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Effects of *Meconopsis aculeata* Royle Extracts on Leukocytosis and Eosinophilia Induced by Milk in Albino Mice: Anti-asthmatic Property

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Abstract

Meconopsis aculeata Royle is a herb with medicinal value, traditionally used by the local people of the Himalayan range for its anti-asthmatic potential. The present work aims to determine the effects of the methanolic whole plant extract of *M. aculeata* for the management of allergy (asthma) in mice as less work has been done on this potential of the plant. We also determined the *in vitro* antioxidant and anti-inflammatory potential and identification of the plant's active phytoconstituents through GC-MS. It was found that *M. aculeata* extracts inhibited (89.68 %) free radicals in a better way when compared with standard ascorbic acid (73.47 %) at a higher concentration of 250 µg/mL. Plant extract inhibited (85.52 %) denaturation of protein when compared with standard aspirin (76.47 %) at a higher concentration of 250 µg/mL. Methanolic extracts (at 100-200 mg/kg, intraperitoneally) significantly decreased elevated leukocyte and eosinophil count in mice that were induced by milk. The anti-asthmatic potential of this plant may be due to identified phytoconstituents like Stigmast-5-en-3-ol; Protopine; 9,12-Octadecadienoic acid; and 9,12-Octadecadienoic acid (Z, Z), methyl ester as these possesses anti-asthmatic and antihistaminic properties. Thus, *M. aculeata* can be used to treat allergies like asthma, and inflammation and reduce oxidative stress.

Keywords: Anti-inflammatory; Antioxidant; Eosinophilia; GC-MS; Leukocytosis; *Meconopsis aculeata*.

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1. Introduction

Meconopsis aculeata is a plant of the Papaveraceae family and its common name is Himalayan Blue Poppy. It is a thorny herb having blue flowers found at an altitude of 3000-4000 m growing in the alpine area, rock crevices, light woodlands, and moist soils. In the traditional system, this species was used to cure febrifuge, analgesic, cough, asthma, and rheumatic pains, to cure the bones, especially around the ribs, and possesses antioxidant properties [1].

Free radicals viz reactive oxygen species (ROS) and reactive nitrogen species (RNS), unavoidable by-products of aerobic metabolism are generated by the process of oxidative stress. The increased oxidative stress causes the development of various diseases like

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neurological, cancer, cardiovascular, and inflammatory diseases. Bioactive phytoconstituents of plants like polyphenols have antioxidant properties and fruits, vegetables, etc. are the natural source of polyphenols, so these can reduce oxidative stress. [2].

Inflammation is the first response of the immune system to defend the body from foreign antigens, invasion of harmful microbes, damage to cells, etc. Although inflammation could repair and restore the homeostasis of the damaged tissue, chronic and persistent inflammation can cause the development of diseases like rheumatoid arthritis, inflammatory bowel disease, DNA damage, tumor progression, cancer, atherosclerosis, osteoporosis, chronic heart failure, asthma, diabetes mellitus, etc. Various mediators control the inflammatory process in a regulated way to return the inflamed physiological state of the cells to normal [3,4]. Nowadays, the major goal of the research is to discover the biologically active compounds in plants that can be used in place of synthetic products which are much safer for mankind with fewer side effects on the body. Secondary metabolites of plants show various pharmacological activities like tannins, phenols, and flavonoids account for their anti-inflammatory and antioxidant potential [5].

Allergy and allergic diseases are increasing in recent years, which include asthma, allergic rhinitis, atopic eczema, contact dermatitis, atopic dermatitis, etc. Allergic diseases can be caused by the interaction between genetic and environmental factors. These diseases can be classified into IgE mediated (in response to allergens, there is the production of IgE antibodies) or non-IgE-mediated (associated with specific T-cell response). Examples of IgE-mediated diseases are asthma, eczema, dermatitis, rhinoconjunctivitis, etc., and an example of a non-IgE-mediated disease is contact dermatitis [6]. Asthma is a chronic inflammatory disorder of the airways that show intermittent and variable symptoms like shortness of breath, wheezing, cough, bronchospasm, and reversible airflow obstruction. These symptoms occur due to the exposure of the body to allergens, exercise, viruses, or irritants in the environment [7]. Asthma degrades the quality of life, work efficiency, physical activities, etc. Corticosteroids, bronchodilators, and expectorants are generally used to make orthodox medicines used to cure asthma. Due to their side effects, there is a growing interest in the treatment of asthma by using herbal products, and medicinal plants. Medicinal plants which treat allergies are immune-modulatory, relax smooth muscles, and show anti-inflammatory, and antihistaminic activities, can be used to cure asthma [8].

