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Original article

Phytochemical inspection and anti-inflammatory potential of *Euphorbia milii* Des Moul. integrated with network pharmacology approach

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ABSTRACT

The inflammatory reaction is induced by several factors like stimulation of different enzymes, oxidative stress, and production of various proinflammatory mediators. Here, we elucidated the phytochemical profile of *Euphorbia milii* ethanol extract (EMEE) then, we studied its anti-inflammatory potential using a carrageenan-induced paw edema model in rats. It was found that the average paw edema weight substantially declined ($p < 0.05$) by treatment with EMEE (100 mg/kg and 200 mg/kg). Also, it was noticed the immunostaining with cyclooxygenase-2 and tumor necrosis factor-alpha antibodies decreased to a mild positive (score 1) after treatment with EMEE (200 mg/kg). This is along with improvement in the histological findings after examination of the paw tissues with hematoxylin and eosin as it revealed a decrease in the inflammation. Also, EMEE (200 mg/kg) resulted in a significant decline ($p < 0.05$) in the levels of the proinflammatory mediators (granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1, inducible nitric oxide synthase, and interleukin-5). Interestingly, it resulted in a substantial increase ($p < 0.05$) in the levels of the anti-inflammatory interleukins (IL-10 and IL-12). Using the Swiss Target Prediction database, 127 genes were related to the identified metabolites in EMEE, of which 55 target genes were associated with 11 inflammatory disorders, as demonstrated by the DisGeNET database. The PI3K-Akt signaling route, the MAPK signaling pathway, and the Ras signaling pathway were identified as the dominant signaling pathways by the study of annotated genes. Therefore, EMEE requires further clinical investigations as an anti-inflammatory agent.

1. Introduction

The inflammatory process is carried out by the human cells as a defensive response against different inducers such as injury of tissues, specific chemical compounds, and infections. During this protective

mechanism, particular reactions are provoked via mediators of the inflammatory response (Sychrová et al., 2020). Interleukin-5 (IL-5), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) are examples of these proinflammatory mediators (Kaneko et al., 2019) and they can launch and boost the inflammatory process. Moreover, certain

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Table 1
Phytochemical profiling of EMEE by LC-MS/MS analysis (negative mode ESI).

Metabolite Name	RT. (min.)	Molecular Formula	Precursor <i>m/z</i> (Telesford et al., 2015) ^c	Error	MS/MS	Class
Homogenetic acid	1.385	C ₈ H ₈ O ₄	167.03	-12.5	99.01,124.01, 125.02,152.01, 167.03	2(hydroxyphenyl) acetic acids
Quercitrin	7.274	C ₂₁ H ₂₀ O ₁₁	447.09	4.3	163.02, 256.93, 285.02, 296.91, 301.02, 334.90, 402.89, 447.08	Flavonoid-3-O-glycosides
Kaempferol-3-O-alpha-L-rhamnoside	7.964	C ₂₁ H ₂₀ O ₁₀	431.09	1.5	112.98, 227.03, 284.05, 431.09	Flavonoid-3-O-glycosides
Astragaln	8.877	C ₂₁ H ₂₀ O ₁₁	447.094	-0.5	447.09, 285.18, 284.03	Flavonoid-3-O-glycosides
1-Methyl-6-hydroxy-1,2,3,4-Tetra hydro isoquinoline-3-carboxylic acid	10.370	C ₁₁ H ₁₃ NO ₃	206.082	-0.5	206.08	Isoquinoline-3-carboxylic acid
Baicalein-7-O-glucuronide	10.468	C ₂₁ H ₁₈ O ₁₁	445.1552	-7.7	261.08, 263.10, 445.12	Flavonoid-7-O-glucuronides
6,7-Dihydroxycoumarin	10.481	C ₉ H ₆ O ₄	177.05	-5.5	117.03, 119.00, 145.02, 177.04	6,7-dihydroxycoumarins
Quercetin-3-O-(6"-galloyl)-β-galactopyranoide)	10.770	C ₂₈ H ₂₄ O ₁₆	615.099	1.5	151.01, 178.02, 301.04, 331.06, 615.09	Flavonoid-3-O-glycosides
Sinapyl aldehyde	10.852	C ₁₁ H ₁₂ O ₄	207.0665	-1.0	133.02, 149.02, 161.02, 177.01, 179.03, 188.93, 192.04, 207.06	Methoxyphenols
Luteolin	10.865	C ₁₅ H ₁₀ O ₆	285.18	5.7	285.04	Flavones
3'-methoxy-4',5,7-trihydroxyflavonol	11.530	C ₁₆ H ₁₂ O ₇	315.04	3.6	243.01, 246.89, 269.25, 297.08, 300.02, 314.89	Flavonols
Gamma-Linolenic acid	11.875	C ₁₈ H ₃₀ O ₂	277.1451	-0.6	59.01, 102.95, 120.02, 121.03, 205.15, 208.92, 233.14, 277.17	Lineolic acids and derivatives
(+/-)-Taxifolin	13.559	C ₁₅ H ₁₂ O ₇	303.1588	1.8	190.95, 258.93, 285.14, 303.15	Flavanonols
3, 5, 7-Trihydroxy-4'-methoxyflavone	14.564	C ₁₆ H ₁₂ O ₆	299.09	42.3	177.02, 178.02, 281.21, 299.09	Flavones
3,4,5'-Trihydroxy-3'-glucopyranosylstilbene	19.296	C ₂₀ H ₂₂ O ₉	405.2002	0.5	405.16	Stilbene glycosides
7-Hydroxy-4-methylcoumarin	27.004	C ₁₀ H ₈ O ₃	174.9572	-5.5	86.98, 130.96, 146.96, 174.95	7-hydroxycoumarins

