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Isolation and identification of pharmacologically active compounds in fruit of *Pyrus pashia*

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ABSTRACT

The aim of this work is to investigate pharmacological active compounds analysis and their anti-inflammatory activity of Pyrus pashia fruit. In this work some pharmacological active compounds were isolated by column chromatographic techniques and identification of their structure by spectroscopic (NMR, IR, Mass, UV, etc.) methods. Lupeol, β-Sitosterol and β-Sitosterol –ß-D glucoside compounds were isolated. These compounds firstly were isolated from Pyrus pashia fruit. The anti-inflammatory activity of these compounds has been investigated with in vivo study. In this study, it has been shown that Pyrus pashia isolated compounds showed potent anti-inflammatory activity. The active ingredient in the extract that reduces the inflammation is not known at present. There is ongoing research to isolate and characterize the bioactive compound (s) responsible for the anti-inflammatory activity of Pyrus pashia.

Keywords: *Pyrus pashia*, Mahal, Lupeol and Anti-inflammatory activity.

INTRODUCTION

Medicinal plants represent a rich source of potent and powerful drugs. The treatment of human and animal disease depends mainly on natural products derived from plants, animals, microorganisms and minerals. The wild edible fruits have show's different types of activity i.e, anti-inflammatory, antioxidant, antimicrobial, digestive disorder, heart disease, skin disease, astringent, diuretic and anti-dysenteric properties [1].

Pyrus pashia (Moraceae) commonly known as Mahal is a popular fruit in Uttarakhand. The fruit is employed frequently in the traditional medicine as supplementary food, minerals, vitamins, polyunsaturated fatty acids, certain phytochemical and dietary fibres [2]. *Pyrus pashia* is employed frequently in the traditional medicine as antimicrobial, antioxidant, stomachic and hypoglycemic activities [3, 4]. The aim of this study was to evaluate isolation and structural identification of pharmacological active compounds in fruit of Mahal and acute anti-inflammatory activity studies were performed following the Carrageenan induced hind paw edema.

MATERIALS AND METHODS

Collection and Identification

The materials included fresh and dry fruits of *Pyrus pashia* were collected from district Chamoli, Uttarakhand during August-September 2010. These plants were authenticated from Taxonomy Laboratory, Department of Botany, HNB Garhwal University, Srinagar. The voucher specimens GUH 8267 were deposited in the University herbarium for future records.

Preparation of Plant Extract
The air dried fruits ground to moderately fine powder and soxhlet extracted with petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water using soxhlet apparatus [5]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The extracts thus obtained were stored in air tight container at 4^oC until further analysis. The hexane fraction was chromatographed on column chromatography eluting with hexane with increase amount of ethyl acetate to give fractions of 200 ml each. Fractions which gave similar spots on the thin layer chromatography (TLC) with appropriate solvent system were combined.

Experimental animals
The wistar rats (100-150g) were obtained from the Animal House, National Centre of Fungal Taxonomy, New Delhi, India. They were housed at a temperature 24 ± 1^oC, 12 hour light/dark cycle 35-60% humidity, in polypropylene cages and fed a standard rodent diet with water ad *libitum.* The animals were deprived of food but not water 4 hours before the experiment. The experimental design was approved by the ethical committee for animal experimentation of faculty of Pharmaceutical Science (S.B.S Ballawala Dehradun Uttarakhand) bearing the number 273/PP/SCEA.

Acute Toxicity Study
To determine the minimum lethal dose, acute oral toxicity studies were performed as per OECD guidelines [6]. Adult albino rats of either sex weighing 100-150gm were used. The animals were divided into five groups of six each. Group I was given 2 ml of 1% saline and group II received 2 ml of 1% vanillin both acted as control. The other three groups were administered isolated components. All the experimental rats were fasted overnight. They were observed continuously for any gross behavioral changes and toxic manifestations like hyperactivity, grooming, convulsions, sedation, hypothermia and mortality during the first three hours. Thereafter the animals were continuously monitored at regular intervals for 7 days. No adverse effect or mortality was detected in this study up to 500 mg/kg between doses. Hence sub-lethal doses of 50-150 mg/kg between doses of the isolated components were selected for the following experiments.

Chemicals & Experimental instruments
All the chemicals and reagents of analytical grade such as Indomethacin (Merck, Bangalore, India) and Carrageenan (Sigma Chemicals, St. Louis, MO, USA) were procured from the respective companies and were used in the study. Melting points were determined on a Kofler hot-stage microscope melting point apparatus and were uncorrected, infrared and ultraviolet spectra were obtained on Perkin-Elmer lambda model 1330 and 20 spectrometers respectively. ^1H and ^{13}C -nuclear magnetic resonances were recorded on JEOL JNM-GSX 400 spectrometer. Mass spectra were obtained using GCMSQP 5050 A Shimadzu mass spectrometer, column chromatography and analytical thin layer chromatography was carried out using Merck 77491 and Merck 7730, 60 F-254.