In this study, through GC-MS analysis various phytoconstituents were identified in the methanolic plant extract of *M. aculeata* which possesses therapeutic and pharmacological properties like antioxidant, anti-inflammatory, antidiabetic, anticancer, antiallergic, etc. Antioxidant and anti-inflammatory properties of the plant were determined by the *in vitro* methods. The effects of the extracts of *M. aculeata* were determined by the assay of increased leukocytes and eosinophils induced by milk in the albino mice.

2. Material and Methods

2.1. *Plant collection and extract preparation*

Collected whole plant of *M. aculeata* from the Great Himalayan National Park, Kullu, Himachal Pradesh in August 2021 and authenticated at B.S.I (Botanical Survey of India), Dehradun, Uttarakhand (Identification Number: BSI/NRC.Tech./Herb(Ident.)/2022-23/145). The whole plant was washed properly, shade dried, and powdered. Further, it was extracted with methanol solvent, kept on the shaker for 24 h or more, filtered using filter paper (Whatman No. 1), and kept under normal conditions for drying out.

2.2. *Preliminary phytochemical screening*

Qualitative screening of methanolic plant extracts for the phytochemicals was done to know whether different bioactive chemical constituents are present or not [9].

2.3 *Antioxidant property determination by DPPH assay*

The ability of plant extracts was determined to scavenge the radical DPPH (1,1-diphenyl-2-picrylhydrazyl) which was taken as a positive control. Methanol was taken as blank and standard ascorbic acid. From 2 mL of plant extract, concentrations from 50 µg/mL-250 µg/mL were taken, then 2.7 mL methanolic solution of DPPH was added to test tubes. Dark conditions were maintained. Absorbance was noted after 30 min. spectrophotometrically (517 nm) [10]. Percent inhibition of free radicals was measured as.

$$\frac{Abs.of\ control - Abs.of sample}{Abs.of\ control} \times 100 \quad (1)$$

Where *Abs.*= Absorbance. The experiment was repeated three times (n=3).

2.4. *Anti-inflammatory property determination*

Here the assay to inhibit the protein denaturation was followed. Extract concentrations (50-250 µg/mL) and 1 % protein Bovine albumin fraction (aqueous solution) were poured into each test tube. Pour a few drops of 1 N HCl into the reaction mixture to adjust the pH. Incubated the reaction mixture for 20 min (37 ºC), and the heat was provided at a temperature of 57 ºC for another 20 min. The absorbance of the turbid solution was noted with the help of a spectrophotometer (660 nm) after cooling the mixture [11]. % Inhibition formula is:

$$\frac{Abs.of\ control - Abs.of sample}{Abs.of\ control} \times 100 \quad (2)$$

The experiment was performed in triplicate (n=3).

2.5. *Characterization of bioactive compounds by GC-MS*

It was carried out with GCMS-QP Ultra Spectrophotometer (2010) equipped with an RTX-5MS 30 m, 0.25 mm, 0.25 µm capillary column. The oven temperature was adjusted from 70 ºC (5 min hold time) to 310 ºC (10 min hold time). The injection size was 1.0 µL, injection temperature 250 ºC, Helium as a carrier gas, and sampling time was 1 min, and GC-MS total running time was 1 h for the plant extract. The unknown component spectrum was compared in the NIST (National Institute of Standards and Technology) library database with the help of retention time, CAS number, molecular formula, and weight.

2.6. *Animal model*

Swiss albino mice (Male) of 20-25 g have been procured to evaluate the leukocytosis and eosinophilia induced by milk. Standard conditions in the lab were maintained for the mice and provided water and food. The experimental protocol was sanctioned by the Institutional Animal Ethical Committee. Sanction No. is PU/45/99/CPCSEA/IAEC/2019/316.

2.7. *Evaluation of acute toxicity*

This was followed up per the Organization of Economic Co-operation and Development guideline 423. Divided the animals into 5 groups (n=6), 5 % Tween 80 solution (aqueous) was given to the 1st control group, and the 2nd, 3rd, 4th, and 5th groups were given 500, 1000, 1500, and 2000 mg/kg dosage orally. After 1, 4, and 24 h of extract administration for 14 days, general behavior, weight, signs of mortality, toxicity, and other physiological activities were observed [12].

