

# Effects of methanol and aqueous extracts of *Acacia xanthophloea*, *Strychnos heninningsii* and *Microglossa pyrifolia* on Immunoglobulin E using asthma induced mice model

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## Abstract

**Background:** The increasing prevalence of asthma in developing countries during the last decade continues to represent a significant public health problem, causing both economic and social burdens. It remains an area of considerable unmet medical need which affects 235–330 million and kills about 300,000 people worldwide. Low and middle income countries make up more than 80% of the mortality and the prevalence of Asthma in Kenya is 15.8%. Treatment of asthma still remains far from being satisfactory, which is severely limited by undesirable adverse effect and high cost. Methanol and aqueous extracts of *Acacia xanthophloea*, *Strychnos heninningsii* and *Microglossa pyrifolia* have shown efficacy on antimicrobial and antioxidant properties but have not been investigated for anti-asthmatic activities. **Objectives:** This study was aimed at evaluating the anti-asthmatic activities of extracts of *Acacia xanthophloea*, *Strychnos heninningsii* and *Microglossa pyrifolia* on asthma induced mice. **Materials and Methods:** Female Swiss Albino mice aged 8 weeks old and weighing 20 +/- 2g, were asthma induced by using 1% Ovalbumin (grade VI; Sigma, Steinheim, Germany) followed by treatment using methanol and water extracts of *A xanthophloea*, *S heninningsii* and *M pyrifolia* in concentrations of 50, 100 and 200mg/kg body weight except for positive control group of mice which was induced and not treated. Standard reference drug control group was given 10mg/kg Prednisolone. After treatment, serum total Immunoglobulin E (IgE) levels were determined using mouse OVA specific IgE Enzyme Linked Immunosorbent Assay (ELISA) (BioLegend). Cytotoxicity screening of the plant extracts was also done using Vero E6 cells and MTT dye. Data were analyzed and expressed as Means and Standard Deviation and the parametric data was statistically analyzed using one way Analysis of Variance (ANOVA) followed with unpaired student's t-test at p-value < 0.001. **Results:** The results showed that the extracts were able to reduce the serum total IgE levels by up to 100% in reference to the positive control. **Conclusion:** The extracts tested have the ability to reduce IgE levels in an asthmatic attack. The results can be used for future possible large scale implementation in an effort to solve the burden of asthma as well as the current anti-asthmatic drug side effects.

**Keywords:** Anti-asthma, Immunoglobulin E, *Acacia xanthophloea*, *Strychnos heninningsii*, *Microglossa pyrifolia*

## 1. Introduction

The prevalence of asthma has increased during the last two decades in both the developed and developing countries [3]. According to the [11], over 300 million individuals are affected with asthma worldwide, and there are approximately 250,000 deaths attributed to the disease each year. The current rising trend it is estimated that the number of people with asthma will grow by a further 100 million by 2025 [8].

Asthma is a chronic inflammatory disorder of the lungs that is characterized by recurrent episodes of airflow obstruction, bronchial inflammation, mucus hyper secretion and airway hyper responsiveness (AHR) [2]. Patients with asthma typically experience shortness of breath, wheezing, coughing and difficulty in breathing particularly after exposure to an allergen or non-specific irritants with symptoms often worsening at night. The most general form of asthma is allergic asthma which is attributable to airway inflammation triggered by an environmental allergen. Allergic asthma can be defined by the positivity to skin prick test or via the presence of IgE antibodies to common environmental allergens. Other forms of asthma are non-allergic asthma where the cause of airway inflammation is unclear and mixed-type asthma where there is a combination of allergic and non-allergic factors. It is estimated that allergic asthma patients account for slightly more than half of all asthmatics [6].

Asthma is associated to T-helper (Th) type 2 cells response, immunoglobulin (Ig) E-mediated mast cell activation, and other inflammatory factors, including eosinophils, B cells, cytokines and chemokines [7]. Despite the availability of a wide range of anti-asthmatic drugs, the relief offered by them is mainly symptomatic and short lived. Moreover their side effects are also quite disturbing. Hence a continuous search is needed to identify effective and safe remedies to treat bronchial asthma [1]. Medicinal plants such as *Acacia xanthophloea*, *Strychnos heninningsii*, and *Microglossa pyrifolia* have shown anti-bacterial, anti-cancer, anti-oxidant and anti-plasmodial activity but have not been investigated against anti-inflammatory activity even though they contain bioactive compounds that are known to show anti-inflammatory activity. This study was therefore aimed at determining the effects of aqueous and methanol leaf extracts of *A. xanthophloea*, *S. heninningsii*, *M. pyrifolia* on IgE in Asthma induced mice.

## 2. Materials and Methods

### 2.1 Plant material preparation and extraction

#### 2.1.1 Plant material preparation

The plant materials: (stem barks of *Acacia xanthophloea* and leaves of *Strychnos heninningsii* and *Microglossa pyrifolia*) were carefully washed under running tap water to remove dust and any other foreign materials and left to drain off, this was followed by air-drying at room temperature under shade for 14 days and grinding using a laboratory mill (Christy & Norris Ltd., Chelmsford, England) at the Center for Traditional Medicine and Drug research, KEMRI. The resultant plant powders were packed in air tight polythene bags.

#### 2.1.2 Extraction

**Water extraction:** A 150g of the powdered plant material were extracted with 1500 ml of distilled water in a water bath at 60°C for 1 hour. The extract were then decanted into a clean dry 3000ml conical flask and filtered through 2 layers of sterile gauze. The filtered extract were freeze dried in 200ml portions using a Freeze Dryer (Edwards freeze dryer Modulyo). The freeze dried powder was weighed, labeled and stored in an air tight bijou bottle at 4 °C until used. For *in vitro* cytotoxicity assay sterilization was done using 0.22µm Millex<sup>R</sup> syringe driven filter unit after diluting the freeze dried powder with PBS.

**Methanol extraction:** A 150g of each plant powder were weighed and put in a flat-bottomed conical flask, methanol was then added to cover the plant material completely and left to stand for 24 hours. Filtration was done as stated above. The acquired filtrate was concentrated using a rotary evaporator (Buchi Rotavapor R-114) and stored in a cool dry place until use. Further sterilization was done as stated above but with first diluting in 0.1% DMSO before final dilution with PBS

### 2.2 Induction of Asthma and measurement of serum total Immunoglobulin E (IgE) levels

#### 2.2.1 Induction of Asthma

Female healthy Swiss Albino mice aged 8 weeks old and weighing 20±2g were placed in 9 groups with 9 mice per group and each group had 3 different concentration to be administered hence a total of 3 mice were used per concentration. The groups were composed of the methanol and aqueous extracts of *Acacia xanthophloea*, *Strychnos heninningsii*, *Microglossa pyrifolia* and mix extract. The 9<sup>th</sup> group was composed of 3 subgroups; Positive control, Baseline control and standard reference subgroup. A total of 81 mice were used.

