

Paw Edema and Bronchoconstriction Induced by Mediators: How They Were Inhibited *In vivo* by Partially Purified Extract from Dichloromethane Leaf Extract of *Labisia pumila*

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Abstract: This present study investigated inhibitory effect of partially purified leaf extract of *Labisia pumila* on histamine, serotonin, arachidonic and bradykinin induced paw edema and histamine and acetylcholine induced bronchoconstriction. Oral administration of partially purified fractions A, C, E and fractions A & E (100mg/kg), inhibited significantly ($p < 0.05$, $p < 0.001$), histamine and serotonin induced paw edema at the fifth hour respectively, while fraction D inhibited the first 2hrs of serotonin induced paw edema. Fractions A, B, D, E inhibited bradykinin-induced paw edema at the 60 mins. The highest inhibition was observed in fraction A, histamine; 5.07% (0.674 ± 0.002 mm), serotonin 4.24% (0.678 ± 0.002 mm) and bradykinin 9.59% (0.622 ± 0.002 mm), compared to the negative control (0.710 ± 0.003 mm). Partially purified fractions A-E at (100mg/kg), significantly ($p < 0.001$), inhibited bronchoconstriction induced by histamine and acetylcholine at the 4th hour. The highest protection was observed in fraction A; 55% (123 ± 0.000 sec.) and 58% (123 ± 0.000 sec), 48.17% (100.333 ± 12.454 sec.) and 45.70% (85.333 ± 8.667 sec.) respectively, compared to the negative control (45 ± 0.000 sec.) and (39.333 ± 0.667 sec.). For the 14 days continuous treatment with histamine and acetylcholine- induced bronchoconstriction, partially purified fractions A (100mg/kg), showed a significant ($p < 0.001$) protection of 68.84% (134.980 ± 5.025 sec.) and 51.21% (110.260 ± 1.750 sec.) respectively, compared to the negative control (35.600 ± 1.140 sec.) and (34.180 ± 1.385 sec.). The present study confirmed that the extracts were able to inhibit paw edema induced by histamine, serotonin and bradykinin and bronchoconstriction induced by histamine and acetylcholine.

Key words: Paw Edema • Bronchoconstriction • *Labisia pumila* • Histamine • Serotonin • Bradykinin

INTRODUCTION

Labisia pumila (Kacip Fatimah) (LP) is a sub-herbaceous plant with creeping stems. It is widely spread in the rainforest lowland and hills of Malaysia, Thailand, Indochina, Philippines and New Guinea [1, 2]. In Malaysia, the water extract from the roots or the whole plant is used to treat women's ailments associated with child birth, firming and toning of abdominal muscles, breasts, tightening vaginal muscles and inflammatory disorders such as rheumatism [3], and as an energy drink as well as other medical usage [4]. Men from different ethnic groups in Sarawak use LP to maintain and increase stamina [5]. We have

earlier reported paw edema and bronchoconstriction, anti-inflammatory, anti-histaminergic, anticholinergic and gastro-protective effect of crude extract of LP [6-8].

MATERIALS AND METHODS

Plant Material: The leaves of LP (Kacip Fatimah) were purchased from University Putra Malaysia, Serdang, Selangor Darul Ehsan. The plant was specifically identified by Mr. Shamsul Khamis, a research officer (plant taxonomy) from the Laboratory of Natural Products (NATPRO), Institute of Bioscience in University Putra Malaysia.

Preparation of Extracts: The preparation of crude extract and partial purification of the crude extract into fraction A to E was done according optimized method [4,5,6, 11, 12].

Animal Preparation: Ninety (90) male Sprague Dawley rats of 6-8 weeks of age, weighing 150- 200g, thirty-five male albino mice (35) weighing 30-40g and seventy (70) male guinea pigs (350- 400), were purchased from Institute for Medical Research (IMR). They were fed with standard pellet diet and water *ad libitum*. They were housed in groups of five (5) in standard cages in animal holdings units, UCSI University, Kuala Lumpur, Malaysia and maintained under standard environmental condition. They were acclimatized for 2 weeks before starting the experiment. The animals were deprived of food for 24 h before the beginning of experiments with free access to tap water. The animals were kept under conditions approved by the UCSI research ethics committee.