MATERIALS AND METHODS

Method of isolation
The 20.0 gm of the hexane fraction of the ethanolic extracts of the fruits of *Pyrus pashia* were subjected to column chromatography over Merck silica gel PF_{254} (70-230 Mesh ASTM) and eluting with petroleum ether, petroleum ether/hexane, hexane and hexane/ethyl-acetate to give 50 fractions. Whereas thin layer chromatography was performed on commercially TLC plastic sheets precoated with Merck silica gel 60 F-254 (0.2 mm thickness).

Carrageenan induced rat paw edema assay
Acute anti-inflammatory activity studies were performed following the Carrageenan induced hind paw edema suggested [7]. The rats were divided into five groups of six rats each. Group 1 acted as control and was given 1% saline, Group 2 received 100 mg/kg between of standard reference drug – Indomethacin, Group 3, 4 and 5 were administered 150 mg/kg between of the isolated components with 2 ml of 1% vanillin respectively. 0.1 ml of 1% solution of Carrageenan was injected intradermally to the rats into the plantar surface of the right hind limb to induce paw edema. The paw volume was measured plethysmographically before induction (0 H) and after at one hour intervals for four hours. The paw volume in group II, III and IV were compared with that of the control. Percentage inhibition was calculated using the formula,

$\%\ \text{Inhibition} = (V_C - V_T/V_C) \times 100$
Where, V_C= Paw volume in control group,

V_T= Paw volume in drug treated group.

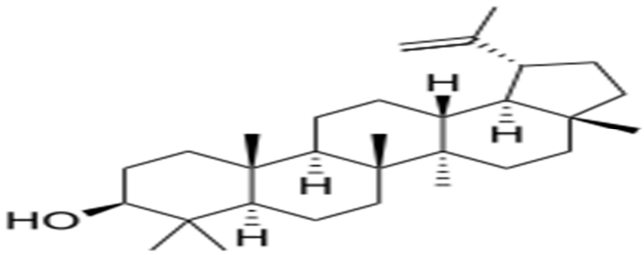
Statistical analysis

The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Tukey's t-test was used as the test of significance. P value<0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software [8].

RESULTS

Compound (I)

Structure - Lupeol



State - white needles

M. P. – 120-122 ^{0}C

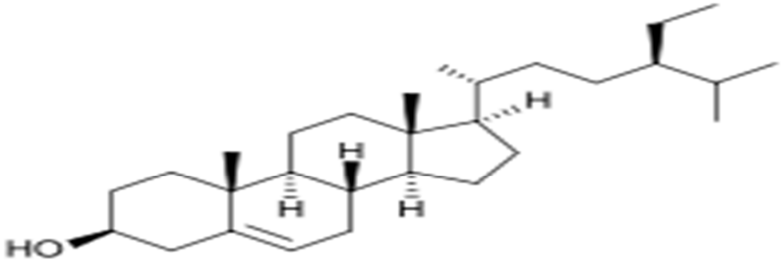
Molecular formula - $C_{30}H_{50}O$

Molecular weight - 426.72 gmol^{-1}.

IR (V_{max} CCl_4) cm^{-1}: 3056, 2929, 2313, 1593, 1435, 1265, 898, 741; ^{1}H-NMR ($CDCL_3$, 400 MHz): δ 4.70, 4.55(2H, s, H-29a, 29b), 3.2(1H, m, H-3), 0.77, 0.79, 0.85, 0.94, 0.97, 1.05, 1.65 (each 3H, S); ^{13}C-NMR ($CDCL_3$, 100 MHz): δ 151.0 (C-20), 109.0(C-29), 79.0 (C-3), 55.5(C-5), 50.5(C-9), 48.3(C18), 48.0(C-19), 43.0(C-17), 42.9(C-14), 40.9(C-8), 40.0(C-22), 38.9(C-4), 38.7(C-1), 38.1(C-13), 37.2(C-10), 35.5(C-16), 34.2(C-7), 29.9(C-21), 28.0(C-23), 27.4(C-2), 27.1(C-15), 25.2(C-12), 21.0(C-11), 19.5(C30), 18.5(C-6), 18.0(C-28), 16.1(C-25), 16.0(C-26), 15.5(C-24), 14.8(C-27).