All mice except for the baseline control subgroup were sensitized by intraperitoneal (IP) injection of each mouse with 100µl of 20µg ovalbumin (grade VI; Sigma, Steinheim, Germany) emulsified in 2mg Aluminum hydroxide (Alum) (Pierce, Rockford, IL, USA) in 200µl phosphate-buffered saline (PBS) (Gonzalo et al., 1996; Yoo n-Seok et al., 2005) using a 29 gauge needle and syringe on day 0, 7 and 14 for induction of asthma. This was followed by ovalbumin challenge on day 21 and 22, where exposures to nebulizer 1% OVA in PBS (in a 5.9 L Pyrex glass box) for 30 minutes each day [4][5][12]. A once per day treatment with 0.2ml of the desired drug (the plant extracts or standard reference drug; Prednisolone) was administered intraperitoneally on day 22, 23, 24 and 25 for all mice except those in Positive control and Baseline control subgroups. The plant extracts (methanol or water extracts) were administered in the concentrations of 50, 100, and 200mg/Kg body weight with 3 mice receiving each of the concentration. The standard reference subgroup received 10mg/kg Prednisolone. All treated

mice were also observed for basic asthmatic symptoms including reduced physical activity, general discomfort, wheezing and difficulty in breathing 10 minutes before and after nebulization as well as one hour after each treatment exposure.

### 2.2.2 Mouse Ovalbumin specific IgE Enzyme linked immunosorbent Assay

Blood was obtained from mice (anesthetized by intraperitoneal injection of 50mg/kg body weight of sodium pentobarbital - sagatal - conc. 0.5v/v distilled water) by Cardiac puncture and allowed to stand at room temperature for 45minutes to clot and thereafter serum was obtained after centrifugation at 1000 xg for 10minutes at 20°C. The serum was freeze at -80°C until analysis using ELISA kit (LEGEND MAX™).

All reagents were prepared prior to use and brought to room temperature. A 500µl of the 20ng/ml top standard was prepared by diluting 50 µl of standard stock solution in 450 µl of Assay Buffer A. Six two-fold serial dilutions of the 20ng/ml top standard was performed in separate tubes using Assay Buffer A as diluent. The final mouse OVA specific IgE standard concentrations in the tubes were 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml and 0.313ng/ml, respectively. Assay Buffer A served as the zero standard (0ng/ml). Washing of the plate was done 4 times with 300 µl of 1x Wash Buffer per well and any residual buffer was blot by firmly tapping the plate upside down on absorbent paper. All subsequent washes were performed similarly. A 50 µl of Matrix A was added to each well that was to contain the standard dilutions while Assay Buffer A of the same amount was added to wells that were to contain samples. This was followed by addition of 50 µl of standard dilutions or samples to the appropriate wells. The plates were then sealed with a Plate Sealer and incubated at room temperature for 2 hours while shaking at 200rpm. After incubation the plate content was discarded and the plate washed 4 times as described above. A 100 µl of Avidin-HRP D solution was added to each well, plate sealed and incubated at room temperature for 30 minutes while shaking. After incubation the content of the plate was discarded and washing was done 5 times as described above with soaking of wells in 1x Wash Buffer for 45 seconds for each wash. A 100 µl of substrate solution F was added to each well and incubation done for 15 minutes in the dark. The reaction was then stopped by adding 100 µl of stop solution to each well. Absorbance was read at 450 nm within 10 minutes.

### 2.3 Cytotoxicity Assay

On day one, Vero E6 cells in T-25 flask were trypsinized and 5 ml of complete Dulbecco's Modified Eagle Medium (Gibco<sup>R</sup> DMEM) was added to trypsinized cells to stop reaction, followed by centrifugation in a sterile 15 ml falcon tube at 300 xg for 5 min at 21°C. Media was removed and the cells resuspended with 1.0 ml complete media (DMEM supplemented with 15% fetal calf serum and 1% penicillin/streptomycin). Cells were then counted and recorded per ml. Dilution of cells was done using complete DMEM medium as per the following principle ( $C1V1=C2V2$ ) to 200,000cells per ml. A 100 µl of cells (20,000 total cells) were added into each well except for column 3, 6, 9 and 12 which served as controls then the plates were incubated for 24hrs at 37°C, 5% CO<sub>2</sub> and 95% humidity. On the second day cells were treated cells with the medicinal plant extracts after removal of the media. The highest concentration of the extract was placed at row H at a concentration of 1000µg/ml then serially diluted upwards until row A. (Serial dilution was done by placing 100µl of complete DMEM media in all the rows except H, then 50µl of the resuspended extract was transferred to the next row from H till A). The final concentration of the extract in each row is shown in the table 1. below. A single 96 well plate was enough to analyze four extracts. Since the first extract takes column 1,2,3, the second 4,5,6, the third 7,8,9 and the fourth extract 10,11,12.

Table 1: Concentration of plant extract in each row

1	2	3	4	5	6	7	8	9	10	11	12
A	0.46µg/ml										
B	1.37µg/ml										
C	4.12µg/ml										
D	12.35µg/ml										
E	37.04µg/ml										
F	111.11µg/ml										
G	333.33µg/ml										
H	1000µg/ml										

The final volume in each well was 100µl. The plates were then incubated at 37°C/95% humidity and 5% CO<sub>2</sub> for 24hrs. On the third day, a 20 µl of 5 mg/ml MTT was added to each well. Including the wells without cells (control wells: 3, 6, 9 and 12). This was incubated for 4 hours at 37°C in culture hood. Media was then removed carefully and 100µl of DMSO was added into each well without disturbing or rinsing the cells with PBS. Then cells were covered with tinfoil and agitated on orbital shaker for 15min. Absorbance was read at 562nm with a reference filter of 690nm. The results were then analyzed using Almar Blue assay software

### 3. Results

The mice exhibited basic asthmatic symptoms including reduced physical activity and general discomfort on the first day of nebulization, followed by wheezing and difficulty in breathing on the second day of nebulization. The symptoms reduced as treatment commenced in most groups with only two deaths recorded (in mice receiving 200mg/kg body weight of *S.heninningsii* methanol extract and *M.pyrifolia* water extract respectively) out of a total of 81 mice.