Paw Edema (Histamine, Serotonin and Bradykinin): The rats were divided into 7 groups (5rats in each group) and their respective drugs were administered orally as follows: Group1 received saline, group2 received the reference drug indomethacin at a dose of 10mg/kg and groups 3-7 received fraction A-E at a concentration of 100mg/kg respectively. After one hour, the rats were given a sub-plantar injection of 0.1ml of 1% histamine and the paw volume was measured at 0hr, 1hr, 2hrs, 3hrs, 4hrs and 5hrs after histamine injection with a plethysmometer. The inhibitory activity was calculated according to equation 1 [13]. This procedure was repeated for serotonin and bradykinin but for bradykinin, the drugs were orally administered together with an anti-peritoneal injection of perindopril arginine and paw volume was measured at 0, 10, 20, 40 and 60 minutes [14].

Equation 1

$$\text{Percentage inhibition} = \left(100 - \frac{T_1}{T_2} \right) \times 100$$

Where;

- T_1 = Average inflammation of treated group (positive control, fractions A-E)
- T_2 = Average inflammation of control group (negative control).

Arachidonic acid induced ear edema: The mice were divided into 7 groups (5 in each group). All the mice

received negative control (acetone) on their left ear and on the right ear, group 1 received saline, group 2 Cimetidine (2mg/kg) while groups 2-7 received Fractions A-E respectively at a concentration of 100mg/kg respectively. 30 minutes after treatment, 20 μ g (2mg/kg) of arachidonic acid was topically applied to the ears of the mice and after one hour, the edema was measured using a plethysmometer. The ear edema was expressed as the difference in ear thickness between test animal and controls [15].

Induced Bronchoconstriction (Histamine and Acetylcholine): The guinea pigs were divided into 7 groups (five in each group). They were fasted overnight and exposed to 0.2% histamine aerosol in an air-tight chamber and the pre-convulsion time (PCT) (time of aerosol exposure to the onset of dyspnoea leading to appearance of convulsion) was recorded and they were immediately removed from the chamber and placed in fresh air to recover. They were left for 24hours to recover. They were given their various treatments as follows: group I received saline, Group II received pyrelamine maleate and groups 3-7 received Fractions A-E respectively at a concentration of 100mg/kg. They were exposed to histamine aerosol at 1hr, 4hrs and 24hr after treatment and PCT was recorded. Because fraction A showed the best result, the animals were treated with fraction A for 14days and were exposed to histamine aerosol. The protection offered by the treatment was calculated using equation 2 [16]. The whole experiment was repeated using acetylcholine aerosol.

Equation 2

$$\text{Percentage protection} = \left(1 - \frac{T_1}{T_2} \right) \times 100$$

Where

- T_1 = Average inflammation of treated group (positive control, fractions A-E)
- T_2 = Average inflammation of control group (negative control)

Phytochemical Screening: Conventional simple standard biochemical protocols [16, 17] for detecting the presence of different phytochemical constituents in the plant extract were employed. The common phytochemical constituents tested include flavonoids, alkaloids, tannins, steroids and saponins.

Statistical Analysis: Data were expressed as the mean \pm S.D. Experiment groups were compared using one way analysis of variance (ANOVA test) followed by the Bonferroni's test. Statistical analysis was performed using Graph-pad Prism 5.0. Values of probability $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The sub-planter administration of histamine and serotonin caused an oedema immediately and increased progressively for 5 hours to a maximum. Indomethacin (10mg/kg) and partially purified fractions A, C, E and fractions A&E (100mg/kg), inhibited significantly ($p < 0.05$, $p < 0.001$), histamine and serotonin induced paw edema at the fifth hour respectively, while fraction D inhibited the first 2hrs of serotonin induced edema. Fraction A and E showed the highest effect, while fractions B, C and D showed the least effect Figure 1&2. Similarly sub-planter administration of bradykinin induced edema immediately and increased progressively for 60 minutes to a maximum. Indomethacin (10mg/kg) and fractions A, B, D, E (100mg/kg) significantly inhibited bradykinin induced paw oedema at the 60th minute. Fraction A and E showed the highest effect, while fractions B, C and D showed the least effect at the 60th minute Figure 3. The topical application of arachidonic acid on the ear of the mice caused ear edema of mice.

The reference drug (10mg/kg) and extract A-E (100mg) showed a significant inhibition of ear edema caused by topical application of arachidonic acid. Fractions B, C and D showed the least effect while Fractions A and E showed best effect Figure 4.

