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LUPEOL, A NOVEL ANTI ASTHMATIC AGENT FROM THE FRUIT PULP OF *AGLAIA ELAEAGNOIDEA* (JUSS.)BENTH

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ABSTRACT

The aim of the study was to evaluate the bronchodilating effect of lupeol, isolated from the fruit pulp of Aglaia elaeagnoidea. The evaluation has been carried out in an isolated goat trachea chain preparation. Histamine induced contraction in isolated goat tracheal chain showed that the lupeol isolated from the fruit pulp of Aglaia elaeagnoidea inhibited the contractile effect of histamine $P<0.05$.

KEYWORDS: *Aglaia elaeagnoidea*, Fruit pulp, Lupeol, Bronchodilator

INTRODUCTION

Plants have been used for medicinal purposes long before prehistoric period. Traditional systems of medicine continue to be widely practiced in many countries, side effects of several synthetic drugs and development of resistance to currently used drugs have increased demand of use of herbs as a source of medicine.

Asthma is a common chronic disorder of the airways that involves a complex interaction of airflow obstruction, bronchial hyperresponsiveness and an underlying inflammation. Acute symptoms of asthma usually arise from bronchospasm and require bronchodilator therapy. Acute and chronic inflammation can affect not only the airway caliber and airflow but also underlying bronchial hyper responsiveness, which enhances susceptibility to bronchospasm[1].

Aglaia elaeagnoidea commonly known as Priyangu[2] is an evergreen tree found in the dense and moist forests in Western Ghats, and also in many drier parts of India. It grows up to 10 m tall, with grayish brown bark. The plant is from Meliaceae family and is found among tropical forests or hills of

Andhra Pradesh, Karnataka, Kerala and Andaman Nicobar Islands[3]. The fruit is cooling, astringent, tonic, antidiarrheal, anti-inflammatory and it is also used for treating skin diseases[4].The seeds are used for painful urination[4]. The fruit is a berry, indehiscent. This berry is globose to ovate and 1 to 1.5 cm across. It is buff-coloured which turns orange when ripe[5]. The white coloured pulp surrounding the seed is edible[6]. Preliminary phytochemical screening shows the presence of alkaloid, steroid, carbohydrate, tannin, flavonoids, saponins, terpenoid, coumarins, phenol, carboxylic acid, amino acids and resins[7].Aglaia species are known for many biological activities like antipyretic, analgesic, anticancer, hepatoprotective, antidiabetic, antiinflammatory, immunomodulatory and insecticidal activity[8]. However anti asthmatic potential of the plant has not been evaluated. Hence in the present study an attempt has been made to evaluate the anti-asthmatic potential of the pulp obtained from the fruits.

MATERIALS AND METHODS

Ripe fruits of *Aglaia elaeagnoidea* were collected from the hilly areas of Pariyaram, Kannur district of Kerala state and Thirunelli hills of Wayanad district of Kerala state. It was then shade dried and its botanical identity was confirmed and the specimen of bearing voucher no. AE (Fr) 27 has been deposited in the department of Pharmacognosy and Phytochemistry, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur District, Kerala state.

Preparation of the extract

The fruits were dried in shade and the pulp was separated and extracted with ethanol .The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample.

Phytochemical studies

Preliminary phytochemical screening[9,10]was carried out by successive solvent extraction technique by preparing petroleum ether extract, benzene extract, chloroform extract, acetone extract, ethanolic extract and aqueous extract. The above extracts were used for phytochemical studies. The extractive value for each extract was calculated and recorded. Chemical tests carried out for different extracts of the fruit pulp of *Aglaia elaeagnoidea* to identify the presence of various chemical constituents.

Alcoholic extract of the fruit pulp of *Aglaia elaeagnoidea*was subjected to column chromatography using silica gel as the stationary phase. The column was prepared using silica gel in petroleum ether. The column was eluted with petroleum ether (100%); graded mixtures of petroleum ether and benzene (10-90%); benzene (100%); graded mixtures of benzene and chloroform (10-90%); chloroform (100%) and finally with graded mixture of chloroform and ethyl acetate (10-50%). Each time 50 ml of the eluate was collected. The components eluted were monitored by thin layer chromatographic studies and visualized with 10% sulphuric acid or in an iodine chamber[10].

The benzene eluate of the alcoholic extract of the fruit pulp of *Aglaia elaeagnoidea* on concentration deposited a white compound. Thin layer chromatographic studies of the compound was carried out which confirmed the presence of a single compound. Melting point of the compound suggested that it may be lupeol**.** Further confirmation as lupeol was made by co chromatography with standard lupeol by HPTLC technique[10].