#### 3.1 Serum total IgE levels after treatments

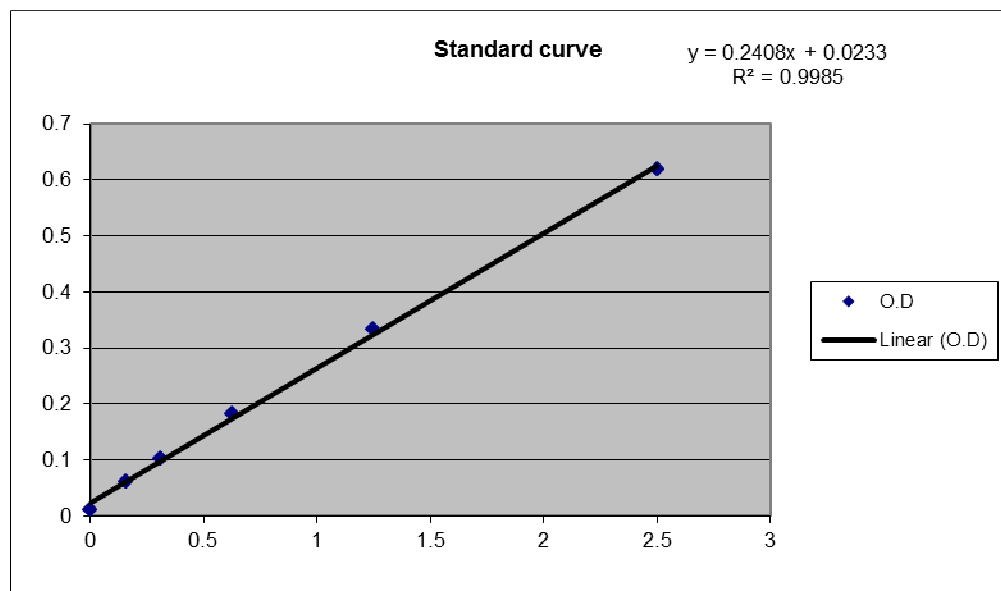
The serum total IgE levels were calculated from the standard curve using the known standard concentrations and their specific optical density read at 450nm. The optical densities of the samples read at the same absorbance were used to calculate their specific serum levels using the following equation obtained from the standard curve in figure 1 below:  $y = 0.2408x + 0.0233$  ( $R^2 = 0.9985$ ), then multiplied by 2 which is the dilution factor for the serum. The positive control group (mice induced with asthma and not treated) exhibited the highest serum total IgE level of 7.25ng/ml. Standard drug reference group (treated with 10mg/kg b.w Prednisolone), base line control group (non-induced and non-treated) and most extract had their serum total IgE at 0ng/ml. The extracts that were able to reduce IgE level to zero include 50mg/Kg b.w of *A.xanthophloea* water and methanol extract, *S.heninningsii* methanol extract, *M.pyrifolia* methanol extract, Mix water extract, 100mg/Kg b.w of *A.xanthophloea* methanol extract, *M.pyrifolia* water and methanol extract, Mix water and methanol extract, 200mg/Kg b.w of *A.xanthophloea* water, *S.heninningsii* water extract, *M.pyrifolia* methanol extract, and Mix water extract. The results for serum total IgE levels are shown in figure 2.a and b below. The percentage reduction of serum total IgE levels was calculated in reference to positive control, see figure 3 below. As high as 100% reduction in serum total IgE was seen in extracts mentioned above with the least reduction being 60.95%.

The final mean optical densities as read at 450nm were compared for all samples (figure 4). The optical densities for separate extracts type (methanol or water) were also compared and analyzed using one way Anova and individual O.D for each concentration of extract compared with positive control value using unpaired student's t-test (figure 5 and 6) The Base line control (non-induced and non-treated) and Standard reference drug control group were also compared to the positive control group (figure 7). Graph pad prism 6 software was used for analysis. There was a significant difference in the extracts optical densities and controls in reference to the positive control (P value <0.001). Figure 5, 6, and 7 below show the mean optical density and standard deviation.

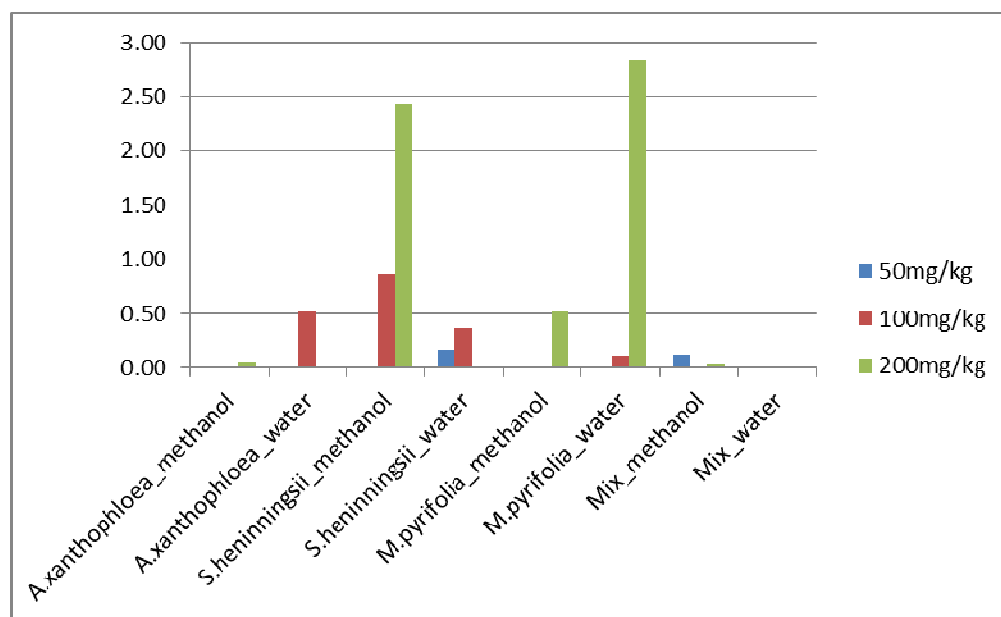
#### 3.2 Cytotoxicity screening of the plant extracts

Cytotoxicity test was done to determine the dosage of the plant extracts that does not kill or damage the mammalian cells after establishing that the plant have the ability to reduce serum total IgE levels in mice. Vero E6 cells derived from kidney tissue of *Cercopithecus aethiops* (Vero ATCC<sup>®</sup> CCL81<sup>™</sup>) were used. The cells

were obtained from the Kenya Medical Research Institute (Kemri) tissue culture lab. The Cytotoxic concentration 50 (concentration with 50% viable cells – also known as Effective dilution; ED) measured with MTT dye and analysed with Almar Blue assay (figure 8a,b,c,d and 9a,b,c) indicated that Methanol extracts of *Acacia xanthophloea*, *Microglossa pyrifolia* and *Strychnos heninningsii* had a CC 50 of 83.79, 278.72, and 540.19µg/ml respectively (figure 8a,b,c), while the water extracts of the same plants indicated a CC 50 of 56.05, 198.60 and 389.08µg/ml respectively (figure 9a,b,c). Mixed methanol extract had no significant loss of viability (8.d).

