Egypt. J. Chem. **Vol. 66,** No. **SI 13, 683-696** (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Chemical Profiling and Antiasthmatic Activity of *Euphorbia mauritanica*: *In vivo* Study

Mona Hetta[1]; Abeer Salama[2]; Rehab Hussein[3]; Marwa Samy[4]; Nemat Yassin[2]

1 Pharmacognosy Department, Faculty of Pharmacy, Fayoum University, 63514, Fayoum, Egypt
2 Pharmacology Department, National Research Centre, Cairo, Egypt
3 Pharmacognosy Department, National Research Centre, Cairo, Egypt
4 Pharmacognosy Department, Faculty of Pharmacy, Al Azhar University - Girls, Egypt

Abstract

The prevalence of asthma has dramatically increased in the last decades. Egyptian children aged 3 - 15 years was estimated to be 8.2%. Of major concern is a 10% annual increase in mortality. And probably, it is the most common cause of hospital admission for a respiratory complaint in adults. The **aim** of the present study is designed to find out safe and effective remedy to prevent asthma attack in susceptible patients from natural source. The total ethanol 70% extract of *Euphorbia mauritanica* L. was defatted using n- hexane. The non-polar fraction was analyzed by GC/MS technique. Metabolomics was performed using LC/HRMS analysis to identify the chemical components of this species. Antiasthma activity was studied in vivo on the extract. Asthma was induced by ovalbumin (OVA) sensitization followed by OVA challenge by intraperitoneal injection followed by intranasal challenge at day 20. Four groups of rats were used: 1st& 2nd groups for negative and positive control; 3rd& 4th groups were treated with the extract at 200 and 400 mg/kg orally concurrent with OVA sensitization and challenge. Measurement of Tidal Volume (TV) and Peak Expiratory Flow (PEF), MDA, GSH,IgE, IL-5, TNF-α and histopathology study were performed. Results of GC/MS analysis showed the presence of seven of the identified compounds are sesquiterpenes, one diterpene and one fatty acid ethyl ester. *β*-Vetivone is the major identified compound. LC/HRMS analysis identified 16 compounds including quercitin glycoside, oleanane terpenoids, phorbol acetate derivative and chlorogenic acid among the components. Results of antiasthma assay proved that *Euphorbia mauritanica* L. extract improved TV, PEF, GSH, reduced MDA, IgE, IL-5, TNF-α and alleviated histopathology changes produced by OVA. It is concluded that the identified components in *Euphorbia mauritanica* L. ethanol 70% extract are correlated with the interesting findings of results suggesting its role as antiasthma natural source.

Keywords: *Euphorbia mauritanica* L., GC/MS, LC/HRMS, antiasthma, *in vivo* study, histopathology

1. Introduction

Asthma is a lower airway inflammation and reversible airway obstruction. It promotes a decrease in the airflow [1]. According to World Health Organization (WHO), about 262 million people have suffered from asthma in 2019 and 461000 died [2]. The combination of inhaled corticosteroids and long-acting β2 agonist (LABA) are asthma preferred treatment in clinics [3] even though the side effects of both [4]. Other options such as leukotriene receptor antagonists, or anti-immunoglobulin E monoclonal antibodies are used in patients whose asthma is not well controlled [5]. Some environmental allergies can induce asthma as dust, food and pollen which can release cytokines as tumor necrosis factor-α (TNF-α), interleukins as IL-5, histamine and leukotriene [6]. The respiratory epithelium basal membrane thickening and sputum exist in severe asthma [7] and exacerbations control cannot be achieved by treatment with standard therapy or if the treatment is reduced, symptoms flare up [8]. In asthma progression, mucosal innate immune system activation, antigen-presenting cells activation and T helper2 (Th2)/mast cell/eosinophil activation are mediating inflammatory pathways, thus, suppression of type 2 cytokines, such IL-5 leads to immune response of Th2-mediated eosinophilic reorganization [9].In addition, antigen-presenting cells release proinflammatory mediator; TNF-α [10] which, in turn produce tissue remodeling that are associated with asthma late stages [11].

The prevalence of asthma and allergies is increasing in both western and developing countries. Among Egyptian children aged 3 - 15 years, the prevalence of asthma was estimated to be 8.2% [12], with the major concern is a 10% annual increase in mortality [13].

*Corresponding author e-mail: berrotec@yahoo.com .; (Abeer Salama).
Receive Date: 01 May 2022, Revise Date: 15 August 2022, Accept Date: 22 May 2023
DOI: 10.21608/EJCHEM.2023.136711.6019

The conventional treatment of asthma has side effects, are highly coasted and not compatible with some patients. Then the herbal remedy and the use of natural source is the choice for these people, especially in the third world countries as a complementary or alternative treatment for asthma [14].

The genus Euphorbia is one of the most diverse genera in the Euphorbiaceae family and exceeding 2000 reported species [15].They are widely distributed in tropical, subtropical, and temperate regions. Euphorbia species showed biological activities as, anti-inflammatory, antioxidant, antimicrobial and cytotoxic activity [16].This genus produce, pathologically, a milky exudate, latex, [17]. Traditionally, the latex and leaves of this genus are used for bronchitis [15]. They contain, chemically, polyphenols [18], steroids, terpenoids as major constituents [19].

Euphorbia mauritanica L., with few reports in literature, includes several varieties and synonyms; it is commonly known as yellow milk bush, golden spurge [20]. The antiproliferative activity of this species in lung cancer showed an efficient role in inducing the death of these cells [21].Mauritanicain, a serine protease, was isolated from the latex of this species and showed high antinflammatory activity [22]. The proteolytic active proteins, isolated from latices of *Euphorbia mauritanica* L. stimulate the production of interleukine-6 and interleukine-8 in keratinocytic HaCaT cells (human adult calcium low high temperature keratinocytes [23]

Euphorbia mauritanica L. are reported to have antiviral and immunogenic properties against pathogens affecting the respiratory tract [24].

The aim of this study is testing the antiasthmatic activity of *Euphorbia mauritanica* L.*in vivo* as a natural source for the first time

2. Materials and methods

Phytochemical study:

Plant material

The botanical specimen was collected from Cactus farm in El Mansouria, Giza, Egypt. It was authenticated by the senoir botanist; Trease Labib at El Orman Garden . A voucher specimen no. FUPD60 was kept at Pharmacognosy Department, Faculty of Pharmacy, Fayoum University.

Preparation of samples

The aerial parts of *Euphorbia mauritanica* L. (790 g) were into small pieces, extracted with 70% ethanol (3L.) by maceration. The solvent was distilled off using a rotatory evaporator under reduced pressure at a temperature not exceeding 50^{o}C. A portion of the dried crude extract was stored in a dark glass container in a - 20^{o}C refrigerator for biological study. The other portion of the crude total ethanol extract (70%) was defatted using *n*-hexane. The *n*-hexane fraction (nonpolar part)was analyzed by GC/MS. The defatted ethanol 70% (polar part) was kept for phytochemical investigation.

GC/MS analysis:

GC/MS was done using a Finngan SSQ 7000 gas chromatograph coupled with a mass spectrometer with the following conditions: Capillary column: DB-5 fused silica (5% phenyl methyl polysiloxane), 30 m length, 0.25 mm id and 0.25 μm thickness. Carrier gas: Helium at 13 psi. Oven temperature: was programmed at 60°C isothermal for 3 min., then heating to 260°C at a rate of 4°C/min. then isothermal at 260°C for 5 min. Injector temperature: 220°C. Ionization energy: 70 eV. Volume injected: 1μl .

Phytochemical investigation of the polar fraction

LC-HR/ ESI-MS analysis

The LC-Q-TOF-MS system, 6530 (Agilent Technologies) equipped with an autosampler (G7129A), a Quat. Pump with an on-line degasser (G7104C), and a thermostatted column compartment (G7116A) were used for chromatographic separation. The injection volume was 2 µL. The analytes were separated on a Zorbax RP-18 column from Agilent Technologies (dimensions: 150 mm × 3 mm, dp = 2.7μm) in a flow rate of 0.1 mL•/min detection was done by a photodiode array detector (DAD), an auto-sampler and an electron spray ionization (ESI) source was interfaced .

The sample (100µg/mL) solution was prepared using high performance liquid chromatography (HPLC) analytical grade solvent filtered using a membrane disc filter (0.2μm) then subjected to LC-ESI-MS analysis. Samples injection volumes (10μL) were injected into the instrument equipped. Mobile phase was filtered using 0.2μm membrane filter and degassed by sonication before injection.

