Study on the Smooth Muscle Relaxation Activity of *Ageratum conyzoides* L. (Khway-thay-pan) Leaf

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Abstract

Ageratum conyzoides L. (Khway-thay-pan) leaf, reputed in folk medicine for its value as an anti asthmatic remedy, was selected for this study. The study of this plant was carried out to investigate the phytoconstituents and smooth muscle relaxation activity in both normal and histamine-induced guinea-pig. The compounds were isolated as stigmasterol, myristic acid, palmitic acid, 9-hydroxy nonan-2-one and kaempferol from *A. conyzoides*. *In vitro* screening of relaxant effect of the different extracts revealed the activity in the order of Khway-thay-pan (70 % EtOH) (74.2%) > Khway-thay-pan (H_2O) (59.6 %) of relaxation response. Maximum dose for both extracts was found to be 2 mg per cm^3 bath concentration. Isolated compounds exhibited anti asthmatic effect in the order of stigmasterol (56.5%) > kaempferol(49.3%). From these results, it is suggested that this plant should be used as a remedy for the treatment of bronchial asthma.

Keywords: Bronchial Asthma, Smooth Muscle Relaxation Activity, Histamine, Guinea pig, Aqueous and 70% ethanolic extracts

Introduction

Indigenous medicine is widely practised in Myanmar due to its long and deep rooted tradition and also due to the trust placed by the people in its therapeutic qualities. The uses of medicinal plants for treatment of the disease are different in different areas of our country. The number of asthmatics has increased during the last decades, mainly due to air pollution and improper ventilation (Barnes, et al., 1988). There are many traditional medicines and medicinal plants used for the treatment of Asthma. Commonly used medicinal plants include *Clerodendrum indicum* (L.) Kuntze (Nga-yant-padu), *Ageratum conyzoides* L. (Khway-thay-pan), *Piper betle* L. (Kun-ywet), *Ocimum sactum* L. (Pin-zein), *Piper longum* L. (Peik-chin), *Coelus aromaticus* Benth (Ziyar-ywet-htu), *Adhatoda vasica* Nees (Mu-ya-gyee), *Aegle marmelos* Correa (Okshit) and *Mimosa pudica* L. (Kon-hti-ga-yon). They are employed for treating conditions such as bronchitis, sore throat, tightness of chest, cough and expectorant.

Among these, three of medicinal plants such as *Piper betle* L. (Kun-ywet), *Coelus aromaticus* Benth (Ziyar-ywet-htu) and *Ageratum conyzoides* L. (Khway-thay-pan) have been reported to have relaxation effect on isolated guinea pig tracheal smooth muscle (Aye Than *et al*., 1995). The extracts of leaves of *Adhatoda vasica* Nees (Mu-ya-gyee) contain vascinone alkaloid which produced relaxation of isolated tracheal smooth muscle of guinea-pig (Mya Mya Than, 1983). The bronchodilating activities of some medicinal plants were also reported by Indian Council of Medical research. Alcoholic extract of *Tylophora indica* (Burn. F.) Merr. (Oo-pa-tha-ka) showed no specific smooth muscle relaxant action on isolated guinea pig tracheal smooth muscle. The extracts of the leaves of *Acorus calamus* L. (Lin-nay), *Acalypha indica* L. (Kyaung-yo-thay), *Hedychium spicatum* Ham. (Kun-sa-gamon-gyi) have been reported to bring about significant relief in bronchospasm and other clinical signs and symptoms in patient with bronchial asthma (Chandra, 1980). The aqueous extracts of *Coelus aromaticus* Benth (Ziyar-ywet-htu) (Aung Htay Oo, 1996) have presented the antiasthmatic action against histamine or acetylcholine induced bronchospasm in guinea pig and rabbit. Therefore, in this study comparison between different extracts and its isolated compounds having smooth muscle relaxation activity on trachea is illustrated. The result obtained from this

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study will enhance the existing knowledge of medicinal plants, and also will go a long way to helping the poor folks in our locality who tend to prefer herbal therapy to conventional medicine to manage asthma at lower costs.

Materials and Methods

Plant materials

The leaves of *A. conyzoides* L. (Khway-thay-pan) were collected from Yangon Region. This sample was identified by an authorized botanist, lecturer in Department of Botany, Yangon University. The collected sample was cleaned, air-dried and pulverized into powder using a grinding mill and then stored in the screw-capped bottles.

Preparation of Different Extracts

The dried, purified and powdered sample (ca 50 g) was put into a 1 L conical flask and distilled water was added up to 600 ml. The same amount of each sample was packed in the cotton bag and placed in the Soxhlet extractor, equipped with a round bottomed flask (500 cm^3) containing 250 cm^3 of ethanol. Extractions were done by heating the flask in the water bath. After 6 hours of such extraction, it was cooled to room temperature and filtered. The filtrate was then evaporated to dryness over a water bath using evaporator porcelain basin.