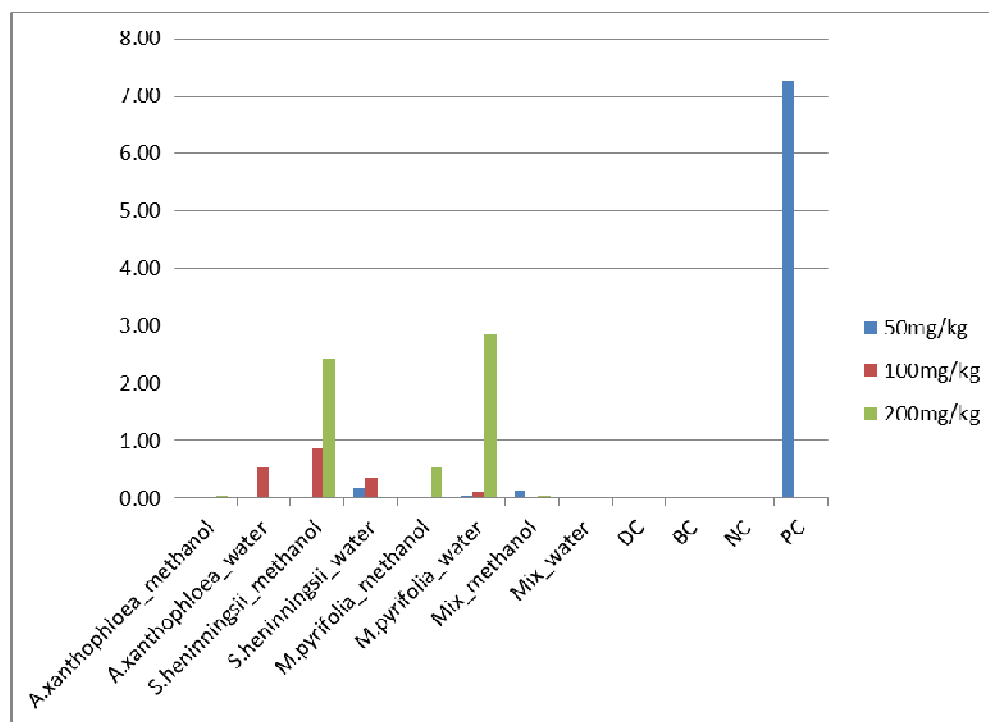


**Figure 1:** Standard curve obtained from standards Immunoglobulin E (IgE) concentrations against their optical densities. The y-axis represents the optical density (O.D) read at 450nm while the x-axis represents the standards IgE concentration in ng/ml.

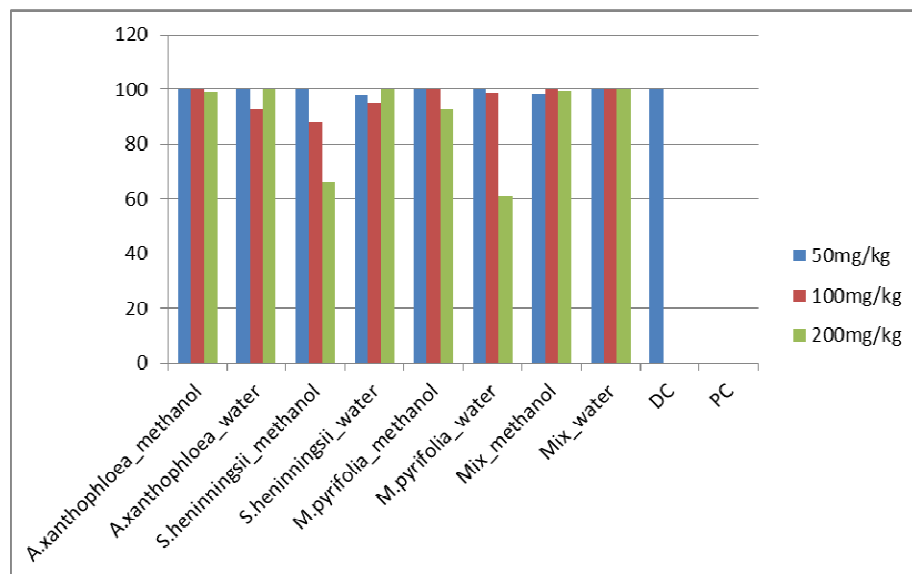


**Figure 2.a:** Serum total IgE levels in mice treated with the plant extracts in concentrations of 50, 100, or 200mg/ml. The IgE levels were calculated using the equation  $y = 0.2408x + 0.0233$  derived from standard curve in figure 1. The unknown 'x' which is the IgE concentration in ng/ml was determined. The y value is the optical

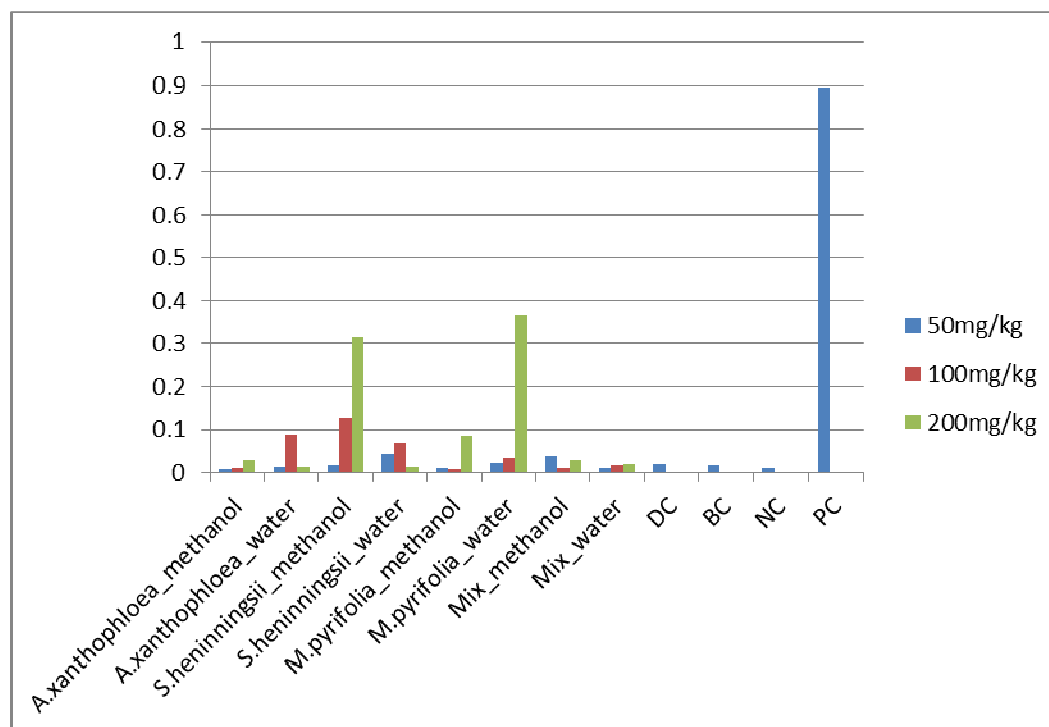
densities obtained from the absorbance readings. The resultant IgE concentration was multiplied by two since the serum was double diluted in the experiment.