The mobile phase consisted of a combination of solvent A (0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid). The gradient elution was as follows: t = 0 min, 30% B; t = 10 min, 45% B; t = 15 min, 65% B; t = 25 min, 80% B; t = 30 min, 90% B; t = 32 min, 30% B. Mass spectra were simultaneously acquired using ESI in positive ionization mode with a capillary voltage of 4000 V. The mass spectra were recorded in the m/z range of 40 to 1500 m/z. The gas temperature and drying gas flow were 350^{0}C and 10 L•/min, respectively. The skimmer and fragmentation voltages were set at 65 and 130 V, respectively and collision energy was 10V. The nebulization pressure was 35 psig. The peaks and spectra were processed using 6200 series TOF/6500 series Q-TOF B.09.00 (B9044.0) software and tentatively identified by comparing its retention time (Rt) and mass spectra with reported data [25]; [26]; [27]; [28], mass spectra databases available online http://www.massbank.jp/ and the apparatus mass library.

Pharmacological study:

Animals

Male and female Swiss mice with an average weight of 20-30 g and adult male Wistar Albino rats, weighting (130–170 g), were purchased from the National Research Centre (NRC), Giza, Egypt. Rats were kept under a controlled temperature (22–23°C), on 12-h light/dark cycles with access to standard food and tap water ad libitum in the laboratory animal house of NRC, Cairo, Egypt. The present study was carried out according to the guidelines of the Ethics Committee, NRC, which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory animals.

Drugs, chemicals and reagent kits

Ovalbumin (OVA; grade III) and aluminum hydroxide (Al $(OH)_3$) were purchased from Sigma Aldrich Chemical Co. (USA).

Immunoglobulin-E (IgE), interleukin -5 (IL-5), and tumor necrosis factor-alpha (TNF-α) ELISA kits were obtained from SunRed Biotechnology, Company.

Malondialdehyde (MDA) and reduced glutathione (GSH) kits were purchased from Bio diagnostic, Egypt. All chemicals, solvents, and reagents used were obtained from certified sources and were of analytical grade.

Experimental design

Acute toxicity study

Acute toxicity was carried out according to OECD guideline for the evaluation of the safety and efficacy of herbal medicines. Forty Swiss mice were divided into four groups as follows: Groups 1 and 2: Control male and female mice (ten mice each) were administered saline orally. Groups 3 and 4: Male and female mice (ten mice each) were administered single oral dose of *Euphorbia mauritanica* (5g/kg). The animals were observed daily for signs and behavioral changes until two weeks [29].

Induction of bronchial asthma

The rats were randomly allocated into 4 groups (n=8). Asthma was induced by OVA sensitization followed by OVA challenge. Animals were sensitized by i.p. injection of 1 mg/kg OVA/100 mg$Al(OH)_3$ suspended in normal saline at days 1, 2, 3 and 11. At day 20, the animals were subjected to intranasal (i.n) challenge with 1.5 mg of OVA/150 mg $Al(OH)_3$/kg, for 3 consecutive days [30; 31]. Induction of asthma was done in all groups except the 1st group where the rats received saline instead of OVA to serve as negative control.2nd group was left untreated to serve as positive control. 3rd and 4th groups: rats received daily total ethanol 70% extract of *Euphorbia mauritanica* (200 and 400 mg/kg), orally administered for 3 weeks concurrent with OVA sensitization and challenge.

Measurement of Tidal Volume (TV) and Peak Expiratory Flow (PEF)

The rats were placed in a specific body plethysmograph made of plexi glass. Rats head protruded through a neck collar made of a dental latex dam into a head exposure chamber that ends with a flow head connected to spirometer (ADInstruments spirometer, ML140) which is a precision differential pressure transducer for measuring respiratory variables, such as Tidal volume (TV): the volume of air moved in and out of the lung during quite breathing (ml) and Peak expiratory flow (PEF): maximum speed achieved during maximum forced expiration (ml/min) [32].

Preparation of lung homogenates

At the end of the experiment, the animals were sacrificed by decapitation and the two lungs were dissected and weighted separately. One lung was used for histopathological study and the other lung was homogenized in ice-cold phosphate buffer (pH 7.4) to prepare 20% w/v homogenate using a homogenizer (Heidolph, DIAX 900, Germany)[33]. Lung homogenates were centrifuged at 2000 xg for 20 min at 4°C then stored at -80ºC for analysis of MDA, GSH, IgE, IL-5 and TNF-α

Biochemical measurements

The lung contents used for the assessment of lipid peroxidation (LPO) as MDA and GSH according to the methods described by [34]. Another part of the lung contents used for estimation of IgE, IL-5 and TNF-α by enzyme-linked immunosorbent assay (ELISA) kit. We followed the manufacturer's instructions of SunRed Biotechnology Company for calculating the results. Standards and samples were pipetted into wells with immobilized antibodies specific for each parameter and then horseradish peroxidase-conjugated streptavidin was pipetted into the wells and incubated 60 min at 37ºC, which were aspirated and washed 5 times with kit washing solution. chromogen A and B solutions were added to the wells and incubated 15 min at 37ºC; color developed proportionally to the amount of IgE, IL-5 and TNF-α bound. Color development was discontinued (Stop Solution) and after 10 min color intensity was measured at 450 nm [35]. Using Multisacn FC ELISA reader, Model 1545, Thermo Scientific, China.

Statistical analysis

Data are expressed as mean ± S.E. Data analysis was done using one way analysis of variance (ANOVA) followed by Tukeys (HSD) test for multiple comparisons. Difference was considered significant when p is less than 0.05. SPSS (version 11) program was used to carry out these statistical tests.

3. Results

Phytochemical study

An aliquot of n- hexane (non-polar) fraction was analyzed by GC/MS which proved the presence of nine major peaks starting from the min 27.69 and ending at the min 56.79 (Figure 1). Names of the identified compounds, their retention times, relative retention times, molecular weights, area under curve

(concentration) and formulas are illustrated in Table 1. Seven of the identified compounds are sesquiterpenes, one diterpene and one fatty acid ethyl ester. β-Vetivone is the major identified compound detected at 53.66 min.

Table 1: Identified compounds in *Euphorbia mauritanica* L.non-polar fraction, their retention times, relative retention times, molecular weights and formulas:

No. of peak	Name of compound	Rt (min.)	RRT	Relative AUC	Molecular weight	Molecular formula
1	Oxidohima-chalene	27.693	0.5	4.26	218	C15H22O2
2	Nootkatin	28.363	0.52	4.55	232	C15H20O2
3	Cedryl methyl ketone	29.163	0.54	6.99	246	C17H26O
4	Nuciferol acetate	31.356	0.58	11.43	260	C17H24O2
5	Ethyl hexadecanoate	35.026	0.65	12.01	282	C18H36O2
6	α-Vetivone	41.744	0.77	48.18	218	C15H22O
7	Zizanal	43.786	0.81	41.72	218	C15H22O
8	β-Vetivone	53.666	1	100	218	C15H22O
9	Laurenene	56.796	1.05	46.13	272	C20H32

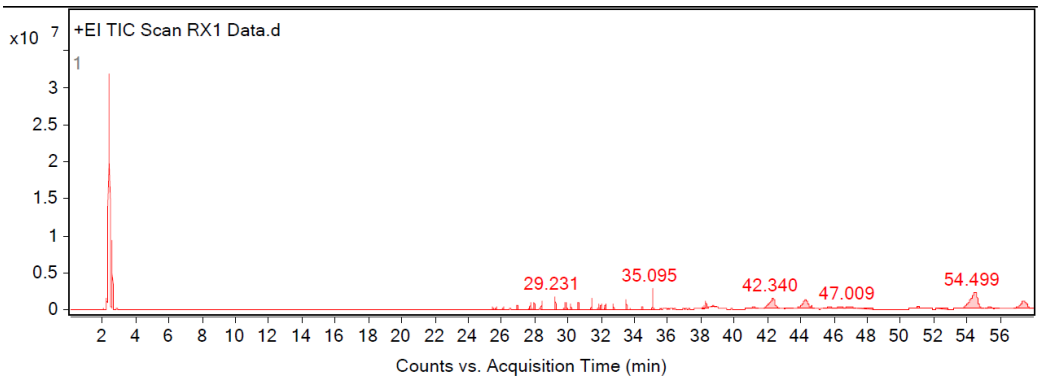


Figure 1: Total ion chromatogram of GC/MS analysis of the non-polar fraction of *Euphorbia mauritanica* L.

Metabolomics was used to identify the different chemical components of the species in order to correlate the biological activity with the chemical content of the tested extract. An alikot of total ethanol 70% extract (polar fraction) was analyzed using LC-HR/ESI-MS technique resulted in the separation and identification of 16 compounds (Figures 2 and 3 & Table2).