Compound (II)

Structure – β-Sitosterol



State - White powder

M.P. – 136-140 ^{0}C

Molecular formula – $C_{29}H_{50}O$

Molecular weight - 414.71 gmol$^{-1;}$ Rf 0.47 (silica gel/methanol: chloroform =1;49); IR $^{\theta}$max (KBr): 3500-3200 (O-H), 3020 (C=CH), 2940, 2860, 1640, 1460, 1380, 1060, 1020, 970, 960, 800 cm^{-1}. ^{1}H-NMR ($CDCL_3$) δ(ppm): 0.68-2.32 (m, C,CH,CH_2CH_3), 3.52 (b, OH), 5.09 (t, CH=CH) 5.35 (d, =CH) ^{13}C-NMR ($CDCL_3$) δ(ppm): β-Sitosterol : 11.87(C-18), 11.87 (C-29), 18.74 (C-26), 19.02 (C-21), 19.39 (C-19), 19.82 (C-27), 21.07 (C-11), 23.02 (C-28), 24.27 (C-15), 26.06 (C-25), 28.22 (C-16), 29.09 (C-23), 31.46 (C-2), 31.85 (C-7), 31.85 (C-8), 33.90 (C-22), 36.14 (C-20), 36.46 (C-10), 37.21 (C-1), 39.76 (C-12), 42.26 (C-4), 42.26 (C-13), 45.78 (C-24), 50.11 (C-9), 56.02 (C-17), 56.72 (C-14), 71.73 (C-3), 121.68 (C-6), 140.75 (C-5).

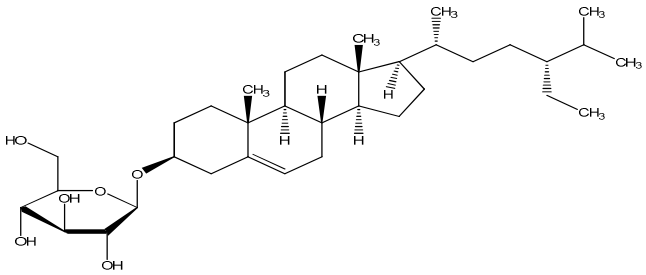
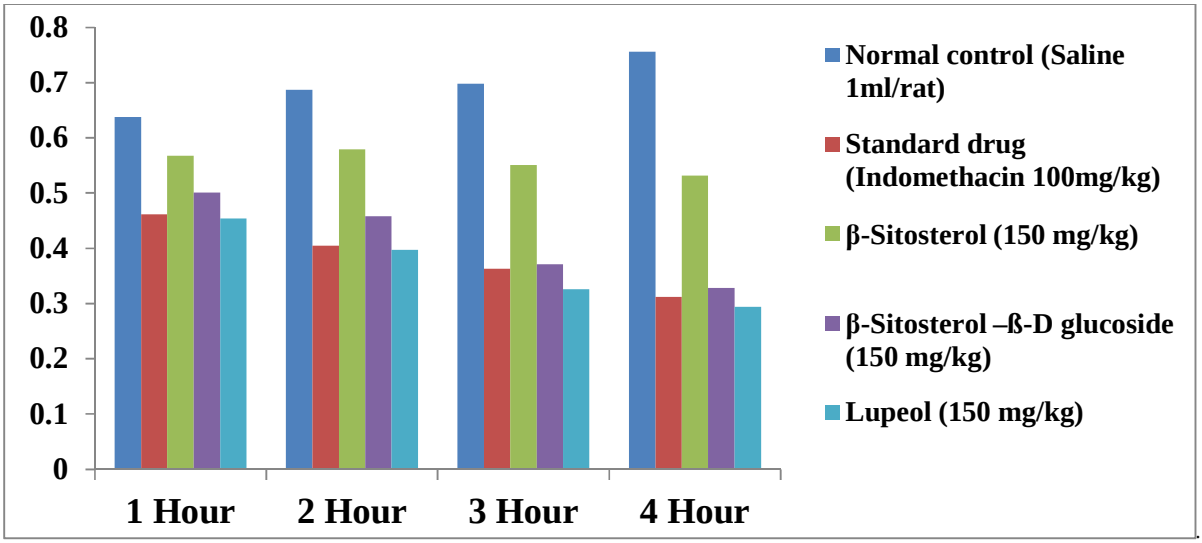