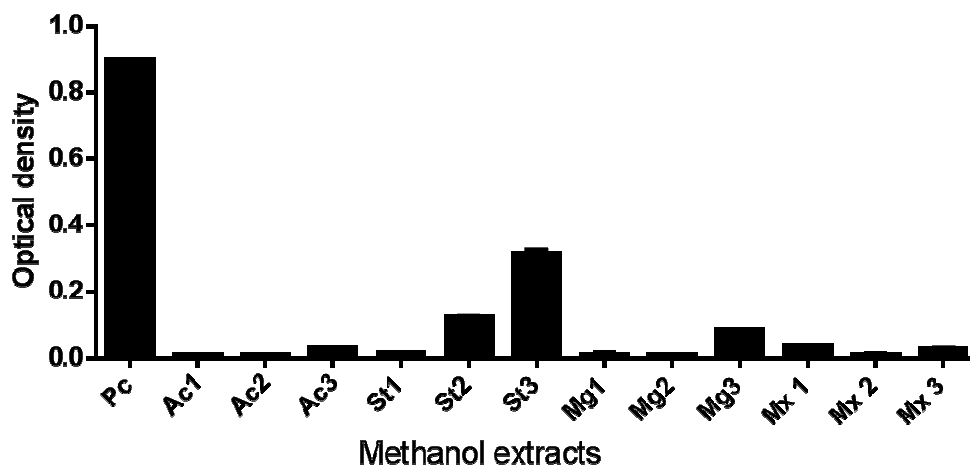
**Figure 2.b:** Serum total IgE levels in mice treated with the plant extracts in concentrations of 50, 100, or 200mg/ml and the controls including the standard reference drug control (DC) which is prednisolone, the Baseline control (BC) which are mice which were non-induced and non-treated, Negative control (NC) which is blank and the Positive control (PC) which are mice induced with Asthma and not treated. The IgE levels were calculated using the equation  $y = 0.2408x + 0.0233$  derived from standard curve in figure 1. The unknown 'x' which is the IgE concentration in ng/ml was determined. The y value is the optical densities obtained from the absorbance readings at 450nm. The resultant IgE concentration was multiplied by two since the serum was double diluted in the experiment.



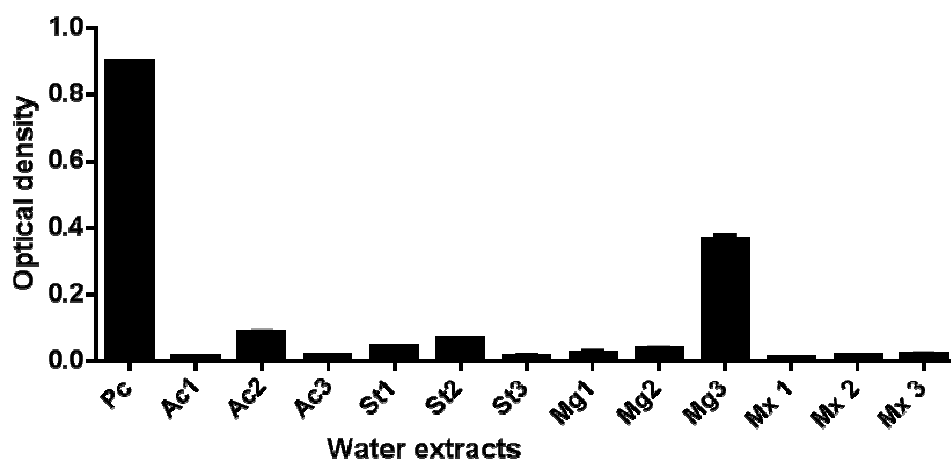
**Figure 3:** Percentage reduction in serum total IgE levels in reference to Positive control. The percentage reduction was calculated by determining the difference between the serum IgE levels of extracts group mice or drug control group mice and the positive control group then dividing by the serum IgE level of the positive control group and finally multiplying by 100 to get the percentage.



**Figure 4:** comparison of the optical densities as read at 450nm

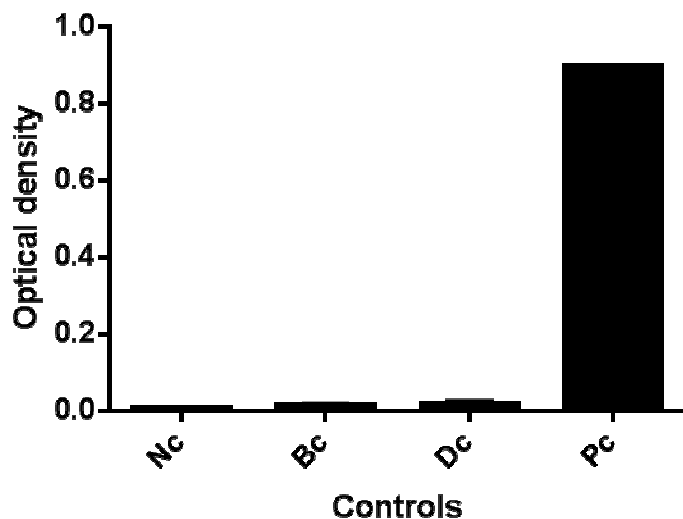


**Figure 5:** Methanol extracts; *Acacia xanthophloea* (Ac), *Strychnos heninningsii* (St), *Microglossa pyrifolia* (Mg) and Mixed extract (Mx) in concentrations 1, 2, and 3 representing 50, 100 and 200mg/Kg b.w respectively against their optical densities at 450nm. Pc represents positive control. There is a significant difference ( $P < 0.001$ ) between the methanol extracts and the positive control analysed using one way Anova, F is 6881. The individual extracts are also significantly different from the positive control ( $P < 0.001$ ) as analysed by unpaired student's t-test

