre-contractions with more potency against the former (fig. 2B), with respective  $EC_{50}$  values of 3.52 (3.261-3.812; n=3) and 5.53 mg/mL (5.112-5.823; n=3).

The extracts on preliminary phytochemical analysis indicated the presence of saponins, flavonoids, alkaloids, terpenoids, anthraquinones and condensed tannins.

## DISCUSSION

The purpose of this study was to explore the pharmacological basis for the use of *Gaultheria trichophylla* in airway and vascular conditions.

This study indicates that Gt. Meth possibly possess chemical (s) having anticholinergic activity, which may be responsible for the selective inhibition in the carbachol induced contractions. However, partial inhibitory effect against the high  $K^+$  pre-contractions indicates presence of additional relaxant component (s).

The components which cause the blockade of  $Ca^{++}$  channel results in non-parallel shift of CCh concentration response curves and maximum response is suppressed (Kamei and Kasuya, 1992).

Potassium is known to increase in intracellular  $Ca^{++}$  which occurs either via influx through voltage-dependent  $Ca^{++}$  channels (VDCs) or its release from sarcoplasmic reticulum. This causes periodic depolarization and regulates smooth muscle tone. The depolarization appears as a rapid influx of  $Ca^{++}$  through VDCs (Brading, 1981). Thus, the relaxant effect of Gt. Hex and Gt. Chlor against

the high  $K^+$  pre-contractions suggest their inhibitory effect on  $Ca^{++}$  moment, possibly, through VDCs. Verapamil, a standard calcium channel blocker (God frained *et al*; 1986) also caused inhibition of the high  $K^+$  pre-contractions, as expected from a  $Ca^{++}$  channel blocker (CCB). The data indicates that hexane and chloroform fraction of *G. trichophylla* possess verapamil like  $Ca^{++}$  channel blocking constituents, which may be partly responsible for the smooth muscle relaxation in the rabbit trachea. This finding may provide a possible pharmacological rationale for the potential use of *G. trichophylla* in hyperactive respiratory disorders, like asthma, as  $Ca^{++}$  channel blockers are considered effective in hyperactive airways diseases (Boushley, 2006; Ghayur *et al.*, 2007).

Gt. Chlor also exhibited a vasoconstrictor effect on high  $K^+$  pre-contractions at lower concentrations while at higher concentrations caused inhibition of the aortic pre-contractions. Both the vasoconstrictors and vasodilators types of constituents are present in the both fractions of *G. trichophylla* as clearly indicated by the above data. The less potent nature of the methanol and n-Hexane extracts suggests the presence of vasoconstrictor constituents that interfered with their vasodilator effect. The chloroform fraction exhibited dual vascular effect, a vasodilatation against PE pre-contractions and combination of vasoconstriction, at lower concentration and vasodilatation at higher concentration against high  $K^+$  pre-contractions of rabbit aorta.

## CONCLUSION

The data indicates that extracts of *G. trichophylla* possesses combination of anticholinergic and  $Ca^{++}$  channel blocking constituents, which possibly explain its potential medicinal use in respiratory and vascular or cardiovascular disorders.

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