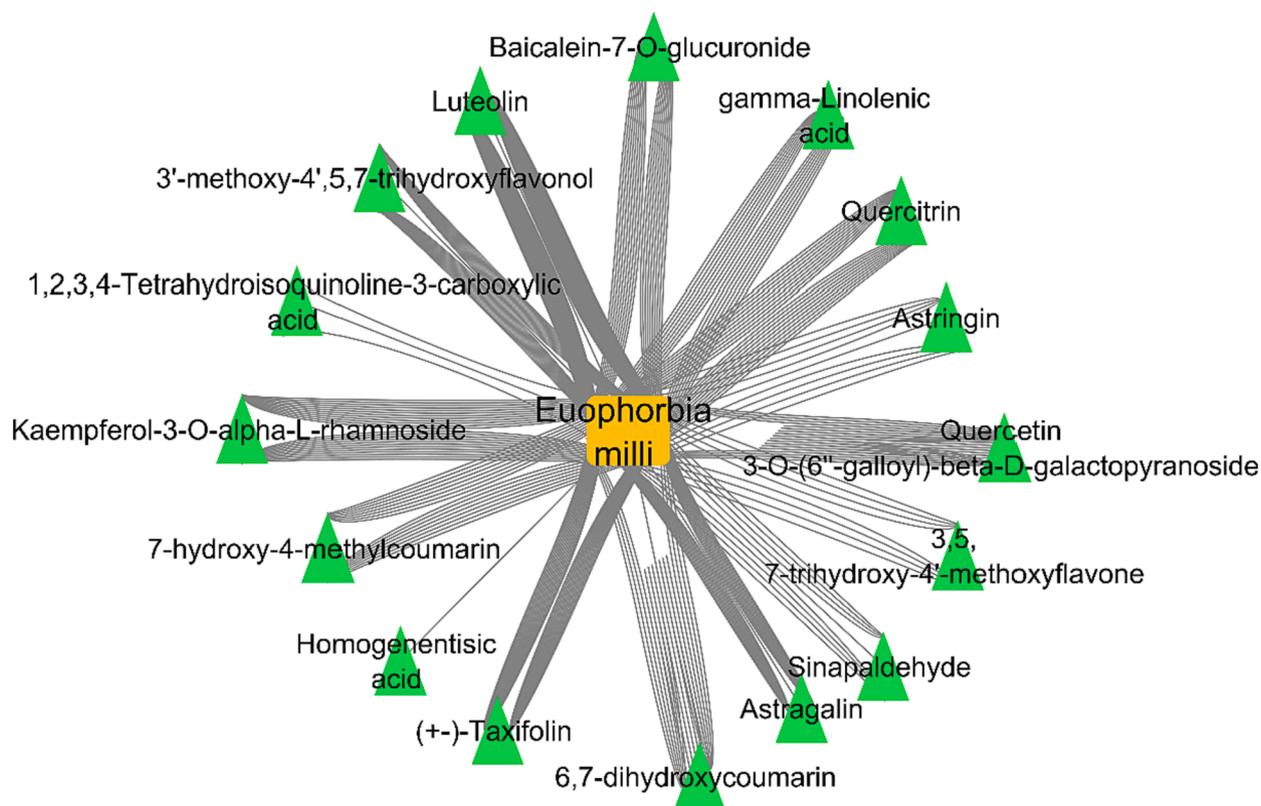


Fig. 1. Plant-metabolite network; a network illustrates the identified compounds from *Euphorbia milli* plant, the orange square is the plant name, the green triangles represent the identified metabolites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enzymes are involved in this inflammatory process, such as cyclooxygenase-2 (COX-2) which can manage the prostaglandin (PGs) production (Rea et al., 2018). Also, reactive oxygen species (ROS) are

considered important supporters of the inflammatory process. Such molecules are released by the inflammatory cells and can exaggerate the process of oxidative stress (Lin et al., 2021, Elekhawy et al., 2021).