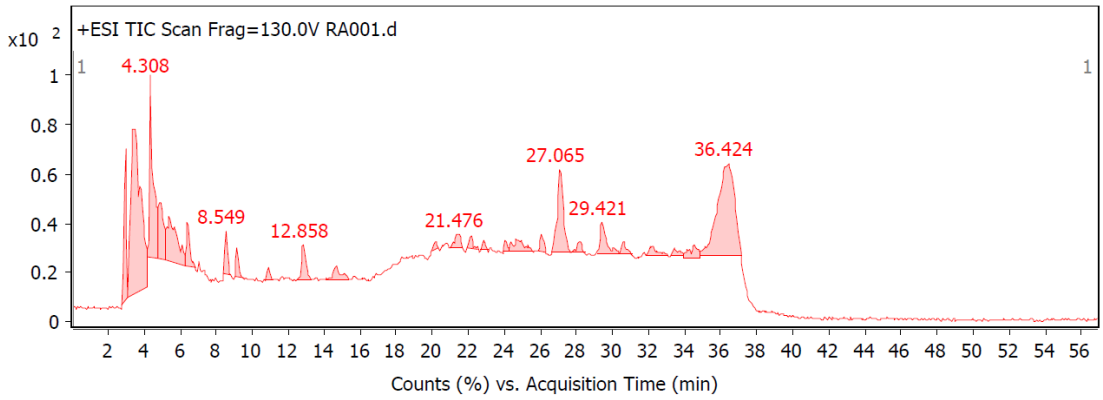


Figure 2: Total ion chromatogram of *Euphorbia mauritanica* L. polar fraction

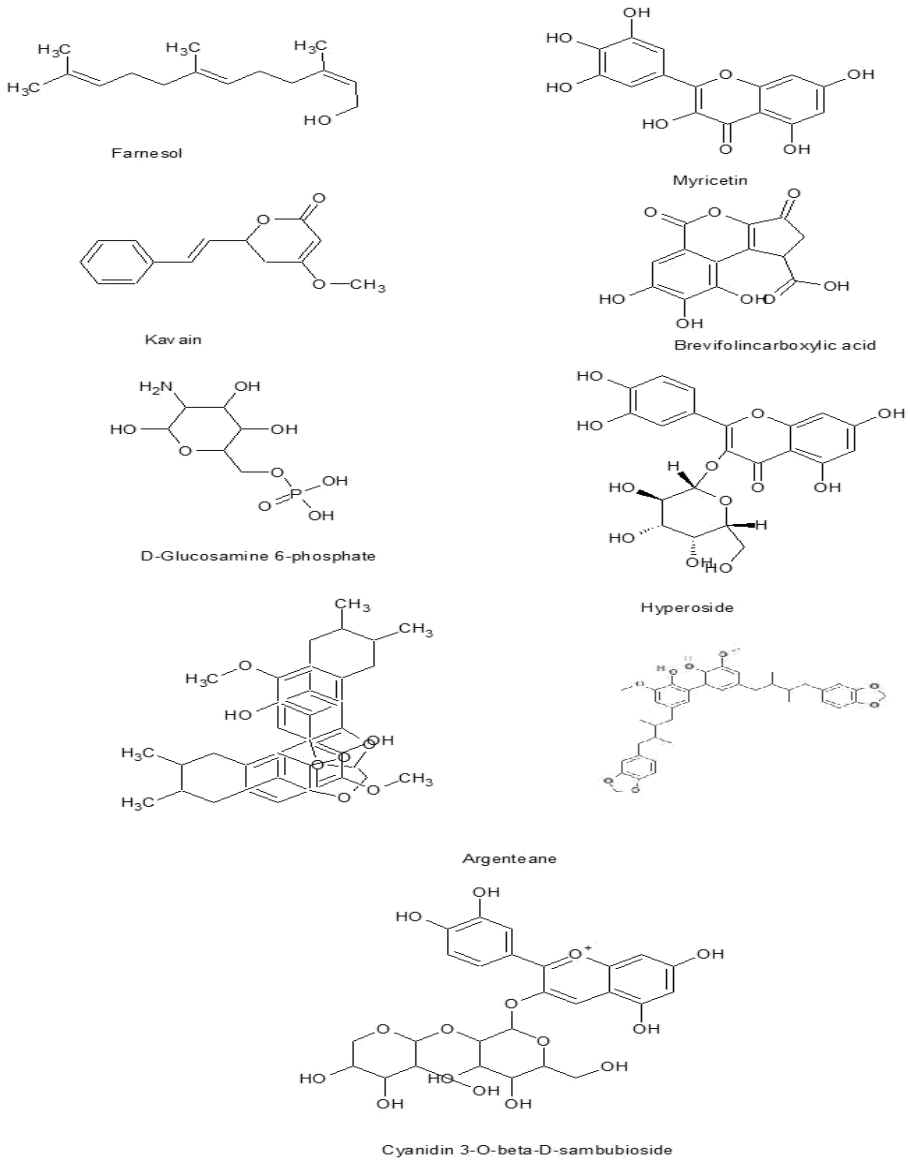


Table 2: Compounds identified by LC-DAD/ESI-MS in the polar fraction of *Euphorbia mauritanica* L., their retention times, major ion peaks, molecular weight and High raise mass:

	Compound	Rt (min)	m/z	Molecular weight
1	Farnesol $[M+H]^+$	2.961	223.9889	222.36634
2	Myricetin$[M+H]^+$	3.432	319.0645	318.2351
3	Kavain $[M+H]^+$	3.769	231.083	230.25916
4	Brevifolincarboxylic acid $[M+H]^+$	4.442	292.0171	292.19782
5	D-Glucosamine 6-phosphate $[M+H]^+$	4.981	259.0199	259.151022
6	Hyperoside (Quercetin 3-galactoside)	5.587	463.0898	464.3763
7	Argenteane (M+2Na)+2[-H2O]	6.462	341.1446	654.78844
8	Sambicyanin (Cyanidin-3-O-(2"-O-beta-xylopyranosyl-beta-glucopyranoside)) $[M]^+$	8.684	581.2286	581.4989114
9	Resiniferatoxin $[M]^+$	12.926	628.7, 515.0385	628.7081
10	Ingenol-3,20-dibenzoate$[M]^+$	14.609	458.1876	556.64544
11	Euphorbia factor L8 $[M+1]^+$	20.265	525.2124	523.61728
12	20-oxo-phorbol-12,13-dibutyrate $[M]^+$	24.910	525.2, 437.1788	504.6124

13	12-deoxyphorbol-13-acetate (prostratin) $[2M+H_2O]^+$		26.190	795.3457, 414.2, 352.3	390.47
14	19,28-Epoxy-3-oleananol		27.065	443.1490	442.7168
15	Chlorogenic acid $[M+1]^+$		28.209	352.3406	354.31
16	Euphorbia factor L1 $[M+1]^+$		29.421	553.2617	552.6552

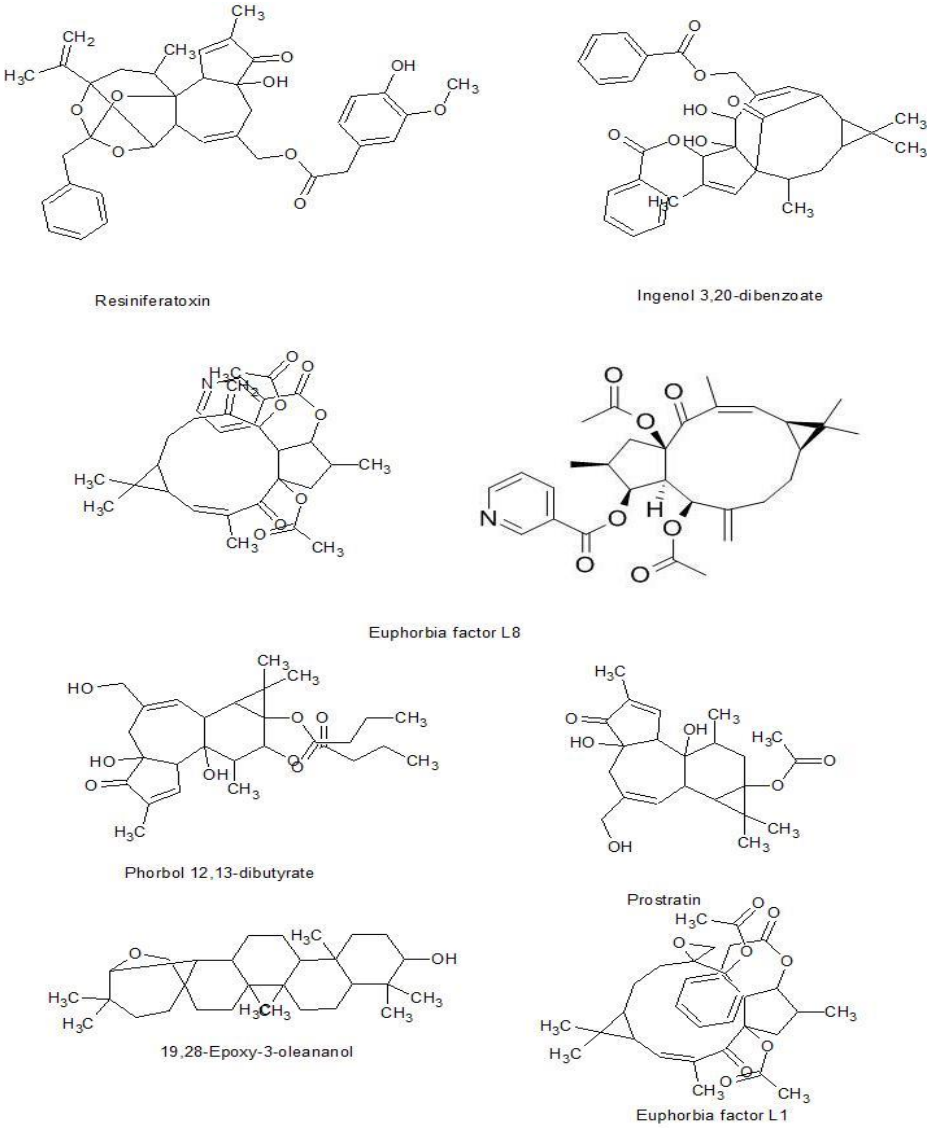


Figure 3: Structures of identified compounds by LC/HRMS of polar fraction of *Euphorbia mauritanica* L.