2.8. *Evaluation of anti-asthmatic activity*

Here, the assay followed was leukocytosis and eosinophilia induced by milk in mice. The animals were categorized into 5 groups (n=6), and blood was taken from the retroorbital plexus of each animal after being anesthetized with diethyl ether. 1st control Group was given distilled water (10 mL/kg) by oral route, 2nd group was injected with 4 mL/kg milk (boiled, then cooled) by the subcutaneous route, 3rd and 4th group was given plant extracts (100-200 mg/kg, intraperitoneally), 5th group injected with drug dexamethasone (50 mg/kg, intraperitoneally). The 3rd to 5th group was given milk (4 mL/kg, subcutaneously) after 30 min of treatment. Each group counted the total leukocyte and eosinophil numbers before plant extract treatment and after 24 h of milk dosage. The difference was calculated between the total count of leukocytes and eosinophils before treatment and after 24 h of extract administration [13].

2.9. *Statistical evaluation*

The values of the result were represented as mean ± SEM (standard error of the mean) by using Excel 2016. For the *in vivo* test, variations between groups were investigated with the help of the one-way ANOVA (analysis of variance) test, then by Dunnett's test. SPSS (16.0) software was used. The significance level was taken as $P<0.05$.

3. Results and Discussion

This study aims to determine the antioxidant and anti-inflammatory activities by *in vitro* method and the antiallergic property of the *M. aculeata* extracts by *in vivo* method. The result revealed that the plant extracts exhibit good antioxidant property and prevent inflammation and allergy (using an animal model).

3.1. *Preliminary screening of phytochemicals*

The phytochemical screening revealed that bioactive compounds like alkaloids, glycosides, phenols, flavonoids, tannins, terpenoids, steroids, and saponins are present in the plant extracts (Table 1). It has been reported that saponins and flavonoids have antioxidant properties [3]. Saponins exhibit mast cell stabilizing activity, several flavonoids exhibit bronchodilator and smooth muscle relaxant activity, and flavonoids like luteolin and apigenin inhibit neutrophil beta-glucuronidase release and basophil histamine release, and thereby exhibit antiallergic property [13]. It has also been reported that flavonoids can prevent anaphylactic shock triggered by IgE responses [14]. Polyphenols, the natural antioxidants, act as efficient radical scavengers due to their redox properties and also exhibit strong anti-inflammatory and anticancer properties and cure neurodegenerative diseases [15].

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Table 1. Qualitative analysis of *M. aculeata* methanolic extract.

Phytochemical constituents	Name of the test	Methanolic extract of *M. aculeata*
Alkaloid	Mayer's test	+
Glycoside	Fehling's test	+
Phenol	$FeCl_3$ test	+
Flavonoid	H_2SO_4 test	+
Tannin	$FeCl_3$ test	+
Steroid	Foam test	+
Terpenoid	Liebermann Burchard test	+
Saponin	Liebermann-Burchard test	+

+ specifies the presence of phytoconstituents

3.2 *Antioxidant property determination*

There is an increase in interest in natural antioxidants in medicinal plants, vegetables, beverages, cereals, spices, and herbs. Antioxidants are free radical scavengers as these prevent the oxidative chain reaction initiation or continuation and prevent diseases and destruction caused by oxidative stress [16]. Antioxidants prevent the damaging effects of

free radicals in the body. Free radicals are formed in our body by the oxidation of food or due to environmental exposures like radiation or smoke. These destroy cells, DNA, RNA, lipids, and proteins, causing many diseases like inflammation, cancer, cardiovascular diseases, allergy, and many more [17]. Hassan *et al*. [18] revealed the antioxidant property of various extracts of *M. aculeata*. Many antioxidant mechanisms exist in all human cells to protect them from oxidative damage, but occasionally they are not enough to stop the harm done by free radicals. So, natural food supplements are employed as oxidation protection [19]. In the present study, the antioxidant activity of the extract may be due to the identified secondary metabolites through GC-MS screening. Plant extract capacity to scavenge radicals was calculated by the percentage inhibition formula, and the maximum inhibition percentage at 250 µg/mL of plant extract was 89.68 % when compared to the standard, which shows 73.47 % inhibition (Table 2). The results suggest that *M. aculeata* has good antioxidant potential.

Table 2. Percent inhibition of DPPH by the extract of *M. aculeata*.