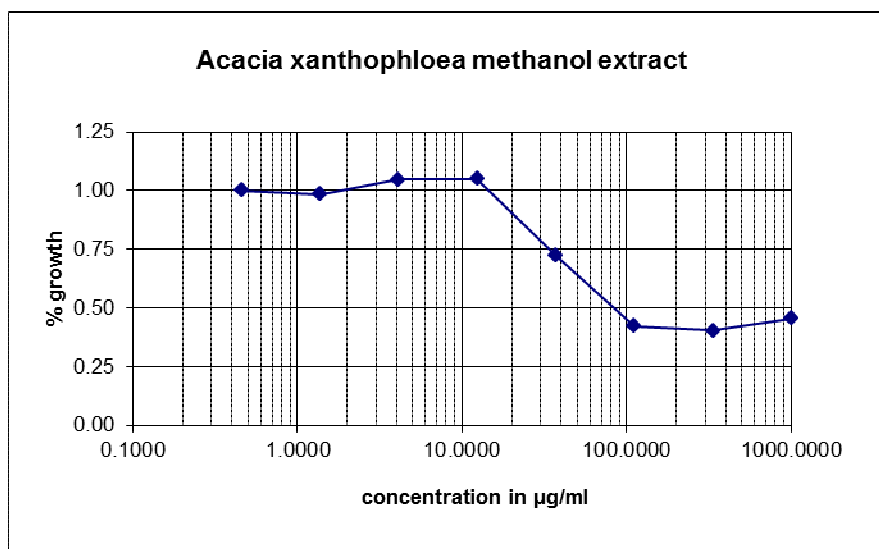


**Figure 6:** Water extracts; *Acacia xanthophloea* (Ac), *Strychnos heninningsii* (St), *Microglossa pyrifolia* (Mg) and Mixed extract (Mx) in concentrations 1, 2, and 3 representing 50, 100 and 200mg/Kg b.w respectively against their optical densities at 450nm. Pc represents positive control. There is a significant difference ( $P < 0.001$ ) between the water extracts and the positive control analysed using one way Anova, F is 5979. The individual extracts are also significantly different from the positive control ( $P < 0.001$ ) as analysed by unpaired student's t-test

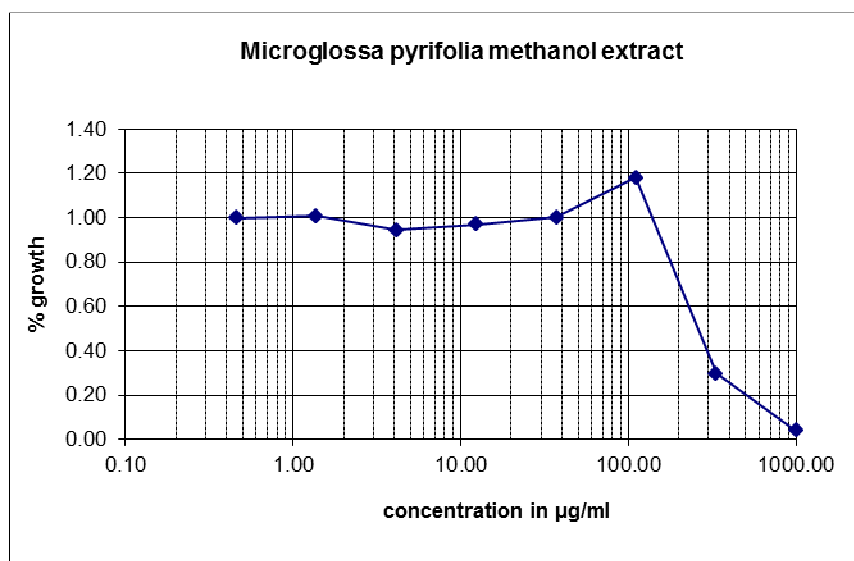




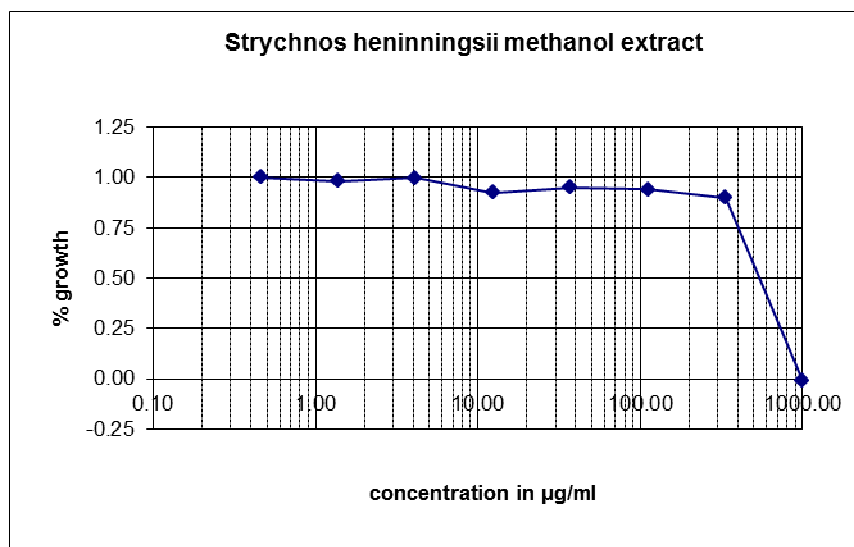
**Figure 7:** Controls; Negative control (Nc), Baseline control (Bc) and Drug control (Dc) and Positive control (Pc). There is a significant difference between the individual controls and the positive control ( $P < 0.001$ ) analyzed with unpaired student's t-test.