Acute toxicity study

Treatment of both male and female mice with a single dose of 5 g /kg of total ethanol 70% extract *Euphorbia mauritanica* L. extracts did not show any sign of toxicity in both sex in mice (no mortality, no hair loss, no diarrhea, and no abnormalities on behavior).

Effect of total ethanol 70% extract of *Euphorbia mauritanica* L. on lung functions of tidal volume (TV) and peak expiratory flow (PEF) in asthmatic rats

OVA sensitization followed by OVA i.n challenge produced a significant reduction in TV and PEF by 75% and 81%, respectively as compared with the negative control group. Both doses of the extract (200 and 400 mg/kg) increased TV by 104% and 205% as well as PEF by 116% and 269%, respectively as compared with OVA group (Table 3).

Table 3: Effect of *E. mauritanica* L. total ethanol 70% extract on lung functions of TV and PEF in experimentally induced asthmatic in rats

	Negative group	Asthmatic group	*Extract* (200 mg/kg)	*Extract* (400 mg/kg)
TV(ml)	0.095±0.001	0.023±0.001a	0.048±0.001 ab	0.072±0.002 ab
PEF(ml/min)	16.72±0.24	3.26±0.22a	7.04±0.26ab	12.04±0.19ab

TV: tidal volume; PEF: peak expiratory flow; Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons; a Significantly different from negative control at $p<0.05$; b Significantly different from asthmatic group at $p<0.05$.

Effect of total ethanol 70% extract of *Euphorbia mauritanica* L. on lung contents of MDA and GSH in asthmatic rats.

A significant increase in lung contents of MDA and a decrease in GSH lung contents were observed in OVA group by 123% and 30% respectively, as compared with the negative control group. Two doses of total ethanol 70% extract of *E. mauritanica* (200 and 400 mg/kg) administration significantly reduced lung contents of MDA by 38% and 46% respectively, and increased GSH by 26% and35% respectively, as compared with OVA group (Table 4).

Table 4: Effect of *E. mauritanica* L. total ethanol 70% extract lung contents of MDA and GSH in experimentally induced asthma in rats

	Negative group	Asthmatic group	*E. mauritanica* (200 mg/kg)	*E. mauritanica* (400 mg/kg)
MDA (nmol/ml)	27.86±0.46	62.20±0.45a	38.59±0.55ab	33.31±0.60ab
GSH (mmol/L)	0.42±0.004	0.29±0.004a	0.37±0.001ab	0.39±0.003ab

MDA:Malondialdehyde; GSH: reduced glutathione; Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons; a Significantly different from negative control at $p<0.05$; b Significantly different from asthmatic group at $p<0.05$.

Effect of total ethanol 70% extract of *Euphorbia mauritanica* L. on lung contents of IgE, IL-5, and TNF-α in asthmatic rats

A significant elevation in lung contents of IgE, IL-5, and TNF-α was observed in OVA group by 137%, 114% and 391% respectively, as compared with the negative control group. Administration of two doses of *E. mauritanica* extract (200 and 400 mg/kg) significantly reduced lung contents of IgE by 39% and 51%, IL-5 by 18% and 35%, TNF-α by 14% and 68% respectively, as compared with OVA group (Figures 4).

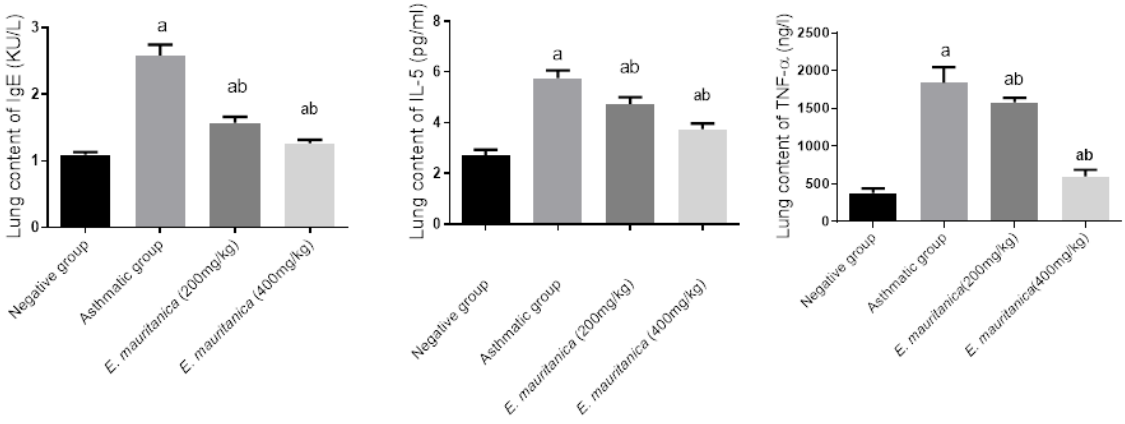


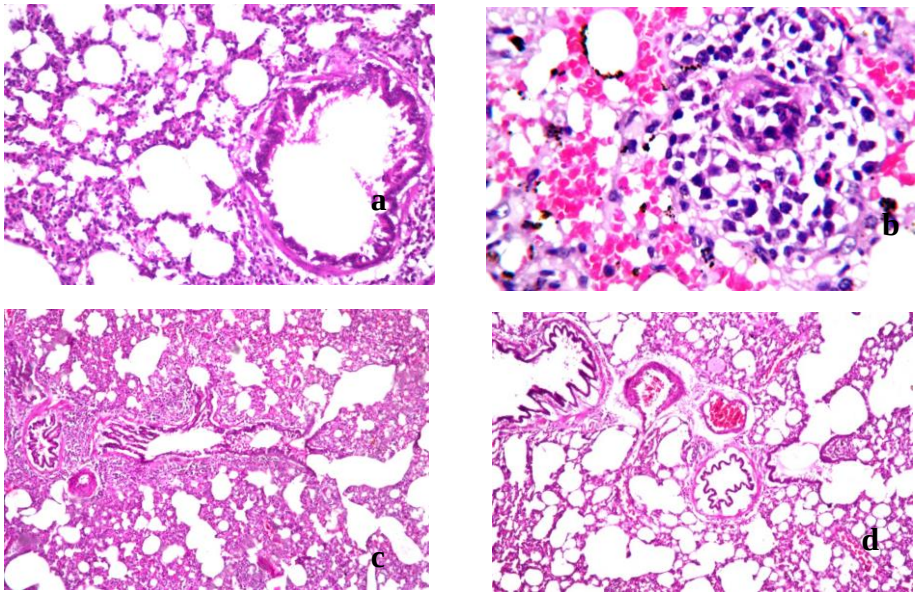
Figure 4: Effect of *E. mauritanica* L. total ethanol 70% extract lung contents of IgE, IL-5 and TNF-α in experimentally induced asthma in rats

Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons; a Significantly different from negative control at $p<0.05$; b Significantly different from asthmatic group at $p<0.05$.

Histopathological study

Control group showed no histopathological alteration in the bronchioles and surrounding air alveoli in the parenchyma, while Group of experimentally induced asthmatic rats showed epithelial bronchiolar thickening with perivascular leucocytes inflammatory association with epithelial bronchiolar thickening and sclerosis in the wall of the blood vessels . In addition, Group of experimentally induced asthmatic rats and cells infiltration mainly macrophages, lymphocytes and eosinophils were detected. Group of experimentally induced asthmatic rats and treated by 200 mg/kg *E. mauritanica*L. total ethanol 70% extract showed Collapse in the air alveoli in

treated by 400 mg/kg *E. mauritanica*L. total ethanol 70% extract exhibited mild bronchiolar thickening and congestion in the blood vessels (Figures 5).