Histamine and Serotonin is important inflammatory mediator, potent vasodilator substance and causes increases vascular permeability [18, 20]. Histamine is present in bound form in mast cells or basophils and is released when these mast cells are activated. They act through specific receptors on nearby vasculature and induce plasma extravasation [19]. While Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter in the brain, synthesized, from the amino acid L-tryptophan, in brain neurons and stored in vesicles. Serotonin is inflammation mediator, potent vasodilator substance and causes increases vascular permeability [20]. This causes increased blood flow and leakage of plasma exudates to the inflamed area thereby causing swelling and redness of the inflamed area. It has also been reported to be a bronchoconstrictor. From the result, the fractions A, C, D, E, were able to reduce the paw oedema caused by histamine and serotonin injection. This means that the extracts are effective at the early phase of inflammation. The efficacy of the drug is pharmacologically similar to that of the reference drug. From the result, it can be suggested that the extract exhibits anti-inflammatory function either by inhibiting the synthesis, release or action of histamine and serotonin.

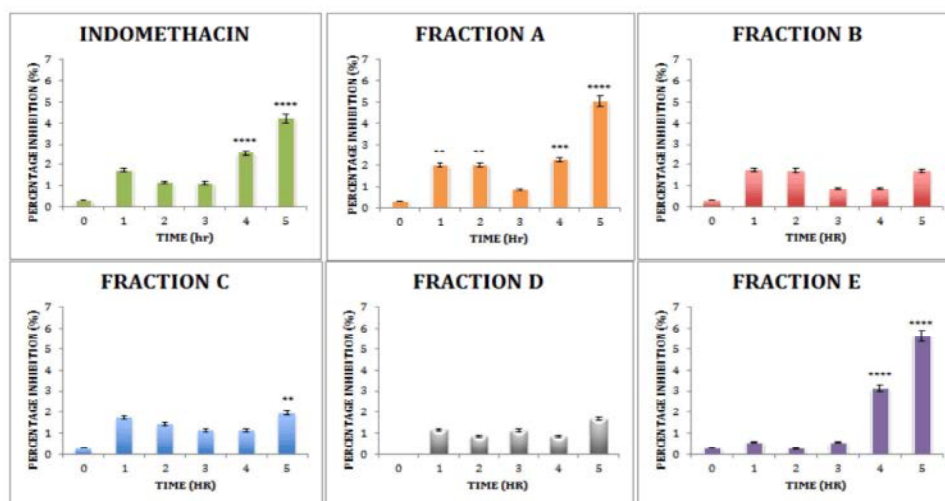


Fig. 1: PERCENTAGE INHIBITION OF HISTAMINE-INDUCED PAW EDEMA TREATED with indomethacin 10mg/kg and fractions a-e 100mg/kg by p.o. values were expressed as mean \pm sd (n = 5). Statistical significance ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$) was calculated by anova followed by bonferroni's test.

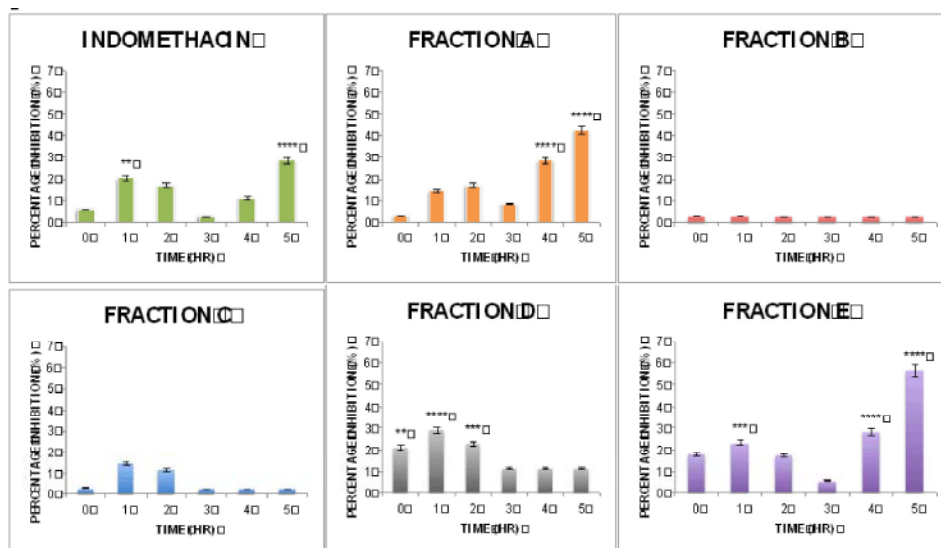


Fig. 2: PERCENTAGE INHIBITION OF SEROTONIN-INDUCED PAW EDEMA in rats by treatment of reference drug indomethacin at a dose of 10mg/kg and partially purified fractions a-e from the leaves of *Labisia pumila* plant at doses 100mg/kg by p.o. values were expressed as mean \pm sd (n = 5). Statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) was calculated by ANOVA followed by bonferroni's test.