Effect of plant extracts on tracheal chain isolated from Guinea pig

(a) Preparation of isolated tracheal chain from Guinea pig

The isolated trachea chain from guinea pig was done according to the method described by Castillo and De Beer (1947).

A guinea pig was killed by a blow on a head and cutting the throat. The neck and upper thorax were opened and the muscles surrounding the trachea were cleared. A length of trachea about 4-6 cm was dissected out from guinea pig and placed in a oxygenated Krebs solution in a petridish. By transverse cuts, each tracheal ring containing two cartilage bands were made. Tracheal rings were tied with a fine thread in a series so that the smooth muscle was in a longitudinal plane and consecutive rings have muscle on opposite sides. About 8-10 tracheal rings were tied to obtain a chain.

The chain was placed in an organ bath, a thread from one end of the chain was fixed to the tissue holder and a thread from the other end connected to a simple lever which was attached with a writing point. The organ bath was filled with Krebs solution and aerated with oxygen and temperature of the bath kept at 37 °C. The magnification of the lever for recording was 10-12 folds and a constant tension on tissue was kept between 0.4-0.6 g. An appropriate passive tension was applied and the strip was allowed to equilibrate for half an hour.

(b) Action of extracts on tracheal chain isolated from Guinea pig

Following the equilibrating period, the effect of extracts on isolated tracheal chain was studied at different doses i.e., 0.5 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg per cm^3 bath. Each dose of extracts was left in contact with tissue for exactly 5 minutes and the effect was recorded. Then the organ bath was washed 3 times and allowed to rest for 15 minutes or more until the tissue recovered to the normal base line.

The same procedure was carried out on the strip which was applied with different concentration of different extracts. Each concentration of different extracts was tested for 3 times.

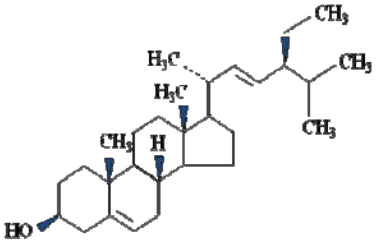
(c) Action of extracts and isolated compounds on histamine induced tracheal chain

Following the equilibrating period, the strip was allowed to contract by adding histamine (1 μg/cm^3 bath conc.) to the organ bath, left for 5 minutes, washed 3 times and allowed to rest for 15 minutes or more until the tissue recovered to the normal base line. After that histamine (1 μg/cm^3 bath conc.) was added to the organ bath, left for 3 minutes and followed by extract (1 μg/cm^3 bath conc.) without washing the bath and left for another 2 minutes before washing and the effect was recorded. Then the organ bath was washed 3 times and allowed to rest for 15 minutes or more until the tissue recovered to the normal base line. The same procedure for each extracts and compounds was carried out for 3 times.

Results and Discussion

Some chemical constituents of the leaves of plants

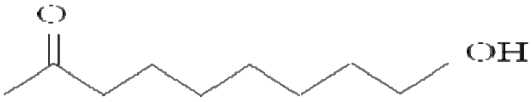
Some phytoconstitutents were also isolated from the leaves of these plants. From PE extract of *A. conyzoides* leaves, stigmasterol (0.028 %) was isolated by column chromatographic separation using silica gel. Besides, myristic acid (0.018%), palmitic acid (0.019%), 9-hydroxy nonan-2-one (0.013%) and Kaempferol (0.0382%) were obtained from EtOAc extract of defatted leaves of *A. conyzoides* (Figure -1). These compounds were structurally identified by the modern spectroscopic methods such as UV-visible, FT IR, ^{1}HNMR, GC-MS spectroscopy and by joint application of their physicochemical properties, and also by comparing with the reported data.



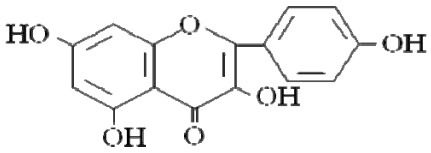
stigmasterol

myristic acid

palmitic acid



9-hydroxy nonan-2-one



Kaempferol

Figure (1) Chemical constituents isolated from PE and EtOAc extracts of *A. conyzoides* leaf.

Different extracts from the samples

In this experiment, the aqueous extract of *A. conyzoides* (10 g) was obtained. And then, the ethanolic extract was obtained as 8.5 g of dry 70% ethanolic extracts of *A. conyzoides.*

Effect of extracts on normal contraction of isolated tracheal chain

Table (1) shows the relaxation effect of the plant sample extracts with different doses (0.5 mg, 1 mg, 2 mg, 3 mg, 4 mg and 5 mg per cm^3 bath) on normal tracheal chain. The maximum dose for all aqueous and alcoholic extracts was found to be 2 mg/cm^3. From the data of these two extracts, it was observed that the alcoholic extract of *A. conyzoides* (75.3%) was slightly more potent than the aqueous extract (72%).