.

Figure 5a : Group of rats kept as control there was no histopathological alteration in the bronchioles and surrounding air alveoli in the parenchyma.
b : Group of experimentally induced asthmatic rats shows epithelial bronchiolar thickening with perivascular leucocytes inflammatory cells infiltration mainly macrophages , lymphocytes and eosinophils were detected .
c: Group of experimentally induced asthmatic rats and treated by 200 mg/kg *E. mauritanica* L. total ethanol 70% extract on lung . Collapse was detected in the air alveoli in association with epithelial bronchiolar thickening and sclerosis in the wall of the blood vessels .
d: Group of experimentally induced asthmatic rats and treated by 400 mg/kg *E. mauritanica* L. total ethanol 70% extract on lung .There were mild bronchiolar thickening and congestion in the blood vessels .

4. Discussion

The use of natural products, as plants, for treatment of asthma is known for more than 5000 years as traditional medicine. The plants chemical components, as essential oils, are widely known to be used as a complementary medicine due to their bioactive components which are effective as anti-inflammatory by inhibiting COX-2 and COX-5. They also reduce cytokine levels of IL-4, IL-5, IL-13 and regulate NF-kB pathway which cause modulation of the function of the immune cells ([36]; [37]; [38]).

The chemical profiling of the non-polar fraction of *Euphorbia mauritanica* L. revealed seven sesquiterpenes, one diterpene and one fatty acid ethyl ester where *β*-Vetivone displayed highest prevalence. However, metabolomics identified in the polar fraction constituted 16 compounds belonging to different classes of compounds. Among the polyphenol family; Chlorogenic acid and three flavonoids; myricetin, sambicyanin and hyperoside (quercetin 3-galactoside) flavonoids were identified. Brevifolincarboxylic acid was also identified. It belongs to isocoumarins that can be found in pomegranate. Brevifolincarboxylic acid was isolated along with twenty chebulic acid and brevifolincarboxylic acid derivatives, from an EtOH extract of the aerial parts of Euphorbia hirta [39]. A dibenzylbutane lignan; argenteane was also identified

which was previously detected in some herbs and spices like nutmeg [40]. Kavain is one of the identified compounds in the polar extract of *Euphorbia mauritanica* L. Kavain is the main kavalactone found mostly in the roots of the kava plant (*Piper methysticum)* it is thought to be responsible for its anxiolytic effect (Chua et al., 2016).

Terpenoids constitutes a major class of compounds to which belong eight out of the sixteen identified compounds in the polar extract of *Euphorbia mauritanica* L. One acyclic sesquiterpene alcohol; farnesol, three phorbol derivatives, three diterpenoids and one triterpenoid were identified.Phorbol is a member of the tigliane family of diterpenes found in many plants, especially those of the Euphorbiaceae and Thymelaeaceae families [42]. Herein, 20-oxo-phorbol-12,13-dibutyrate and 12-deoxyphorbol-13-acetate (prostratin) were identified in addition to ingenol; a diterpenoid related to phorbol.

Resiniferatoxin is the irritant principle of the Moroccan cactus-like plant, *Euphorbia resinifera*, and was first isolated and identified in 1975 [43]. It is a diterpenoid of the Rhamnofolane and daphnane classes. Euphorbia factor L8 and euphorbia factor L1, are diterpenoids isolated from *Euphorbia lathyris* [44] [45]. Last, an oxygenated pentacyclic triterpenoid; 19,28-Epoxy-3-oleananol was also identified.

Bronchial asthma (BA) is a chronic inflammation accompanied by allergic (eosinophilic) mechanism, inducing acute bronchoconstriction and TV and PEF reduction [46].In the present study, the protective effect of total ethanol 70% extract of*E. mauritanica* in two doses (200 and 400mg/kg) was studied, *in vivo*, on experimental OVA-induced bronchial asthma in experimental rats. OVA sensitization followed by OVA i.n challenge was produced with a significant reduction in TV and PEF as compared with the negative control group in the current study. *E. mauritanica* administration for 3 weeks, significantly elevated TV and PEF. The anti-inflammatory activities of oleanane terpenoids[47] and sesquiterpenoids [48] to which belong the major constituents of *E. mauritanica* namely; 19,28-Epoxy-3-oleananol and *β*-Vetivone, respectively. The latex in *E. mauritanica* contains mauritanicain, protease, and phorbol-12-myristate -13-acetate (diterpene) compounds which exert an anti-inflammatory activity response [20]. Quercitrin bioflavonoid proved to be efficient as anti-inflammatory and antioxidant. It is converted to quercitin which is active as anti-inflammatory and antiasthma [49].The sterols as *β*-sitoterol and 24- methylene artenol and triterpenes have been reported also for their anti-inflammatory activity [50].

Evolution of reactive oxygen species (ROS) is one of the main features of bronchial asthma which involved in the progression of asthma and playing a key role in its pathogenesis (Fujisawa, 2005. Results of the present investigation showed that in OVA group significant decrease in GSH and increase in MDA as compared to negative control rats, indicating the role of oxidative stress in bronchial asthma, that is similar to what was early described [51].Administration of *E. mauritanica* extract reduced the lung content of MDA and elevated GSH as compared to OVA group. The aqueous extract of genus Euphorbia as *E. hirta* reported to have an antioxidant and free radical – scavenging activity [52].Other Euphorbia species reported for their antioxidant activity include *E. heterophylla* L, *E. milii* Des Moul and *E. thymifolia* L [53].

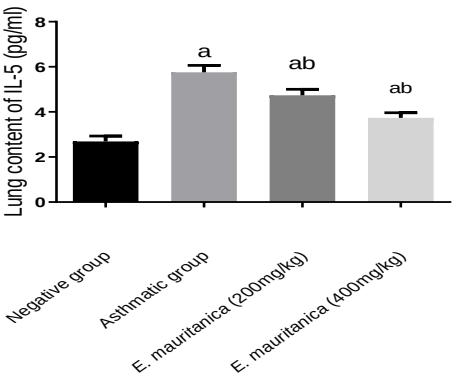


Figure 5: Effect of *E. mauritanica* L. total ethanol 70% extract lung contents of IL-5 in experimentally induced asthmatic in rats

Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons; a Significantly different from negative control $p<0.05$; b Significantly different from asthmatic group at $p<0.05$.

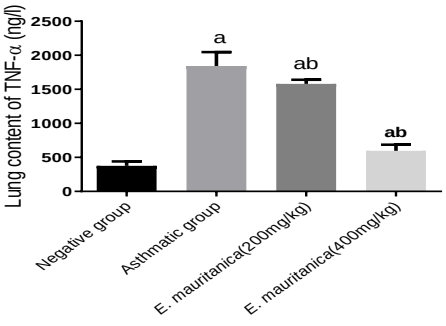


Figure 6: Effect of *E. mauritanica* L. total ethanol 70% extract on lung contents of TNF-α in experimentally induced asthmatic in rats

Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons; a Significantly different from negative control $p<0.05$; b Significantly different from asthmatic group at $p<0.05$.

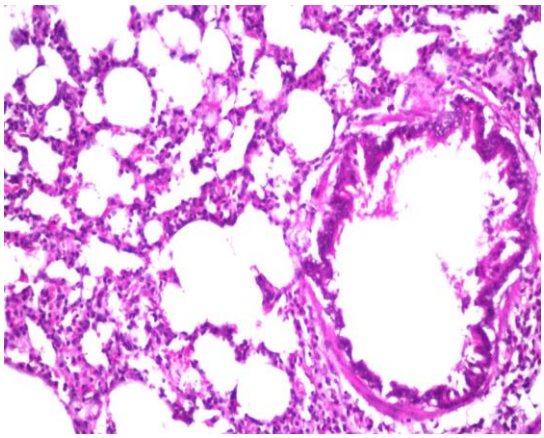


Figure 7 : Group of rats kept as control there was no histopathological alteration in the bronchioles and surrounding air alveoli in the parenchyma .

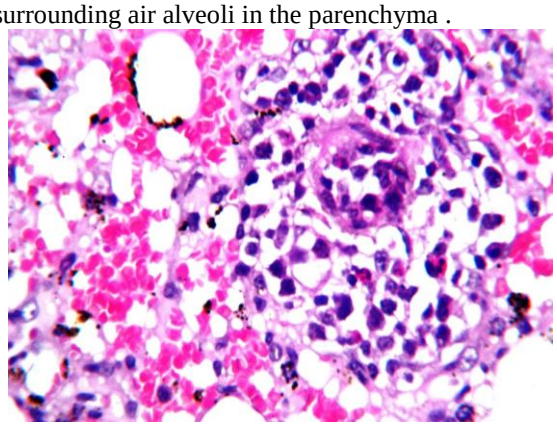


Figure 8 : Group of experimentally induced asthmatic rats shows epithelial bronchiolar thickening with perivascular leucocytes inflammatory cells infiltration mainly macrophages , lymphocytes and eosinophils were detected .