Antiasthmatic screening

Isolated goat trachea chain preparation

Isolated adult goat tracheal tissue was obtained immediately after slaughter house of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Krebs solution and was continuously aerator at 37+0.5°C. Dose response curve of histamine in plane Krebs solution and in 800 µg/ml ethanolic extract of the fruit pulp of *Aglaia elaeagnoidea* act in Krebs solution was taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of drug extract[11,12.]

Statistics

The statistical analysis was performed by using One way analysis of variance (ANOVA) followed by Dunnett's test for individual comparison of groups with the control. The p values less than 0.05 were considered as significance.

RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of mainly alkaloids, glycosides, phenolic compounds, amino acids, phytosterols, flavonoids etc.

Antiasthmatic screening

Histamine contracts the trachea-bronchial muscle of guinea pig, goat, horse, dog and man[13] Histamine (10µg/ml) was taken in different dose level and dose response curve was plotted. It was observed that ethanolic extract of the fruit pulp of *Aglaia elaeagnoidea* inhibits contraction produced by histamine in these tissue preparations. Dose dependent response relationship was observed during the evaluation. It can also be noted that the fruit pulp exhibits significant ($p<0.01$) percentage decreased contraction at concentration 800 µg /ml in goat tracheal chain preparation.

Phytochemical investigation of alcoholic extract of the fruit pulp of *Aglaia elaeagnoidea*.results in isolation of a single compound.The compound isolated and recrystallized is colorless and having needle like shape. It soluble in organic solvents like; ether, benzene, chloroform and alcohol, and insoluble in water, acid and alkali. It shows positive to Liebermann-Burchard's test indicating the presence a compound having

terpenoid nature. The melting range of the compound was found to be 212-215°C and the R_f value was found to be 0.43.

Isolated goat tracheal chain is selected for the evaluation because it is very easy to handle and prepare and also have much more sensitivity than guinea pig. In the present study, there is a right side shift of dose response curve of histamine in the presence of ethanolic extract of the fruit pulp of *Aglaia elaeagnoidea* indicating antiasthmatic action.

In addition from the fruit pulp of the plant, lupeol is isolated.Lupeol is a triterpene which is also known as fagarsterol. Various studies have shown that lupeol can cause the reduction of cellularity and eosinophils in the broncho alveolar lavage fluid. In a recent study by Vasconcelos *et al.,* where lupeol was tested for the treatment of inflammation in a mouse model of bronchial asthma. It is well established that asthma is a chronic inflammatory disease of the airways associated with a Th2 immune response. This study showed that lupeol administration causes a significant reduction in cellularity and eosinophil levels in the broncho-alveolar fluid. Treatment of lupeol was also found to reduce the production of mucus and overall inflammation in the lungs. The plant seems to be having a promising role in the treatment of bronchial asthma could be due to the presence of flavonoid glycosides. Eventhough, various studies of flavonoids[14]. in asthmatic models have shown their beneficial effects, whereas the evidence in epidemiological studies and human clinical trials is currently limited. Hence the antiasthmatic effect of the alcoholic extract of the fruit pulp of the *Aglaia elaeagnoidea* could be due to the presence of its lupeol and flavonoid glycosidal content.

Table.1 Extractive values of various extracts of the fruit pulp

Sl.No	Solvents	Percentage of extract (w/w)
1	Petroleum ether	0.89
2	Benzene	0.75
3	Chloroform	0.70
4	Acetone	0.69
5	Ethanol	3.52
6	Chloroform water	4.19

Table.2 Qualitative Preliminary Phytochemical Screening of extracts of the fruit pulp

Sl. No	Test	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloids	-	-	-	-	+	+
2	Carbohydrates	-	-	-	-	+	+
3	Phytosterols	++	-	-	-	-	-
4	Glycosides	-	-	-	-	-	-
5	Fixed oils & fats	-	+	-	-	-	-
6	Saponins	-	-	-	-	-	-
7	Phenolic compounds and Tannins	-	-	-	-	+	+
8	Proteins and amino acids	-	-	-	-	-	+
9	Flavonoids	-	-	-	-	+	+
10	Gums and mucilages	-	-	-	-	-	-
11	Resins	-	-	-	-	-	-

(+ Present, - Absent)