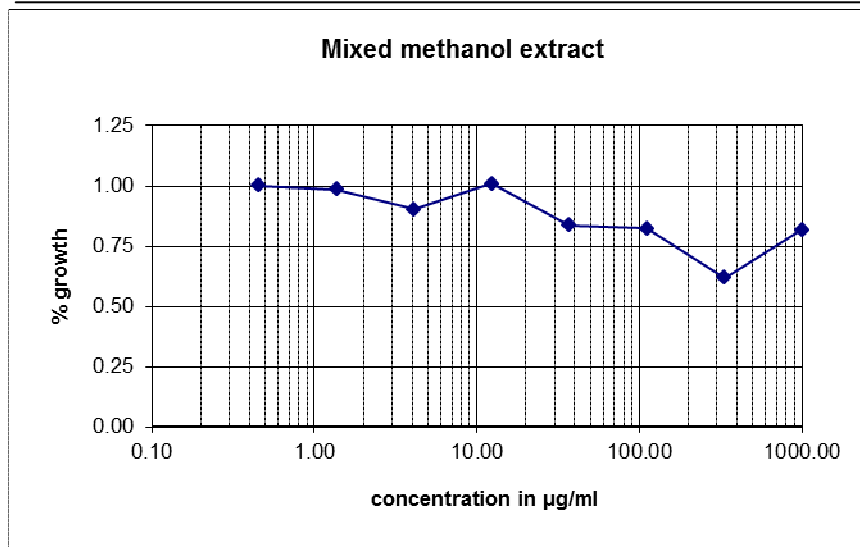
**Figure 8.a:** Cytotoxicity results for *Acacia xanthophloea* methanol extract as tested on Vero E6 cells and viability determined using MTT dye. Absorbances were read at 562nm and a reference wavelength of 690nm was used. The optical densities were analysed by Almar Blue assay. Cytotoxic concentration 50 (CC50) is 87.79 $\mu\text{g/ml}$



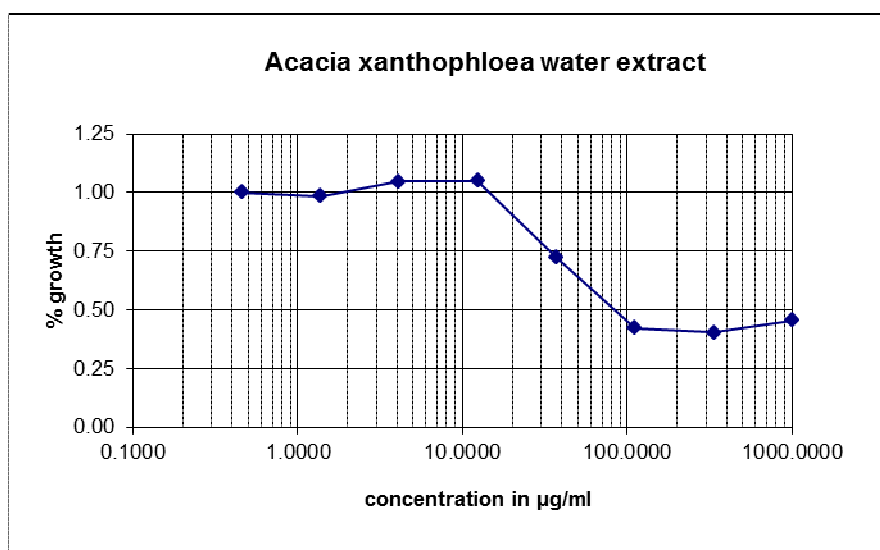
**Figure 8.b:** Cytotoxicity results for *Microglossa pyrifolia* methanol extract as tested on Vero E6 cells and viability determined using MTT dye. Absorbances were read at 562nm and a reference wavelength of 690nm was used. The optical densities were analysed by Almar Blue assay. Cytotoxic concentration 50 (CC50) is 258.72 $\mu\text{g/ml}$



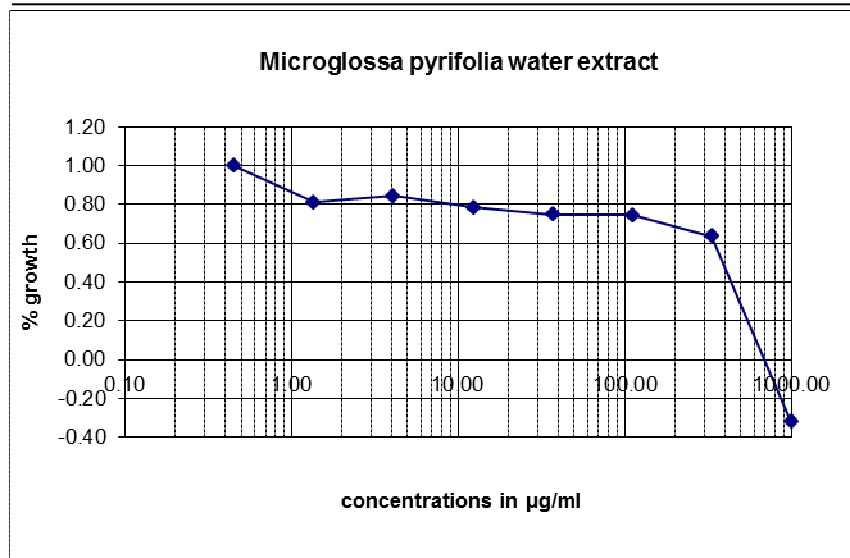
**Figure 8.c:** Cytotoxicity results for *Strychnos heninningsii* methanol extract as tested on Vero E6 cells and viability determined using MTT dye. Absorbances were read at 562nm and a reference wavelength of 690nm was used. The optical densities were analysed by Almar Blue assay. Cytotoxic concentration 50 (CC50) is 540.19 $\mu\text{g/ml}$



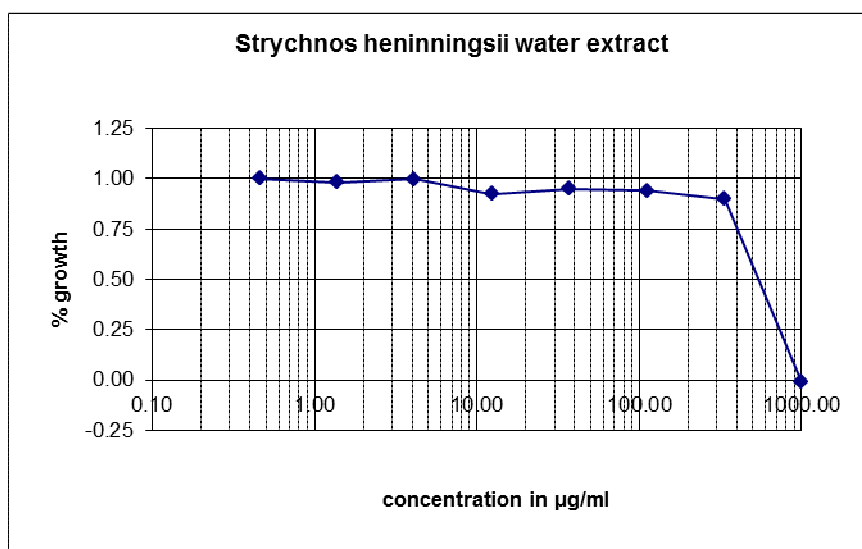
**Figure 8.d:** Cytotoxicity results for mixed methanol extract as tested on Vero E6 cells and viability determined using MTT dye. Absorbances were read at 562nm and a reference wavelength of 690nm was used. The optical densities were analysed by Almar Blue assay. Cytotoxic concentration 50 (CC50) is not applicable for mixed extract since there was no significant loss of viability.