Concentration µg/mL	% Inhibition by plant extract	% Inhibition by ascorbic acid
50	31.37±0.011*	32.56±0.100*
100	52.55±0.009*	41.55±0.033*
150	72.78±0.009*	52.57±0.047*
200	83.56±0.017*	64.26±0.032*
250	89.68±0.025*	73.47±0.020*
IC_{50} value	95.82	136.20

*Values represent mean ± SEM (n=3), to estimate the IC_{50} value, regression analysis (linear) was used.

3.3. *Anti-inflammatory property determination*

In some inflammatory ailments like rheumatoid arthritis, functional changes are due to reactive oxygen species (ROS). ROS causes cytokines release and activates proinflammatory enzymes like lipoxygenase, inducible nitric oxide, and cyclooxygenase [3]. Inflammation is the first response of the immune system that protects the body. Many inflammatory agents, like physical injuries, noxious chemical irritations, heat, microbial infections, etc., induce the inflammatory process in living tissues. In response to inflammation, pathological conditions exist, such as redness, swelling, pain, heat, etc. Though controlled inflammatory response restores normal physiology regulated by a complex molecular cascade, chronic inflammation will give rise to the onset of some diseases like arthritis, cancer, stroke, etc. [20]. In addition to this, persistent and chronic inflammation is associated with chronic heart failure, atherosclerosis, asthma, allergy, diabetes mellitus, Alzheimer's disease, osteoporosis, tumor progression, DNA damage, etc. [4]. Protein denaturation is a fundamental cause of inflammation and will lead to the formation of inflammatory diseases. Well-known models for the study of the property against inflammation by *in vitro* method are denaturation of proteins assay. In the protein denaturation mechanism, external agents like heat, acid, base, or organic solvent are responsible for the loss of secondary and tertiary structure and biological properties of protein molecules [15]. The inhibition of protein denaturation would be related to the

ability of plant phytoconstituents like alkaloids, flavonoids, and tannins which communicate with the aliphatic domain encompassing lysine amino acid on proteins and inhibit the destruction of the molecular structure of the protein [3]. Angmo *et al.* [21] revealed the potential against inflammation of shoot, root, and leaves parts of *M. aculeata*. To evaluate the property of plant extract against inflammation, percent inhibition was determined. The percent inhibition at a higher concentration of 250 µg/mL of the plant extract was found as 85.52 % when compared to the standard, which shows 76.47 % inhibition (Table 3). The present study demonstrates that *M. aculeata* whole plant extracts exhibit a better ability than standard aspirin to inhibit the protein denaturation induced by heat, thus having a pronounced anti-inflammatory effect. The anti-inflammatory property of the plant may be due to some identified phytoconstituents through GC-MS.

Table 3. Consequences of *M. aculeata* extract on heat-induced denaturation of albumin.

Concentration µg/mL	% Inhibition by Plant extract	% Inhibition by Aspirin
50	23±0.010*	47.68±0.020*
100	33.85±0.005*	54.26±0.020*
150	44.71±0.005*	64.13±0.029*
200	73.34±0.005*	68.08±0.028*
250	85.52±0.003*	76.47±0.014*
IC_{50} value	143.63	65.09

*Each value represents the mean ± SEM (n=3), to estimate the IC_{50} value, regression analysis was used.

3.4. *Characterization of bioactive compounds*

Fig. 1. depicts the GC-MS graphic record of *M. aculeata* extract representing different bioactive constituents having relative concentrations at various peak heights, and 40 compounds were identified as confirmed through the NIST library. The major bioactive compounds identified are shown in Table 4, depicting peak number, retention time, relative abundance, molecular formula, and weight.

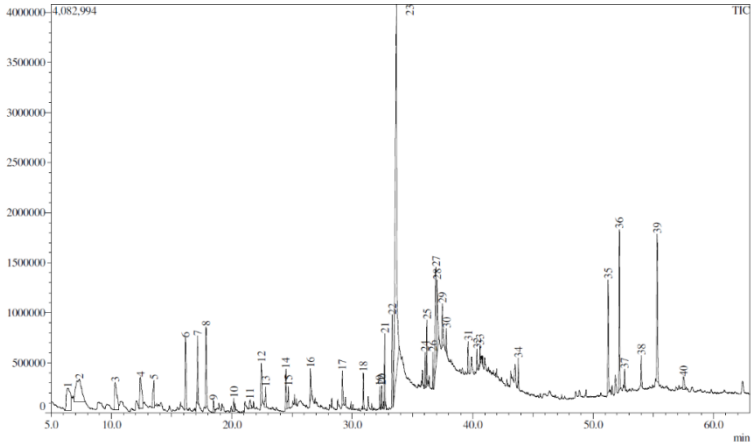

Fig. 1. Chromatogram of *M. aculeata* methanolic extract (GC-MS).