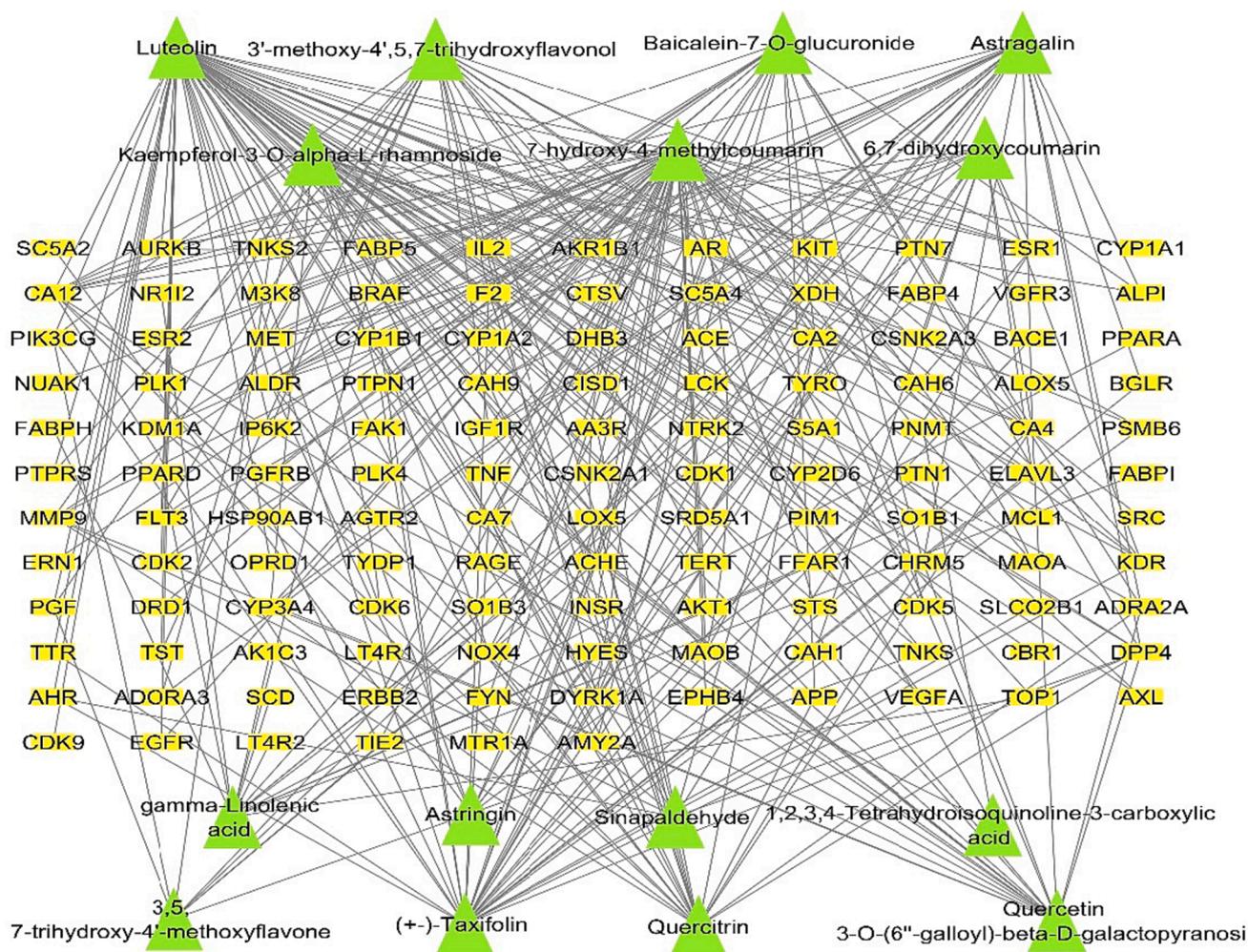


Fig. 2. Metabolites-targets network; a network illustrating the targets of the identified compounds. Green triangles represent the identified compounds; yellow rectangles represent the targets. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Unfortunately, finding safe and efficient anti-inflammatory compounds is challenging. Anti-inflammatory medications currently in use, such as non-steroidal anti-inflammatory agents, have numerous side effects (Bindu et al., 2020). These drugs, when used for a long term, can cause renal, gastrointestinal, and cardiac adverse reactions. Therefore, there is an urgent necessity to identify novel, effective anti-inflammatory products with no or relatively low side effects. Currently, natural agents, like plants, are regarded as a rich source for many bioactive compounds, and consequently, their therapeutic efficacy is being widely inspected. This is attributed to their numerous advantages like efficacy, safety, and biocompatibility (Attallah et al., 2022b).

With about 2000 species, the genus *Euphorbia* is a well-known angiosperm that may be found all over the world, especially in Asia, Africa, Central and South America. This genus's members range in size from tiny annual or perennial herbs to woody shrubs, vines, trees, and even succulent plants. They also produce an irritant milky latex. Generally, the entire plants, stems, leaves, latex, roots, and seeds of the *Euphorbia* species have been the subject of chemical and pharmacological investigation (Zhao et al., 2022, Xu et al., 2021).

Euphorbia roots or tubers are well-known for their therapeutic properties, particularly in China. Many of them are utilized as Chinese herbal medicines (Zhao et al., 2022, Salehi et al., 2019). Additionally, a recent study showed distinctions between the geographic distribution of *Euphorbia* plants used in traditional medicines around the world (Ernst et al., 2015). Three uses in particular were found to be the most