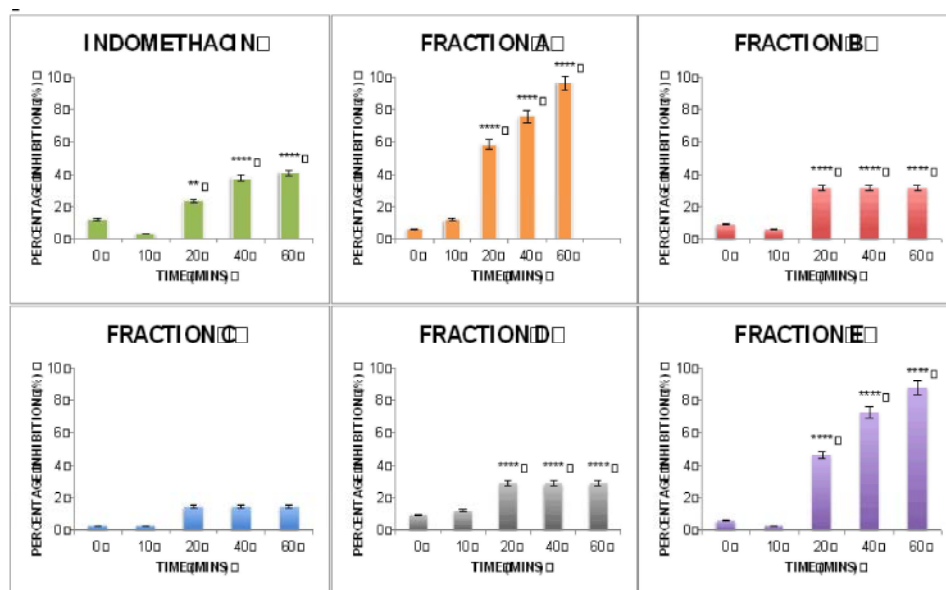


Fig. 3: PERCENTAGE INHIBITION OF BRADYKININ-INDUCED PAW EDEMA in rats by treatment of reference drug indomethacin at a dose of 10mg/kg and partially purified fractions a-e from the leaves of *Labisia pumila* plant at doses 100mg/kg by p.o. values were expressed as mean \pm sd (n = 5). Statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) was calculated by anova followed by bonferroni's test.

Bradykinin is a potent endothelium-dependent vasodilator, which causes contraction of non-vascular smooth muscle, increases vascular permeability and is also involved in the mechanism of pain. It is a nine amino

acid peptide that has a number of properties, which causes inflammation [21]. Bradykinin is a protein, which means it degrades quickly. This is why perindopril arginine was injected intra-peritoneally before

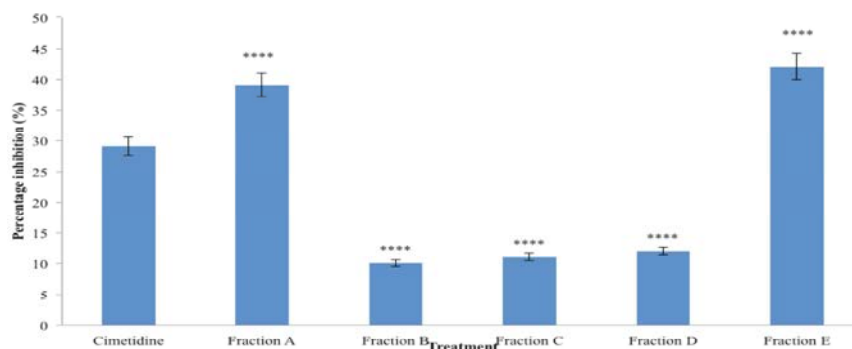


Fig. 4: PERCENTAGE INHIBITION OF ARACHIDONIC ACID-INDUCED EAR EDEMA IN MICE by treatment of reference drug cimetidine at a dose of 1mg/kg and partially purified fractions a-e from the leaves of *Labisia pumila* plant at doses 100mg/kg by p.o. values were expressed as mean \pm sd (n = 5). Statistical significance ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$) was calculated by ANOVA followed by bonferroni's test.

administration of bradykinin injection and why it was measured for 60 minutes instead of 5hrs. Perindopril arginine is an angiotensin-converting enzyme (ACE), which lowers blood pressure, inhibits bradykinin degradation thereby increasing bradykinin content. From the result, fractions A, B, D, E were able to reduce the paw edema caused by bradykinin injection. This means that the fractions are effective at the late phase of inflammation. The efficacy of the drug is pharmacologically similar to that of the reference drug. This result is in agreement with our earlier finding, which has shown that dichloromethane extract of *Labisia pumila* (DELP), was able to inhibit the first phase which involves release of histamine and serotonin and second phase which involves release of prostaglandins cyclooxygenase products leukotriene and kinin [7].