**Figure 9.a:** Cytotoxicity results for *Acacia xanthophloea* water extract as tested on Vero E6 cells and viability determined using MTT dye. Absorbances were read at 562nm and a reference wavelength of 690nm was used. The optical densities were analysed by Almar Blue assay. Cytotoxic concentration 50 (CC50) is 56.05 $\mu\text{g/ml}$ .



**Figure 9.b:** Cytotoxicity results for *Microglossa pyrifolia* water extract as tested on Vero E6 cells and viability determined using MTT dye. Absorbances were read at 562nm and a reference wavelength of 690nm was used. The optical densities were analysed by Almar Blue assay. Cytotoxic concentration 50 (CC50) is 389.08µg/ml.



**Figure 9.c:** Cytotoxicity results for *Strychnos heninningsii* water extract as tested on Vero E6 cells and viability determined using MTT dye. Absorbances were read at 562nm and a reference wavelength of 690nm was used. The optical densities were analysed by Almar Blue assay. Cytotoxic concentration 50 (CC50) is 198.60µg/ml.

## 4. Discussion

### 4.1 Asthma and IgE levels

Immunoglobulin E (IgE) has long been implicated in asthmatic attack [14] with several cascade developed for the asthmatic process generally termed as allergic cascade. Asthmatic attack is an allergic reaction regarded as type 1 hypersensitivity reaction and IgE has evidently been known to play a key role in this reaction. The allergic cascade has been summarized into three steps, namely; a) Sensitization phase, b) Early allergen response phase-

upon re-exposure to allergen and c) Late allergen response phase. The asthmatic induction process in this study was based on this approach. The sensitization phase is characterized by initial exposure to an allergen in minute doses, the allergen is then presented to T-helper cells by Antigen presenting cells (APC) such as macrophages and dendritic cells, and this triggers the T-helper cells to stimulate B cells to develop into plasma cells which produce IgE antibodies. In this study this process was successfully achieved evident by the detection of as high as 7.25ng/ml of serum total IgE levels in the positive control group (mice induced with Asthma and not treated). The IgE produced is normally specific to a particular allergen used. In our case Ovalbumin was used as an allergen and therefore the IgE antibodies produced were Ovalbumin specific. Mouse Ovalbumin specific IgE Enzyme Linked Immunosorbent Assay (Elisa) was used to detect the serum total IgE levels. The IgE antibodies produced in the sensitization phase have special receptors on mast cells, the bind to mast cells completing the sensitization phase. In the early phase response to allergen, this occurs when there is a re-exposure to the specific allergen. IgE on mast cells binds to the allergen leading to crosslinking of multiple IgE antibodies. As a result the mast cells explode and release histamine and other inflammatory mediators leading to allergic/asthmatic symptoms such as wheezing, sneezing, shortness of breath, coughing, itchy eyes and running nose. Wheezing and difficulty in breathing are some of the symptoms that were evident in the mice used. The late allergen response phase involves the eosinophils. IgE antibodies which have receptors on eosinophils, binds to them leading to more adverse symptoms such as congestion, airway hyper responsiveness and tissue damage in chronic exposures. This phase normally occurs 10 to 24hours after the first allergic reaction.

#### 4.2 Medicinal plants anti-asthmatic property

The medicinal plants used in this study; *Acacia xanthophloea*, *Strychnos heniningsii* and *Microglossa pyrifolia* showed the ability to reduce IgE levels by upto 100% evident by reduction of serum total IgE levels from 7.25ng/ml in positive control to 0ng/ml in most extract concentrations. The extracts that were able to reduce IgE level to 0ng/ml include 50mg/Kg b.w of *A.xanthophloea* water and methanol extract, *S.heniningsii* methanol extract, *M.pyrifolia* methanol extract, Mix water extract, 100mg/Kg b.w of *A.xanthophloea* methanol extract, *M.pyrifolia* water and methanol extract, Mix water and methanol extract, 200mg/Kg b.w of *A.xanthophloea* water, *S.heniningsii* water extract, *M.pyrifolia* methanol extract, and Mix water extract. The anti-asthmatic activity of these extracts can be attributed to presence of anti-inflammatory phytochemical constituents that has not been investigated. Recently, many natural medicines derived from plants, marine organisms, etc. were considered effective and safer for the treatment of various diseases including inflammation and pain [10]. Anti-inflammatory activity in medicinal plants has been accounted to presence of phytochemical bioactive compounds such as flavonoids, saponins, steroids, carbohydrates, phenols, and glycosides [13]

#### 4.3 Extraction Methods

The use of methanol and water extraction led to obtaining optimal amounts of the bioactive compounds. Some of the chemical classes extracted from medicinal plants using the methanol extraction include saponins, tannins, phenols, flavonoids, sugars, amino acids, anthocyanins, terpenoids, xanthoxyllines, totarol, quassinoids, lactones, and polyphenols [9]. Water extraction achieves to obtain bioactive compounds such as saponins, tannins lectins, terpenoids, sugar, anthocyanins, starches, and polypeptides.

#### 4.4 Cytotoxicity of the medicinal plants

The medicinal plants were tested to be cytotoxic to mammalian cells (Vero E6 cells) when used in higher concentrations. The concentrations differ from one extract to another. However mixed methanol extract were not highly cytotoxic showing less than 50% cytotoxicity even at high concentration. The cytotoxic properties of these plants can be attributed to the presence of some amounts of alkaloids and other compounds. Base on this study, the plants should be used at concentrations lower than mention cytotoxic concentration 50 (CC 50). Methanol extracts of *Acacia xanthophloea*, *Microglossa pyrifolia* and *Strychnos heniningsii* had a CC 50 of 83.79, 278.72, and 540.19 $\mu$ g/ml respectively (figure 8a,b,c), while the water extracts of the same plants indicated a CC 50 of 56.05, 198.60 and 389.08 $\mu$ g/ml respectively (figure 9a,b,c). Mixed methanol extract had no significant loss of viability (8.d).

#### 5. Conclusion

Based on this study, *Acacia xanthophloea*, *Microglossa pyrifolia* and *Strychnos heniningsii* have the ability to reduce serum total IgE levels in an Asthmatic attack. The effective and safe dosage of the medicinal plants should be determined before usage. The results in this study can to be used for future possible large scale implementation in an effort to solve the burden of asthma as well as the current anti-asthmatic drug side effects.

## Competing interest

The authors declare that they have no competing interest

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