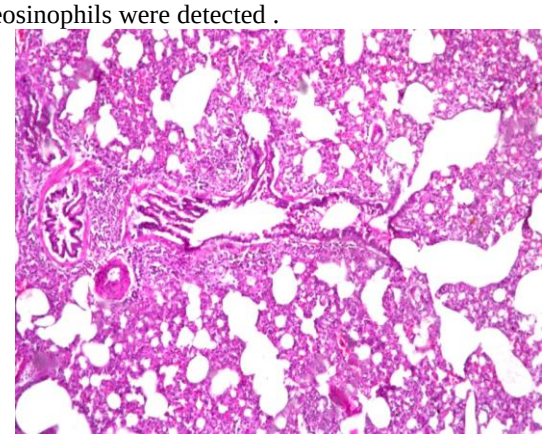


Figure 9: Group of experimentally induced asthmatic rats and treated by 200 mg/kg *E. mauritanica* L. total ethanol 70% extract on lung . Collapse was detected in the air alveoli in association with epithelial bronchiolar thickening and sclerosis in the wall of the blood vessels .

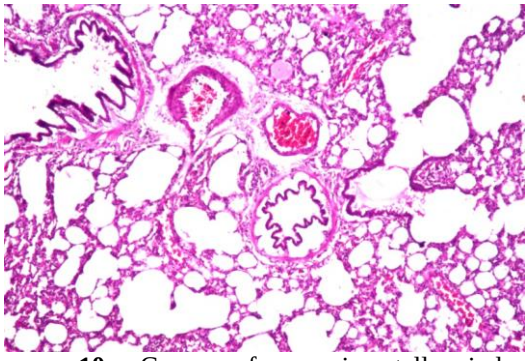


Figure 10: Group of experimentally induced asthmatic rats and treated by 400 mg/kg *E. mauritanica* L. total ethanol 70% extract on lung .There were mild bronchiolar thickening and congestion in the blood vessels .

Chronic airway inflammation is the hallmark features of asthma, through which eosinophils, activated T-helper lymphocytes and mast cells infiltration elevated aggravating the production of various cytokines such as interleukins (ILs) and tumor necrosis factor-alpha (TNF-α). These inflammatory cytokines airway induce obstruction, mucus secretion and airway remodeling (airways structural changes) [54]. In previous research, OVA challenge expressed TNF-α in the lung. TNF-α as a strong pro-inflammatory cytokine influence of eosinophils and neutrophils recruitments provoking airway inflammation [55]. At the same time, OVA challenge expressed IL-5 that controls eosinophils functioning, in addition, Th2 cells regulates immunoglobulin E (IgE) which in turn that also mediates recruitment of eosinophils. In addition, IgE has antigen recognition role of the adaptive immune system triggering rapid pathological responses [56]. Most treatment lines of bronchial asthma depend on anti-inflammatory drugs [57]. The current study exhibited that in OVAsignificantly elevated the lung content of IL-5, IgE and TNF-α as compared to negative control rats, suggesting that bronchial asthma is associated with inflammation that is in a line with previous study [58]. Our results revealed that *E. mauritanica* extract administration down regulated IL-5, IgE and TNF-α in the lung and prevent a series of progressive airways structural changes compared to OVA group. Presence of phenolic compounds, flavonoids, tannins and terpenoids are also correlated as synergistic effect to the antiasthma activity [59].

Conclusion

The interesting findings in current work were that *E. mauritanica* L. extract significantly inhibited the release of MDA, IgE, IL-5, and TNF-α and elevated TV, PEF and GSH suggesting it role involved to control precipitation of asthma in rats. This species may be used as a good herbal remedy for prevention of asthma.

Declaration of interest
The authors declare no conflict of interest.

Author contribution
Mona Hetta: Conceptualization, Resources, Funding acquisition, Review & editing. Abeer Salama: Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing. Rehab Hussein: Methodology, writing -original draft. Marwa Samy: Methodology, Writing - original draft. Nemat Yassin: Conceptualization, Review & editing.

Acknowledgment
This work is funded by Science and Technology Development Fund (STDF) project no. 24229 RS grant.

References

1. Lambrecht, B. N.; Hammad, H. The Immunology of Asthma. *Nature Immunology 2014 16:1* **2014**, *16* (1), 45–56. https://doi.org/10.1038/ni.3049.
2. Abbafati, C.; Abbas, K. M.; Abbasi-Kangevari, M.; Abd-Allah, F.; Abdelalim, A.; Abdollahi, M.Global Burden of 369 Diseases and Injuries in 204 Countries and Territories, 1990–2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *The Lancet* **2020**, *396* (10258), 1204–1222.
3. Chen, C. T.; Shan, C. X.; Ran, J.; Yin, L. M.; Li, H. Y.; Wang, Y.; Xu, Y. D.; Guo, J. L.; Shi, Y. L.; Chen, Y. J.; Yang, Y. Q. Cyclophilin A Plays Potential Roles in a Rat Model of Asthma and Suppression of Immune Response. *J Asthma Allergy* **2021**, *14*, 471–480. https://doi.org/10.2147/JAA.S308938.
4. McKeever, T.; Harrison, T. W.; Hubbard, R.; Shaw, D. Inhaled Corticosteroids and the Risk of Pneumonia in People with Asthma: A Case-Control Study. *Chest* **2013**, *144* (6), 1788–1794. https://doi.org/10.1378/CHEST.13-0871.
5. Gibson, P. G.; Yang, I. A.; Upham, J. W.; Reynolds, P. N.; Hodge, S.; James, A. L.; Jenkins, C.; Peters, M. J.; Marks, G. B.; Baraket, M.; Powell, H.; Taylor, S. L.; Leong, L. E. X.; Rogers, G. B.; Simpson, J. L. Effect of Azithromycin on Asthma Exacerbations and Quality of Life in Adults with Persistent Uncontrolled Asthma (AMAZES): A Randomised, Double-Blind, Placebo-Controlled Trial. *Lancet* **2017**, *390* (10095), 659–668. https://doi.org/10.1016/S0140-6736(17)31281-3.
6. Luster, A. D.; Tager, A. M. T-Cell Trafficking in Asthma: Lipid Mediators Grease the Way. *Nature Reviews Immunology 2004 4:9* **2004**, *4* (9), 711–724. https://doi.org/10.1038/nri1438.
7. Kim, S.; Lee, C. H.; Jin, K. N.; Cho, S. H.; Kang, H. R. Severe Asthma Phenotypes Classified by Site of Airway Involvement and Remodeling via Chest CT Scan. *J Investig Allergol Clin Immunol* **2018**, *28* (5), 312–320. https://doi.org/10.18176/JIACI.0265.
8. Chung, K. F.; Wenzel, S. E.; Brozek, J. L.; Bush, A.; Castro, M.; Sterk, P. J.; Adcock, I. M.; Bateman, E. D.; Bel, E. H.; Bleecker, E. R.; Boulet, L. P.; Brightling, C.; Chanez, P.; Dahlen, S. E.; Djukanovic, R.; Frey, U.; Gaga, M.; Gibson, P.; Hamid, Q.; Jajour, N. N.; Mauad, T.; Sorkness, R. L.; Teague, W. G. International ERS/ATS Guidelines on Definition, Evaluation and Treatment of Severe Asthma. *Eur Respir J* **2014**, *43* (2), 343–373. https://doi.org/10.1183/09031936.00202013.
9. Gubernatorova, E. O.; Namakanova, O. A.; Gorshkova, E. A.; Medvedovskaya, A. D.; Nedospasov, S. A.; Drutskaya, M. S. Novel Anti-Cytokine Strategies for Prevention and Treatment of Respiratory Allergic Diseases. *Front Immunol* **2021**, *12*. https://doi.org/10.3389/FIMMU.2021.601842.
10. Halwani, R.; Sultana, A.; Vazquez-Tello, A.; Jamhawi, A.; Al-Masri, A. A.; Al-Muhsen, S. Th-17 Regulatory Cytokines IL-21, IL-23, and IL-6 Enhance Neutrophil Production of IL-17 Cytokines during Asthma. *J Asthma* **2017**, *54* (9), 893–904. https://doi.org/10.1080/02770903.2017.1283696.
11. Wu, Y.; Zou, F.; Lu, Y.; Li, X.; Li, F.; Feng, X.; Sun, X.; Liu, Y. SETD7 Promotes TNF-α-Induced Proliferation and Migration of Airway Smooth Muscle Cells in Vitro through Enhancing NF-KB/CD38 Signaling. *International Immunopharmacology* **2019**, *72*, 459–466. https://doi.org/10.1016/J.INTIMP.2019.04.043.
12. Elhefny, M.; Nassar, S. I.; Elheneidy, F. M.; Said, M.; El-beleidy, A. S.; Elmarsam, E.; Moustafa, N. A.; Elfalaky, M. M.; Haddad, Z. Epidemiology of Childhood Asthma in Cairo ANI!WL. *Cairo Univ 62* (2), 994.
13. Hossny, E. M.; Hasan, Z. E.; Allam, M. F.; Mahmoud, E. S. Analysis of the Filed Data of a Sample of Egyptian Children with Bronchial Asthma. *Egyptian Journal of Pediatric Allergy and Immunology (The)* **2014**, *7* (2). https://doi.org/10.4314/ejpai.v7i2.
14. Amaral-Machado, L.; Oliveira, W. N.; Moreira-Oliveira, S. S.; Pereira, D. T.; Alencar, É. N.; Tsapis, N.; Egito, E. S. T. Use of Natural Products in Asthma Treatment. *Evidence-based Complementary and Alternative Medicine* **2020**, *2020*. https://doi.org/10.1155/2020/1021258.
15. Mohamed, A.-E.-H. H.; Hegazy, M.-E. F.; M Moustafa, M. F.; El-Sayed, M. A.; Abdel-Farid, I. B.; Esmail, A. M.; Abdelrazik, M. H.;