Table 1. Anti-inflammatory activity of isolated compounds

Group	Dose mg/kg	Paw volume in ml ± SEM and percentage inhibition			
		+1 Hour	+2 Hour	+3 Hour	+4 Hour
I	Control	0.638 ±0.01	0.687±0.03	0.698±0.02	0.756±0.04
II	Indomethacin (100 mg/kg)	0.462±0.02	0.405±0.03	0.363±0.02	0.312±0.01
III	β-Sitosterol (150 mg/kg)	0.568±0.04	0.579±0.03	0.551±0.01	0.532±0.02
IV	β-Sitosterol –ß-D glucoside (150 mg/kg)	0.501±0.02	0.458±0.03	0.371±0.03	0.328±0.02
V	Lupeol (150 mg/kg)	0.454±0.03	0.397±0.04	0.326±0.04	0.294±0.03

Figure 1.1 Anti-inflammatory activity of isolated compounds.



Compound (III)

Structure – β-Sitosterol –ß-D glucoside
State - Buff colour
M. P. – 312-314 ^{0}C
Molecular formula – $C_{35}H_{60}O_6$
Molecular weight – 302.2 gmol^{-1} IR (3600-3400), 2900, 2850, 1720, 1640, 1450, 1240, 900, (830-800) cm-1; ^{1}H-NMR (400 Mz, DMSO-d_6): 7.25 (s, 1-H, –OH), 6.67-6.84 (m, 1H proton of sugar moiety), 5.47 (s, 1H, H-6), 5.35 (dd, 1H, J=12.5 and 8.5 Hz, H-23), 5.03-5.08 (dd, 1H, J= 12.5 and 8.5 Hz, H-22), 4.96 (s, 1H, proton of sugar moiety), 4.85 (s, 1H, anomeric proton), 3.86 (m, 1H, H-3) 2.03 3.31 (m 3H, proton of sugar moiety), 1.24 (s, 3H, H-19), 1.0 (d, 3H, J=6.5 Hz, H-21), 0.97 (t, 3H, J=7.1 Hz, H-29), 8.8 (s, 3H, H-27), 8.7 (s, 3H, H-26), and 0.85 (s, 3H, H-18); ^{13}CNMR (400 Mz, DMSO-d_6): 39.9 (C-1), 29.9 (C-2), 77.3 (C-3), 39.8 (C-4), 55.8 (C-5), 21.6 (C-6),39.2 (C-7), 29.7 (C-8), 48.7 (C-9), 29.4 (C-10), 21.6 (C-11), 27.2 (C-12), 50.8 (C-13), 30.2 (C-14), 62.1 (C-15), 77.3 (C-16), 124.3 (C-17), 118.26 (C-18), 130.2 (C-19), 151.87 (C-20), 178.91 (C-21), 146.47 (C-22), 29.2 (C-23), 14.1 (C-24), 62.12 (C-25), 76.7 (C-26), 63.75 (C-27), 184.9 (C-28), 111.14 (C-29), 121.2(C-30) and the chemical shifts (210.3, 209.45, 130.24, 130.0 and 143.96) ppm are due to the carbon of the sugar moiety.

DISCUSSION

In this study, we evaluated the anti-inflammatory activity of the hexane fraction of the fruits of *Pyrus pashia* by carrageenan-induced paw edema model. The carrageenan-induced paw edema model is a suitable test for evaluating anti-inflammatory activity of a drug in the acute phase of inflammation. Edema induced by carrageenan is believed to be biphasic. The first phase (1 hour) involves the release of serotonin and histamine and the second phase (> 1 hour) is mediated by cyclooxygenase products. Continuity between the two phases is provided by kinin. The result of anti-inflammatory activity of fruits hexane fraction of *Pyrus pashia* against carrageenan induced paw edema is shown in **Table 01**. Paw volume was significantly reduced ($P<0.01$) in all treated groups as compared to control group [**Fig 1.1]**. Lupeol, β-Sitosterol and β-Sitosterol –ß-D glucoside are showed more significant inhibition of edema but less effective than reference standard compound i.e. Indomethacin, table 1 and figure 1.1.

CONCLUSION

It can be concluded that the hexane fraction of the fruits of *Pyrus pashia* possess potent anti-inflammatory activity thus validating the ethno pharmacological claims. This knowledge could be tapped to formulate new agents to treat inflammatory and allergic ailments. Further investigation revealed that isolation and structure determination of the active principle and its mode of action are suggested for the development of a new drug candidate in the treatment of inflammatory diseases. Lupeol has been shown to exhibit various pharmacological activities e.g inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity and β-sitosterol reduces blood levels of cholesterol and inhibits cholesterol absorption in the intestine. Research has indicated that β-Sitosterol –ß-D glucoside may be useful in prevention of inflammation, antioxidant and antimicrobial activity.

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