Table 4. Major bioactive phytoconstituents were identified through GC-MS analysis in *M. aculeata* methanolic extract.

Peak no.	Retention time (min)	Phytoconstituent names	Molecular formula	Molecular weight	Relative abundance (%)
23	33.649	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	27.29
2	7.314	Diglycerol	$C_6H_{14}O_5$	166	6.34
27	36.935	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	5.53
39	55.314	Stigmast-5-en-3-ol, (3β, 24S)	$C_{29}H_{50}O$	414	5.49
36	52.170	10-Nonadecanol	$C_{19}H_{40}O$	284	4.23
1	6.361	2-Heptenal, (Z)-	$C_7H_{12}O$	112	4.21
35	51.242	Protopine	$C_{20}H_{19}NO_5$	353	3.72
28	37.035	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	3.39

Therapeutic properties of some bioactive phytoconstituents of the extract are the following: 2-Heptenal, (Z) exhibits antioxidant and anti-inflammatory properties [22]; Octanoic acid exhibits antifungal and anti-inflammatory properties [23]; Tetradecane possess antimicrobial, antinociceptive, and anti-inflammatory properties [24]; 9-Oxononanoic acid exhibits antioxidant activity [25]; Dodecanoic acid possesses anti-inflammatory, cyclooxygenase-1, and cyclooxygenase-2 inhibitor, hypercholesterolemic, anti-oxidant, and anticancer activities [26]; 9-Octadecenoic acid (Z) treats stroke, respiratory failure, acute neurologic disorder, and anemia [27]; 2-Pentadecanone,6,10,14-trimethyl has the antioxidant and antimicrobial potential [28]; 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione has antioxidant, anti-androgen properties and is the anti-mineralocorticoid agent [29]; Hexadecanoic acid, methyl ester possesses anti-inflammatory, antioxidant, pesticide, nematicide, and antiandrogenic properties [30]; Hexadecanoic acid exhibits anti-inflammatory, antioxidant, hypocholesterolemic, and inhibitor of 5-α reductase activity [26]; 9,12-Octadecadienoic acid (Z, Z), methyl ester exhibits anti-inflammatory, antihistaminic, antieczemic, antiacne, antiarthritic, anticancer, insectifuge, nematicide, and hypocholesterolemic activities [31]; Trans-13-octadecenoic acid, methyl ester has properties to treat inflammation, is the insecticide, antiandrogenic, dermatitigenic, and anaemiagenic [32]; 9,12-Octadecadienoic acid possesses activities like antihistaminic, antieczemic, anti-cancer, antiacne, and prevents damage to the liver, inflammation, hypocholesterolemia, and arthritis [31]; Diisooctyl phthalate has antioxidant, antimicrobial properties [33]; Protopine, an isoquinoline alkaloid possess anti-asthmatic potential [34] and anti-inflammatory, antioxidant, hepatoprotective, anticancer properties [35]; 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl, acetate, (3β, 4α, 5α) has anti-oxidant property [36]; Ergost-5-en-3-ol, (3β) possess anti-oxidant, anti-inflammatory, anti-microbial properties [37, 38]; Stigmast-5-en-3-ol, (3β,24S), a pentacyclic triterpenoid has anti-inflammatory, antioxidant, analgesic, apoptotic, antimutagenic, chemoprotective, anthelminthic, hypocholesterolemic, angiogenic, anti-diabetic, antifungal, antimicrobial, and anti-asthmatic properties [39], and 9,19-Cyclolanost-25-en-3-ol, 24-methyl, (3β,24S) exhibits anti-bacterial potential [40]. Other remaining bioactive phytoconstituents out of 40 identified includes 1,3,5-Triazine-2,4,6-

triamine; 2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one; 2-Decenal, (E); 2,4-Decadienal, (E,E); 1-Undecene, 8-methyl-; 1,8-Nonadien-3-ol; Cyclooctanemethanol; Tetradecanoic acid; 2-Pentadecanone, 6,10,14-trimethyl; 1,2-Benzenedicarboxylic acid, dibutyl ester; Octadecanoic acid, methyl ester; Octadecanoic acid; Hexadecanoic acid, 2-methylpropyl ester; Cyclohexanol, 2-methyl-5-(1-methylethyl), (1α, 2β, 5β); 2,6,6-Trimethylbicyclo[3.1.1] heptan-2-ol and (9E,12E)-9,12-Octadecadienoyl chloride.