Arachidonic acid is a polysaturated fatty acid. It is formed when phospholipase A_2 acts on phospholipids. It can also be formed when phospholipase C cleaves a phospholipid to form diacylglycerol (DAG). DAG is then cleaved by diacylglycerol lipase to form arachidonic acid. Arachidonic acid is a precursor of eicosanoids. Lipoxygenase acts on arachidonic acid to form leukotriene while cyclooxygenase acts on arachidonic acid to form prostaglandin (which increases vascular permeability) and thromboxane. Arachidonic acid causes instant irritation of the mouse ear, which leads to fluid accumulation and edema characteristic of the acute inflammatory response. Suppression of this response is a likely indication of anti-inflammatory effect. Arachidonic acid-induced ear inflammation in mice has been reported to be sensitive in detecting the anti-inflammatory action of lipoxygenase inhibitors. Steroids inhibit arachidonic acid by stabilizing membrane thereby inhibiting

hydrolysis of phospholipids. The fractions inhibited arachidonic acid ear edema, which means that their anti-inflammatory action is either by acting as a lipoxygenase inhibitor, by inhibiting the activity of phospholipase C and phospholipase A_2 or by membrane stabilization [21].

The exposure of the guinea pigs to 0.2% of histamine and acetylcholine aerosol caused bronchoconstriction in the guinea pig reduced the PCT. The reference drug and the extract A-E (100mg/kg) showed a significant protection against histamine and acetylcholine aerosol exposure and extended their PCT after 4hrs of treatment. For both bronchoconstrictor tested fraction B, D and E showed the least effect, while fractions A and C showed the highest effect Figure 5 & 6. From the results, it was seen that the fractions were able to offer protection against histamine-induced bronchoconstriction. This means that the mechanism of action of the extract is by inhibition of airway restriction.

Histamine acts on H_1 receptor, which is a G-protein coupled receptor. Acetylcholine is a neurotransmitter in the parasympathetic nervous system (PNS), it activates M_3 muscarinic receptor, which in turn activates intracellular Gq protein. Both mediators have similar mechanism of bronchoconstriction. They cause the activation of phospholipase C and phosphatidylinositol-4, 5-bisphosphate (PIP_2) pathway (en.wikipedia.org. Accessed 4th October 2012). In this pathway, PIP_2 is cleaved into diacyl glycerol (DAG) and inositol 1, 4, 5-trisphosphate (IP_3). DAG remains bound to the membrane and IP_3 is released as a soluble structure into the cytosol. IP_3 then diffuses through the cytosol to bind to IP_3 receptors, particularly calcium channels in the endoplasmic reticulum (ER). This causes the cytosolic