Mohamed, N. S.; Nenaah, G.; Mohamed, T. A.; Shahat, A. A.; Karchesy, J.; Matsuda, H.; Pare, P. W. International Journal of Phytopharmacology EUPHORBIA HELIOSCOPIA: CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES. *International Journal of Phytopharmacology* **2012**, *3* (1), 78.

16. Barla, A.; Ult¨ur, K. ¨; Ult¨ur, U.; Oks¨uz, S.; Oks¨uz, O. Secondary Metabolites from Euphorbia Helioscopia and Their Vasodepressor Activity. *Turk J Chem* **2006**, *30*, 325.
17. El-Hawary, S. S.; Mohammed, R.; Tawfike, A. F.; Lithy, N. M.; Abouzid, S. F.; Amin, M. N.; Abdelmohsen, U. R.; Amin, E. Cytotoxic Activity and Metabolic Profiling of Fifteen Euphorbia Species. *Metabolites* **2020**, *11* (1), 1–18. https://doi.org/10.3390/METABO11010015.
18. Forero, J. E.; Avila, L.; Taborda, N.; Tabares, P.; López, A.; Torres, F.; Quiñones, W.; Bucio, M. A.; Mora-Pérez, Y.; Rugeles, M. T.; Joseph-Nathan, P.; Echeverri, F. In Vitro Anti-Influenza Screening of Several Euphorbiaceae Species: Structure of a Bioactive Cyanoglucoside from Codiaeum Variegatum. *Phytochemistry* **2008**, *69* (16), 2815–2819. https://doi.org/10.1016/J.PHYTOCHEM.2008.09.003.
19. Shi, Q. W.; Su, X. H.; Kiyota, H. Chemical and Pharmacological Research of the Plants in Genus Euphorbia. *Chemical Reviews* **2008**, *108* (10), 4295–4327. https://doi.org/10.1021/CR078350S/ASSET/CR078350S.FP.PNG_V03.
20. Guenther, F.; Maus, D.; Hedtrich, S.; Melzig, M. F. Serine Protease Mauritanicain from Euphorbia Mauritanica and Phorbol-12-Myristate-13-Acetate Modulate the IL-8 Release in Fibroblasts and HaCaT Keratinocytes. *Planta Med* **2019**, *85* (7), 578–582. https://doi.org/10.1055/A-0735-9911.
21. Thafeni, M. A.; Sayed, Y.; Motadi, L. R. Euphorbia Mauritanica and Kedrostis Hirtella Extracts Can Induce Anti-Proliferative Activities in Lung Cancer Cells. *Mol Biol Rep* **2012**, *39* (12), 10785–10794. https://doi.org/10.1007/S11033-012-1972-6.
22. Flemmig, M.; Domsalla, A.; Rawel, H.; Melzig, M. F. Isolation and Characterization of Mauritanicain, a Serine Protease from the Latex of Euphorbia Mauritanica L. *Planta Med* **2017**, *83* (6), 551–556. https://doi.org/10.1055/S-0042-117645.
23. Guenther, F.; Melzig, M. Proteolytic Active Proteins from Euphorbia Mauritanica L. Stimulate the Production of Interleukine-6 and Interleukine-8 in Keratinocytic HaCaT Cells. *Planta Medica* **2016**, *82* (S 01), P666. https://doi.org/10.1055/S-0036-1596720.
24. Abd-Alla, H. I.; Sweelam, H. T. M.; El-Kashak, W. A.; El-Safty, M. M. Evaluation of Immune Boosting Properties and Combating of Multiple Respiratory Viral Infections by Fifteen Euphorbiaceae Plant Extracts. *Pharmacognosy Journal* **2019**, *11* (6s), 1490–1503. https://doi.org/10.5530/pj.2019.11.230.
25. Yener, İ.; Ertaş, A.; Yilmaz, M. A.; Tokul Ölmez, Ö.; Köseoğlu Yılmaz, P.; Yeşil, Y.; Kaplaner, E.; Öztürk, M.; Temel, H.; Kolak, U.; Topçu, G. Characterization of the Chemical Profile of Euphorbia Species from Turkey by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), and Liquid Chromatography-Ion Trap-Time-of-Flight-Mass Spectrometry (LC-IT-TOF-MS) and Chemometric Analysis. *ANALYTICAL LETTERS* **2019**, *52* (7), 1031–1049. https://doi.org/10.1080/00032719.2018.1512608.
26. Liu, Z.; Zeng, Y.; Hou, P. Metabolomic Evaluation of Euphorbia Pekinensis Induced Nephrotoxicity in Rats. *Pharmaceutical Biology* **2018**, *56* (1), 145. https://doi.org/10.1080/13880209.2018.1435697.
27. Ertas, A.; Yilmaz, M. A.; Firat, M. Chemical Profile by LC-MS/MS, GC/MS and Antioxidant Activities of the Essential Oils and Crude Extracts of Two Euphorbia Species. *Nat Prod Res* **2015**, *29* (6), 529–534. https://doi.org/10.1080/14786419.2014.954113.
28. Nothias-Scaglia, L. F.; Dumontet, V.; Neyts, J.; Roussi, F.; Costa, J.; Leyssen, P.; Litaudon, M.; Paolini, J. LC-MS2-Based Dereplication of Euphorbia Extracts with Anti-Chikungunya Virus Activity. *Fitoterapia* **2015**, *105*, 202–209. https://doi.org/10.1016/J.FITOTE.2015.06.021.
29. el Kady, W. M.; Salama, A. A. A.; Desoukey, S. Y.; Hagag, E. G.; El-Shenawy, S. M.; El-Shanawany, M. A. Comparative DNA Profiling, Botanical Identification and Biological Evaluation of Gazania Longiscapa DC and Gazania Rigens L. *Bulletin of Faculty of Pharmacy, Cairo University* **2015**, *53* (2), 129–145. https://doi.org/10.1016/J.BFOPCU.2015.07.001.
30. Abdel-Fattah, M. M.; Messiha, B. A. S.; Salama, A. A. A. Assessment of the Mechanistic Role of Cinnarizine in Modulating Experimentally-Induced Bronchial Asthma in Rats. *Pharmacology* **2015**, *96* (3–4), 167–174. https://doi.org/10.1159/000438705.
31. A Salama, A. A.; Zaki, H. F.; El-Shenawy, S. M.; S M, E.-D. E.; El-Kassaby Ismaiel, I. Antiasthmatic Effects of Evening Primrose Oil in

Ovalbumin-Allergic Rats. *Scholars Research Library Der Pharmacia Lettre* **2015**, *7* (4), 214–223.