3.5. *Acute toxicity study*

Mortality was not observed in mice by plant extract treatment up to 2000 mg/kg orally while continuously observing for 14 days. Hence, according to acute toxicity results, LD_{50} (lethal dose) can be considered as more than 2000 mg/kg body weight.

3.6. *Evaluation of anti-asthmatic activity*

In this study, a mice model was used to evaluate the assay of leukocytosis and eosinophilia induced by milk. The increased leukocytosis and eosinophilia present a sensitive cellular biomarker, as seen in allergy/asthma patients, and causes hypersensitivity reactions [14]. The most characteristic inflammatory cells are the eosinophils taken from asthma patients in bronchial biopsies, generally seen in epithelial and submucosal layers [41]. Cytotoxic and inflammatory events associated with allergic disorders like rhinitis, urticaria, and bronchial asthma are mediated by eosinophils. During the late phase of allergic asthma development, eosinophils secrete toxic, basic proteins and mediators like cysteinyl-leukotrienes, neurotoxins, cationic proteins, prostaglandins, and tumor necrosis factor, which causes bronchoconstriction, mucus hypersecretion, epithelial layer shed off and promote respiratory airway inflammation (generally allergic) [42]. If the eosinophil count increases abnormally (>4 %) of the total white blood cell count is known as eosinophilia and is linked with allergy and ailments of the respiratory system [41]. In asthma, leukocytes release the mediators, such as histamine, cytokine, and major basic proteins, that, in turn, stimulate the inflammatory process. Leukocyte infiltration results in fewer antioxidant levels due to the release of ROS in surrounding tissues and is linked with various pathogenic features of asthma [42, 43]. Histamine induces mucus secretion and contraction of airway smooth muscle and plays a major role in allergic rhinitis, anaphylaxis, and urticaria [7]. According to the authors, it was reported that local people use the flowers of *M. aculeata* to cure asthmatic problems [1]. It was demonstrated that after 24 h of giving milk subcutaneously to the mice, there is a marked elevation in total leukocytes and eosinophils count, and by administration of an adaptogenic or antistress drug, this traumatic condition can be controlled [43]. In this study, leukocytes and eosinophils count was elevated after 24 hours by the subcutaneous injection of 4 mL/kg milk in albino mice. M. *aculeata* extracts (100-200 mg/kg, intraperitoneally) inhibited the increased leukocyte and eosinophil count at a significant level less than 0.05 ($P<0.05$) according to dosage (Table 5). Dexamethasone, an anti-inflammatory medication (50 mg/kg, intraperitoneally), also declined the rise in

leukocytes and eosinophils count. The identified biologically active constituents like Protopine, 9,12-Octadecadienoic acid; 9,12-Octadecadienoic acid (Z, Z), methyl ester, and Stigmast-5-en-3-ol, (3β, 24S) or (β-sitosterol) suggest the probable usage of the plant to cure the allergic disorders and asthma.

Table 5. Effect of extracts of *M. aculeata* on leukocytosis and eosinophilia induced by milk in mice.

Treatments	Dosage (according to body weight of mice) and administration route	The difference in total leukocyte count (per mm^3)	The difference in total eosinophil count (per mm^3)
Normal control	10 mL/kg (p.o.)[a]	875±79.32	25.33±5.004
Milk intoxicated	4 mL/kg (s.c.)[b]	2475±148.18*	76.83±3.13*
Plant extracts	100 mg/kg (i.p.)[c]	1678.33±98.8*	45.58±2.57*
Plant extracts	200 mg/kg(i.p.)[c]	1495±104.58*	45.15±3.009*
Dexamethasone	50 mg/kg (i.p.)[c]	933.33±130.8	28.66±2.12

Data are represented as mean ± SEM (n=6). *P<0.05 significantly different in comparison with 1st group (One-way ANOVA and *post hoc* Dunnett's test); [a]oral, [b] subcutaneous, and [c] intraperitoneal route of administration.