Table (1) Relaxation Effect of Extracts from *Ageratum conyzoides* L. (Khway-thay-pan) leaf on Tracheal Chain Isolated from Guinea Pig

	Relaxation Response (%)					
	0.5 mg/cm^3 (Bath conc:)	1 mg/cm^3 (Bath conc:)	2 mg/cm^3 (Bath conc:)	3 mg/cm^3 (Bath conc:)	4 mg/cm^3 (Bath conc:)	5 mg/cm^3 (Bath conc:)
KTP(aqueous)	40.7 ± 1.7	62.7 ± 3.7	72.0 ± 6.9	68.7 ± 6.9	69.3 ± 5.2	70.0 ± 5.8
KTP(70% EtOH)	38.7 ± 3.5	56.0 ± 8.3	75.3 ± 2.4	74.7 ± 2.9	74.0 ± 2.3	74.0 ± 3.0

KTP = Khway-thay-pan

Effect of Extracts and Compounds on Histamine Induced Tracheal Chain

When different extracts were added to the organ bath after stimulating the trachealis muscle with histamine (1mg/cm^3), the extracts inhibited the contraction. The relaxation effect of aqueous extract and alcoholic extract from these medicinal plants on isolated tracheal chain is shown in Table (2). The results of the present study have demonstrated that all these extracts possessed anti-histaminic activity. Aqueous extract and 70% EtOH extract of Khway-thay-pan were found to have 59.6% and 74.2% relaxation response.

Thus the beneficial effect of these plants in bronchial asthma and cough expectorant appears to be due to its anti-histaminic activity. So, it could be concluded that both aqueous and ethanolic extracts of this plant studied may possess some active principles with bronchodilating activity on the trachealis muscle.

For Khway-thay-pan leaves, direct relaxant activity of ethanolic extract was observed to be higher than that of aqueous extract. Therefore the constituents present in ethanolic extract may be more active than that in aqueous extract.

Table (2) Relaxation Effect of Aqueous and Ethanolic Extracts of *Ageratum conyzoides* L. (Khway-thay-pan) leaf on Histamine Induced Tracheal Chain Isolated from Guinea Pig

Extracts (1 mg/cm^3 bath conc.)	**Relaxation response (%)**
KTP (watery)	59.6 ± 7.1
KTP (70% EtOH)	74.2 ± 1.8

KTP = Khway-thay-pan

It was observed that many compounds were isolated from the extracts of these plants. Among the isolated compounds, the yield percent of stigmasterol and Kaempferol from Khway-thay-pan was high and then these compounds are major constituents of the leaves. So, further investigation could be continued and direct relaxant activity of these compounds was studied on histamine induced contraction in tracheal chain isolated from guinea pig.

The results of the present study have demonstrated that these compounds possess anti-histaminic activity with relaxation response: 56.5%, and 49.3% after treating with stigmasterol, and kaempferol respectively (Table -3). Stigmasterol is a steroidal compound.

Table (3) Relaxation Effect of Isolated Compounds on Histamine Induced Tracheal Chain Isolated from Guinea Pig

Isolated Compounds (0.1 mg/ cm^3 bath conc.)	**Relaxation response (%)**
Stigmasterol	56.5 ± 1.7
Kaempferol	49.3± 3.55

Most steroidal compounds are used as controllers or anti-inflammatory agents for asthmatic patients. Steroids are the most effective way of controlling inflammation in the lungs. The steroids used in asthma are corticosteroids. They have been widely and safely used to treat asthma for many years.

Kaempferol is a flavonoid compound. According to literature review, flavonoids may contribute to the anti-histamine activity and it can be used as lipoxygenase inhibitor. Flavonoids have shown that H_2 receptor blocking drug can reduce histamine to have an antiasthmatic effect.

Conclusion

From the present chemical and bioactivity investigation on the Myanmar indigenous medicinal plant commonly used in the treatment of bronchial asthma *Ageratum conyzoides* (Khway-thay-pan) leaf, the following inferences can be deduced. By silica gel column chromatographic separation, stigmasterol (0.028%), myristic acid (0.018%), palmitic acid (0.019%), 9-hydroxy nonan-2-one (0.013%) and kaempferol (0.0382%) from PE extract and defatted EtOAc extract of Khway-thay-pan leaf were isolated. Direct relaxant effect of the extracts (water and 70% EtOH) of the leaves of the plant was studied by in *vitro* model of tracheal chain preparation using guinea pig. The maximum dose of all extracts was found to be 2 mg/cm^3. The effect of extracts on histamine induced contraction was screened on isolated tracheal chain. From this study, the relaxation response of histamine induced tracheal chain was observed to be 45-80 % of relaxation response after treating with extracts. Isolated compounds exhibited anti asthmatic effect in the order of stigmasterol (56.5%)> kaempferol (49.3%). Therefore, from these results, it is suggested that this plant should be used as a remedy for the treatment of bronchial asthma.

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