32. Mohamed, S.; Nasr, M.; Salama, A.; Refai, H. Novel Lipid-Polymer Hybrid Nanoparticles Incorporated in Thermosensitive in Situ Gel for Intranasal Delivery of Terbutaline Sulphate. *J Microencapsul* **2020**, *37* (8), 577–594. https://doi.org/10.1080/02652048.2020.1826590.
33. Salama, A.; Hegazy, R.; Hassan, A. Intranasal Chromium Induces Acute Brain and Lung Injuries in Rats: Assessment of Different Potential Hazardous Effects of Environmental and Occupational Exposure to Chromium and Introduction of a Novel Pharmacological and Toxicological Animal Model. *PLoS ONE* **2016**, *11* (12). https://doi.org/10.1371/JOURNAL.PONE.0168688.
34. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. *Anal Biochem* **1979**, *95* (2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.
35. Salama, A.; Fayed, H. M.; Elgohary, R. L-Carnitine Alleviated Acute Lung Injuries Induced by Potassium Dichromate in Rats: Involvement of Nrf2/HO-1 Signaling Pathway. *Heliyon* **2021**, *7* (6). https://doi.org/10.1016/J.HELIYON.2021.E07207.
36. Yang, Z. N.; Su, B. J.; Wang, Y. Q.; Liao, H. B.; Chen, Z. F.; Liang, D. Isolation, Absolute Configuration, and Biological Activities of Chebulic Acid and Brevifolincarboxylic Acid Derivatives from Euphorbia Hirta. *Journal of Natural Products* **2020**, *83* (4), 985–995. https://doi.org/10.1021/ACS.JNATPROD.9B00877/SUPPL_FILE/NP9B00877_SI_007.PDF.
37. Calliste, C. A.; Kozlowski, D.; Duroux, J. L.; Champavier, Y.; Chulia, A. J.; Trouillas, P. A New Antioxidant from Wild Nutmeg. *Food Chemistry* **2010**, *118* (3), 489–496. https://doi.org/10.1016/J.FOODCHEM.2009.05.010.
38. Chua, H. C.; Christensen, E. T. H.; Hoestgaard-Jensen, K.; Hartiadi, L. Y.; Ramzan, I.; Jensen, A. A.; Absalom, N. L.; Chebib, M. Kavain, the Major Constituent of the Anxiolytic Kava Extract, Potentiates Gabaa Receptors: Functional Characteristics and Molecular Mechanism. *PLoS ONE* **2016**, *11* (6). https://doi.org/10.1371/JOURNAL.PONE.0157700.
39. Wang, H. B.; Wang, X. Y.; Liu, L. P.; Qin, G. W.; Kang, T. G. Tigliane Diterpenoids from the Euphorbiaceae and Thymelaeaceae Families. *Chemical Reviews* **2015**, *115* (9), 2975–3011. https://doi.org/10.1021/CR200397N.
40. Finn, D. Resiniferatoxin. *xPharm: The Comprehensive Pharmacology Reference* **2007**, 1–5. https://doi.org/10.1016/B978-008055232-3.62528-0.
41. Jiao, W.; Mao, Z. H.; Dong, W. W.; Deng, M. C.; Lu, R. H. Euphorbia Factor L(8): A Diterpenoid from the Seeds of Euphorbia Lathyris. *Acta Crystallogr Sect E Struct Rep Online* **2007**, *64* (Pt 1). https://doi.org/10.1107/S1600536807066901.
42. Hong, S. E.; Lee, J.; Seo, D. H.; In Lee, H.; Ri Park, D.; Lee, G. R.; Jo, Y. J.; Kim, N.; Kwon, M.; Shon, H.; Kyoung Seo, E.; Kim, H. S.; Young Lee, S.; Jeong, W. Euphorbia Factor L1 Inhibits Osteoclastogenesis by Regulating Cellular Redox Status and Induces Fas-Mediated Apoptosis in Osteoclast. *Free Radic Biol Med* **2017**, *112*, 191–199. https://doi.org/10.1016/J.FREERADBIOMED.2017.07.030.
43. Ahn, K. S.; Noh, E. J.; Zhao, H. L.; Jung, S. H.; Kang, S. S.; Kim, Y. S. Inhibition of Inducible Nitric Oxide Synthase and Cyclooxygenase II by Platycodon Grandiflorum Saponins via Suppression of Nuclear Factor-KB Activation in RAW 264.7 Cells. *Life Sciences* **2005**, *76* (20), 2315–2328. https://doi.org/10.1016/J.LFS.2004.10.042.
44. Leung, C. Y.; Liu, L.; Wong, R. N. S.; Zeng, Y. Y.; Li, M.; Zhou, H. Saikosaponin-d Inhibits T Cell Activation through the Modulation of PKCtheta, JNK, and NF-KappaB Transcription Factor. *Biochem Biophys Res Commun* **2005**, *338* (4), 1920–1927. https://doi.org/10.1016/J.BBRC.2005.10.175.
45. Vasconcelos, J. F.; Teixeira, M. M.; Barbosa-Filho, J. M.; Lúcio, A. S. S. C.; Almeida, J. R. G. S.; de Queiroz, L. P.; Ribeiro-dos-Santos, R.; Soares, M. B. P. The Triterpenoid Lupeol Attenuates Allergic Airway Inflammation in a Murine Model. *Int Immunopharmacol* **2008**, *8* (9), 1216–1221. https://doi.org/10.1016/J.INTIMP.2008.04.011.
46. Matucci, A.; Vultaggio, A.; Maggi, E.; Kasujee, I. Is IgE or Eosinophils the Key Player in Allergic Asthma Pathogenesis? Are We Asking the Right Question? *Respir Res* **2018**, *19* (1). https://doi.org/10.1186/S12931-018-0813-0.
47. da Rocha, C. Q.; Vilela, F. C.; Santa-Cecília, F. v.; Cavalcante, G. P.; Vilegas, W.; Giusti-Paiva, A.; dos Santos, M. H. Oleanane-Type Triterpenoid: An Anti-Inflammatory Compound of the Roots *Arrabidaea Brachypoda*. *Revista Brasileira de Farmacognosia* **2015**, *25* (3), 228–232. https://doi.org/10.1016/J.BJP.2015.03.005.

48. Hegazy, M. E. F.; Hamed, A. R.; Mohamed, T. A.; Debbab, A.; Nakamura, S.; Matsuda, H.; Paré, P. W. Anti-Inflammatory Sesquiterpenes from the Medicinal Herb Tanacetum Sinaicum. *RSC Advances* **2015**, *5* (56), 44895–44901. https://doi.org/10.1039/C5RA07511D.
49. (49) Ekpo, O. E.; Pretorius, E. Asthma, Euphorbia Hirta and Its Anti-Inflammatory Properties. https://doi.org/10.10520/EJC96688.
50. Martínez-Vázquez, M.; Apan, T. O. R.; Lazcano, M. E.; Bye, R. Anti-Inflammatory Active Compounds from the n-Hexane Extract of Euphorbia Hirta. *Revista de la Sociedad Química de México* **1999**, *43*, 103–105.
51. Abdel-Fattah, M. M.; Salama, A. A. A.; Shehata, B. A.; Ismaiel, I. E. The Potential Effect of the Angiotensin II Receptor Blocker Telmisartan in Regulating OVA-Induced Airway Remodeling in Experimental Rats. *Pharmacol Rep* **2015**, *67* (5), 943–951. https://doi.org/10.1016/J.PHAREP.2015.02.010.
52. Kumar, S.; Malhotra, R.; Kumar, D. Euphorbia Hirta: Its Chemistry, Traditional and Medicinal Uses, and Pharmacological Activities. *Pharmacogn Rev* **2010**, *4* (7), 58–61. https://doi.org/10.4103/0973-7847.65327.
53. Mavundza, E. J.; Street, R.; Baijnath, H. A Review of the Ethnomedicinal, Pharmacology, Cytotoxicity and Phytochemistry of the Genus Euphorbia in Southern Africa. *South African Journal of Botany* **2022**, *144*, 403–418. https://doi.org/10.1016/J.SAJB.2021.08.029.
54. Hamid, Q.; Tulic, M. K. Immunobiology of Asthma Proteases in Irritable Bowel Syndrome View Project Systems Biology to Decipher Heterogenous Complex Diseases View Project. *Article in Annual Review of Physiology* **2009**. https://doi.org/10.1146/annurev.physiol.010908.163200.
55. Sherkawy, M. M.; Abo-youssef, A. M.; Salama, A. A. A.; Ismaiel, I. E. Fluoxetine Protects against OVA Induced Bronchial Asthma and Depression in Rats. *Eur J Pharmacol* **2018**, *837*, 25–32. https://doi.org/10.1016/J.EJPHAR.2018.08.026.
56. Asfour, M. H.; Kassem, A. A.; Salama, A.; Abd El-Alim, S. H. Hydrophobic Ion Pair Loaded Self-Emulsifying Drug Delivery System (SEDDS): A Novel Oral Drug Delivery Approach of Cromolyn Sodium for Management of Bronchial Asthma. *Int J Pharm* **2020**, *585*. https://doi.org/10.1016/J.IJPHARM.2020.119494.
57. Walsh, G. M. An Update on Emerging Drugs for Asthma. *Expert Opin Emerg Drugs* **2012**, *17* (1), 37–42. https://doi.org/10.1517/14728214.2012.657625.
58. Salama, A. A. A., H. F. Zaki, S. M. El-Shenawy, EL-Denshary, I. E. K. Ismaiel, and N. M. Sh, Effects of Fish Oil and Dexamethasone in Experimentally-Induced Bronchial Asthma. *Australian Journal of Basic and Applied Sciences* **2012**, *6* (13), 497-506.
59. Kunwar, R. M.; Shrestha, K. P.; Bussmann, R. W. Traditional Herbal Medicine in Far-West Nepal: A Pharmacological Appraisal. **2010**. https://doi.org/10.1186/1746-4269-6-35.