4. Conclusion

This research work shows that *M. aculeata* possesses higher antioxidant and anti-inflammatory potential when compared to their standard ascorbic acid and aspirin. Also, 40 phytoconstituents were identified through the GC-MS analysis which possesses different pharmacological properties. The plant extracts significantly inhibited the elevated leukocytes and eosinophils count in albino mice, thus possessing good anti-asthmatic properties. Anti-asthmatic properties of the plant may be due to identified phytoconstituents like Stigmast-5-en-3-ol; Protopine; 9,12-Octadecadienoic acid; and 9,12-Octadecadienoic acid (Z, Z), methyl ester. Hence, our result is in accordance with the traditional usage of this plant for its anti-asthmatic property. Moreover, to my knowledge, *M. aculeata* is not exploited for its anti-asthmatic property and GC-MS analysis of phytoconstituents. The main aim was to explore the plant for its anti-asthmatic properties, creating awareness among people for treatment by medicinal plants instead of allopathic medicines with various side effects.

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1. A. Bahukhandi, K. C. Sekar, A. Barola, M. Bisht, and P. Mehta, Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. **89**, 1327 (2019). https://doi.org/10.1007/s40011-018-1054-1
2. K. Sridhar and A. L. Charles, Food Chem. **275**, 41 (2019). https://doi.org/10.1016/j.foodchem.2018.09.040

3. T. E. Gonzal, D. N. Flore, A. A. Donatien, N. Y. William et al. Asian Pac. J. Trop. Biomed. **10**, 65 (2020). https://doi.org/10.4103/2221-1691.275421
4. S. Tasneem, B. Liu, B. Li, M. I. Choudhary, and W. Wang, Pharmacol. Res. **139**, 126 (2019). https://doi.org/10.1016/j.phrs.2018.11.001
5. E. Bhattacharya, R. Dutta, S. Chakraborty, and S. M. Biswas, Asian Pac. J. Trop. Biomed. **9**, 484 (2019). https://doi.org/10.4103/2221-1691.270984
6. D. Jenerowicz, W. Silny, A. Danczak-Pazdrowska, A. Polanska, and A. O. Mankowska, Ann. Agric. Environ. Med. **19**, 475 (2012).
7. S. A. Nirmal, A. P. Patel, S. B. Bhawar, and S. R. Pattan, J. Ethnopharmacol. **142**, 91 (2012). https://doi.org/10.1016/j.jep.2012.04.019
8. O. T. Fatokun, T. E. Wojuola, K. B. Esievo, and O. F. Kunle, European J. Pharm. Med. Res. **3**, 82 (2016).
9. G. Visweswari, R. Christopher, and W. Rajendra, Int. J. Pharm. Sci. Res. **4**, 2770 (2013). https://doi.org/10.13040/IJPSR.0975-8232.4(7).2770-76
10. Swati, H. K. Pandey, A. Singh, H. S. Meena, and M. Bala, Def. Life Sci. J. **6**, 117 (2021). https://doi.org/10.14429/dlsj.6.15770
11. N. B. Mirke, P. S. Shelke, P. R. Malavdkar, and P. N. Jagtap, IPP **8**, 28 (2020). https://doi.org/10.31690/ipp.2020.v08i02.003
12. P. Lalitha, S. K. Sripathi, and P. Jayanthi, Asian J. Pharm. Clin. Res. **5**, 59 (2012).
13. D. J. Taur and R. Y. Patil, J. Ethnopharmacol. **136**, 374 (2011). https://doi.org/10.1016/j.jep.2011.04.064
14. S. R. Naik, S. Bhagat, P. D. Shah, A. A. Tare, D. Ingawale, and R. R. Wadekar, Rev. Bras. Farmacogn. **23**, 811 (2013). https://doi.org/10.1590/S0102-695X2013000500014
15. P. Kola, K. Metowogo, S. N. Manjula, G. Katawa et al. J. Ethnopharmacol. **283**, ID 114673 (2022). https://doi.org/10.1016/j.jep.2021.114673
16. D. P. Xu, Y. Li, X. Meng, and T. Zhou, Int. J. Mol. Sci. **18**, 96 (2017). https://doi.org/10.3390/ijms18010096
17. Z. A. Iqbal, M. S. Iqbal, and K. U. Mishra, Asian J. Pharm. Clin. Res. **10**, 12 (2017). http://dx.doi.org/10.22159/ajpcr.2017.v10i12.22303
18. S. Hassan, N. Sajjad, R. Ali, S. A. Ganie, and R. Hamid, IJARSE **7**, 1984 (2018).
19. K. Manna, B. Debnath, A. M. Ikbal, K. Paul, A. Rajkhowa, and R. Majumder, J. Sci. Res. **15**, 547 (2023). http://dx.doi.org/10.3329/jsr.v15i2.62193
20. N. I. Osman, N. J. Sidik, A. Awal, N. A. Adam, and N. I. Rezali, J. Intercult. Ethnopharmacol. **5**, 343 (2016). https://doi.org/10.5455%2Fjice.20160731025522
21. K. Angmo, B. S. Adhikari, and G. S. Rawat, SDRP J. Plant Sci. **2**, 1 (2017).
22. L. Nissen, F. Casciano, and A. Gianotti, Food Funct. **12**, 3159 (2021). https://doi.org/10.1039/D0FO03337E
23. M. Kandula, K. S. Kumar, S. Palanichamy, and A. Rampal, Int. Immunopharmacol. **40**, 443 (2016). https://doi.org/10.1016/j.intimp.2016.09.013
24. O. A. Idris, O. A. Wintola, and A. J. Afolayan, Plants **8**, 51 (2019). https://doi.org/10.3390/plants8030051
25. A. Harun, S. Daud, S. S. Harith, N. E. Rahim, M. A. Jalil et al. GADING J. Sci. Technol. **1**, 52 (2018).
26. J. Saikarthik, S. Ilango, J. Vijayakumar, and R. Vijayaraghavan, Int. J. Pharm. Sci. Res. **8**, 2916 (2017). http://dx.doi.org/10.13040/IJPSR.0975-8232.8(7).2916-21
27. R. Kavitha, Int. J. Pharm. Sci. Res. **12**, 2755 (2021). https://doi.org/10.13040/IJPSR.0975-8232.12(5).2755-64
28. R. M. Romeilah, H. S. El-Beltagi, E. A. Shalaby, K. M. Younes et al. Not. Bot. Horti Agrobot. Cluj-Na. **49**, 1 (2021). https://doi.org/10.15835/nbha49112233
29. A. M. Abdel-Aty, M. B. Hamed, W. H. Salama, M. M. Ali, A. S. Fahmy, and S. A. Mohamed, Biocat. Agric. Biotechnol. **20**, ID 101199 (2019). https://doi.org/10.1016/j.bcab.2019.101199
30. A. A. Reza, M. A. Haque, J. Sarker, M. S. Nasrin, *et al*., Food Sci. Nutr. **9**, 3777 (2021). https://doi.org/10.1002/fsn3.2343