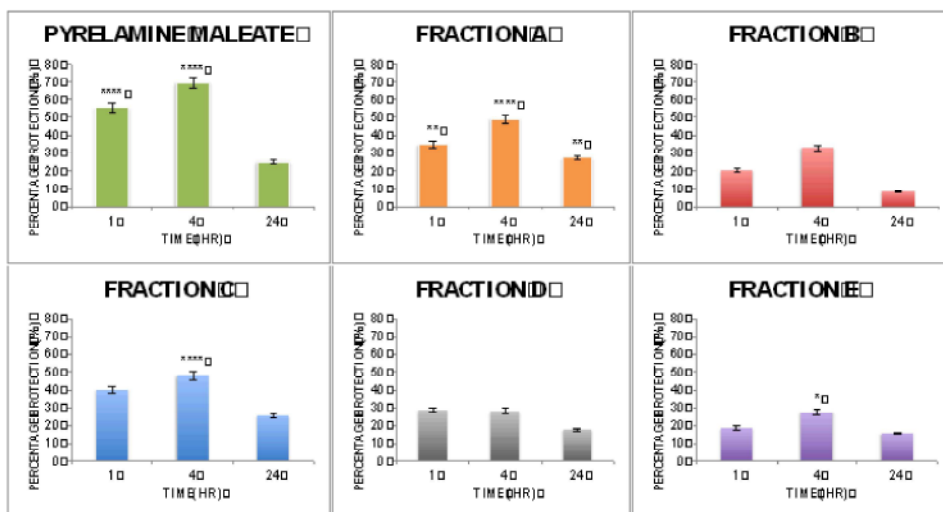


Fig. 5: PERCENTAGE PROTECTION OF HISTAMINE-INDUCED BRONCHOCONSTRICTION IN GUINEA PIGS by treatment of reference drug pyrelamine maleate at a dose of 1mg/kg and partially purified fractions a-e from the leaves of *Labisia pumila* plant at doses 100mg/kg by p.o. values were expressed as mean \pm sd (n = 5). Statistical significance (*p< 0.05, **p< 0.01, ***p < 0.001, ****p<0.0001) was calculated by ANOVA followed by bonferroni's test.

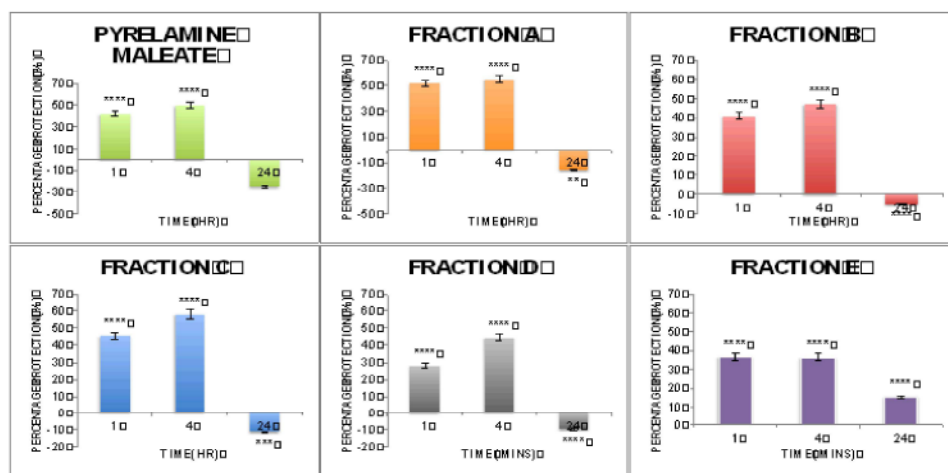


Fig. 6: PERCENTAGE PROTECTION OF ACETYLCHOLINE-INDUCED BRONCHOCONSTRICTION in guinea pigs by treatment of reference drug pyrelamine maleate at a dose of 1mg/kg and partially purified fractions A-E from the leaves of *Labisia pumila* plant at doses 100mg/kg by p.o. values were expressed as mean \pm sd (n = 5). Statistical significance (*p< 0.05, **p< 0.01, ***p < 0.001, ****p<0.0001) was calculated by ANOVA followed by bonferroni's test.

concentration of calcium to increase. An increase in calcium concentration causes smooth muscle surrounding the bronchus to contract leading to airway resistance. This will also cause the release of other mediators such; serotonin, bradykinin and leukotriene which will further enhance the bronchoconstriction effect. It was seen that the fractions were able to offer protection against histamine and acetylcholine-induced bronchoconstriction.

This finding show that the extract may be interacting with H₁ and M₃ receptor as an antagonist [22], blocking the release of histamine and acetylcholine, blocking the calcium channel or PLA₂ inhibitor because of its steroid content.

The phytochemical screening revealed the presence of alkaloids, saponins, flavonoids and steroid. The effect of these fractions may be as a result of

Table 1: Phytochemical Analysis of Partially Purified Fractions

Phytochemicals	A	B	C	D	E
Flavonoids	-	-	+	++	-
Alkaloids	++	+	+	+	+
Tannins	-	+	++	++	-
Saponins	++	++	++	++	++
Steroids	++	+	-	-	-

++ = strongly present; + = weakly present; - = absent.

the presence of these phytochemicals which have been reported previously to have similar effect Table 1.

CONCLUSION

The partially purified leaf extract of *Labisia pumila* was able to inhibit histamine, serotonin, bradykinin induced paw edema, histamine and acetylcholine induced bronchoconstriction. The highest effect was shown by fraction A and E. These effects may be as a result of the presence of phytochemicals; steroids, saponins, flavonoids and alkaloids which have been reported previously to have similar effect. The result showed that fractions A and E could be explored as a possible lead for the development of potential anti-asthmatic drug and this result is in consonance with earlier report on the DELP.

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