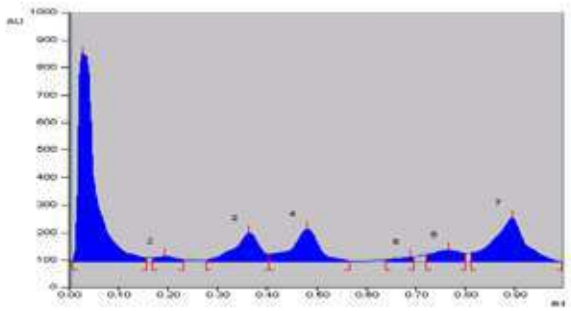

Fig. 1 HPTLC chromatogram of ethanolic extract of the fruit pulp

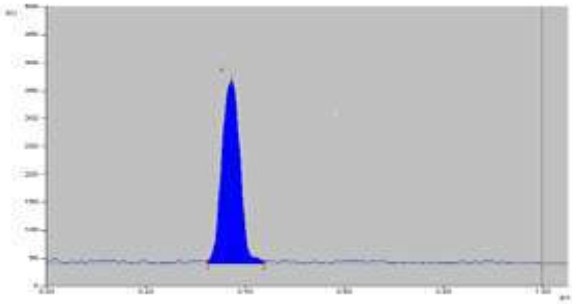

Fig.2 Chromatogram of reference standard lupeol

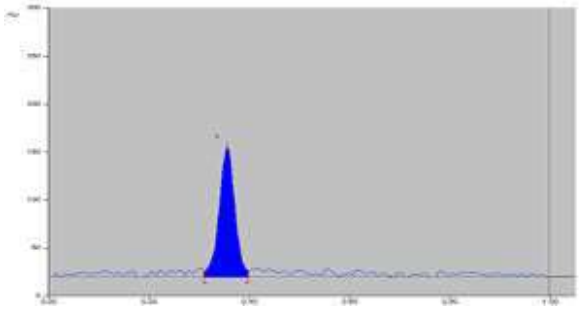

Fig.3 Chromatogram of isolated lupeol

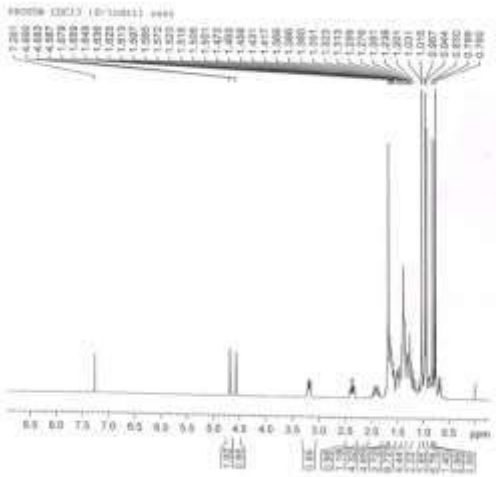

Fig.4 ^{1}H NMR Expanded Spectrum of isolated lupeol

Table .3 Effect of alcoholic extract of the fruit pulp of *Aglaia elaeagnoidea.*on histamine induced contraction on isolated goat tracheal chain preparation

No	Histamine Concentration (10micro grams/ml)	Percentage Response (Mean ± SEM)	
		Test	Control
1	4	15.55±1.58*	39.17±1.29
2	6	24.47±1.88**	44.55±1.55
3	8	32.66±1.22**	59.27±2.25
4	16	44.87±1.15**	66.17±2.98
5	32	56.75±1.40**	79.50±2.11
6	64	61.03±1.25**	88.50±2.44

n = 6 Values are in Mean ± SEM., Dose response curve of Histamine in the absence of alcoholic extract of the fruit pulp of *Aglaia elaeagnoidea.* extract is treated as control and dose response curve of histamine in the presence of extract of the fruit pulp is considered as test. Statistical analysis done by using student's 't'-test. $p<0.01$, significantly different from control.

CONCLUSION

Even though purity, selectivity of action and occurrence of side effects of extracts, are the matters of concern, the easy availability when compared to the synthetic drugs makes the herbal plants the best choice for the treatment of asthma Therefore the use of these plants is primarily done on basis of a balance between activity and toxicity**.** Hence it is necessary for all clinicians to be aware of high prevalence of herbal and ayurvedic interventions available for asthmatics other than using contemporary methods which include the use of steroids and bronchodilators that will give adverse side effects. It is concluded that herbal medicinal products, even though in prevalent use, are of uncertain value in the treatment of asthma. For some herbs there are promising data which warrant further investigation.

Conflict of Interest

The authors declare no conflicts of interest.

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