31. B. Malik, T. B. Pirzadah, I. Tahir, M. Z. Abdin,and R. U. Rehman, J. Pharm. Res. **10**, 715 (2016).
32. O. I. Awonyemi, S. M. Abegunde, and T. E. Olabiran, Eurasian Chem. Commun. **2**, 938 (2020). https://doi.org/10.33945/SAMI/ECC.2020.8.10
33. H. A. Ali, Y. H. Mohammed and H. H. Imad, J. Pharmacogn. Phytother. **8**, 25 (2016). https://doi.org/10.5897/JPP2015.0367
34. G. Liu, P. Wang, C. Li, J. Wang, Z. Sun, X. Zhao, and X. Zheng, J. Mol. Recognit. **30**, (2017). https://doi.org/10.1002/jmr.2613
35. W. Huang, L. Kong, Y. Cao, and L. Yan, Molecules **27**, 215 (2021). https://doi.org/10.3390/molecules27010215
36. S. Srinivasan and V. Priya, J. Med. Plants **7**, 89 (2019).
37. S. Chakraborty, S. Majumder, A. Ghosh, and S. Saha, Bull. Natl. Res. Cent. **45**, 4 (2021). https://doi.org/10.1186/s42269-021-00503-3
38. F. Mujeeb, P. Bajpai, and N. Pathak, BioMed Res. Int. **2014**, 1 (2014). https://doi.org/10.1155/2014/497606
39. G. Parihar and N. Balekar, Int. J. Pharm. Sci. Res. **8**, 1375 (2017). https://doi.org/10.13040/IJPSR.0975-8232.8(3).1375-80
40. N. Fajrih, K. G. Wiryawan, S. Sumiati, S. K. Syahpura, and W. Winarsih, Biodiversitas. **23**, 195 (2022). https://doi.org/10.13057/biodiv/d230125
41. R. G. Mali and A. S. Dhake, J. Adv. Pharm. Technol. Res. **2**, 132 (2011). https://doi.org/10.4103%2F2231-4040.82949
42. S. Nirmal, S. Barwal, V. Dhasade, A. Patil, and B. Latinoam. Caribe PL. **10**, 222 (2011).
43. D. J. Taur and R. Y. Patil, Asian Pac. J. Trop. Biomed. **1**, S40 (2011). https://doi.org/10.1016/S2221-1691(11)60119-6