frequently used in this regard: treating digestive system disorders (very common worldwide with Australasia being an exception); treating infections and infestations (primarily in Southern Africa and America, the Pacific, Asia-tropical, and Asia-temperate); and treating skin/subcutaneous cellular tissue disorders (especially in Australasia, Europe, Asia, and Northern America) (Salehi et al., 2019, Pascal et al., 2017).

One of the most significant medicinal plants in this genus is *Euphorbia milii*, sometimes known as "Christ thrown" or "Christ plant," which has been used traditionally to cure hepatitis, cancer, and warts (Saleem et al., 2019, Yu et al., 2022). Additionally, this plant's antifungal, antinociceptive, antibacterial, and molluscicidal capabilities have been documented (Saleh et al., 2023b, Saleh et al., 2023a).

Network pharmacology is a new approach to virtual biological-chemical interactions that helps in identifying the highly represented genes related to identified or isolated compounds from a specific species in relation to a specific disease under study. It is used in different disorders like inflammatory disorders (Alnusaire et al., 2023), cancer (El-Hawary et al., 2023, Mohammed et al., 2023, Ahmed et al., 2022), wounds (Elmaidomy et al., 2023), neurodegenerative disorders (Baksh et al., 2023) and microbial infections (Alyami et al., 2023). Since this virtual technique can help to save money and effort for identifying target genes, biological pathways, cellular components, biological processes, and molecular functions of natural origin.

The objective of the current study was to look into the anti-inflammatory activity of EMEE in carrageenan-induced paw edoema after elucidating its secondary metabolites by LC/MS/MS. Also, network

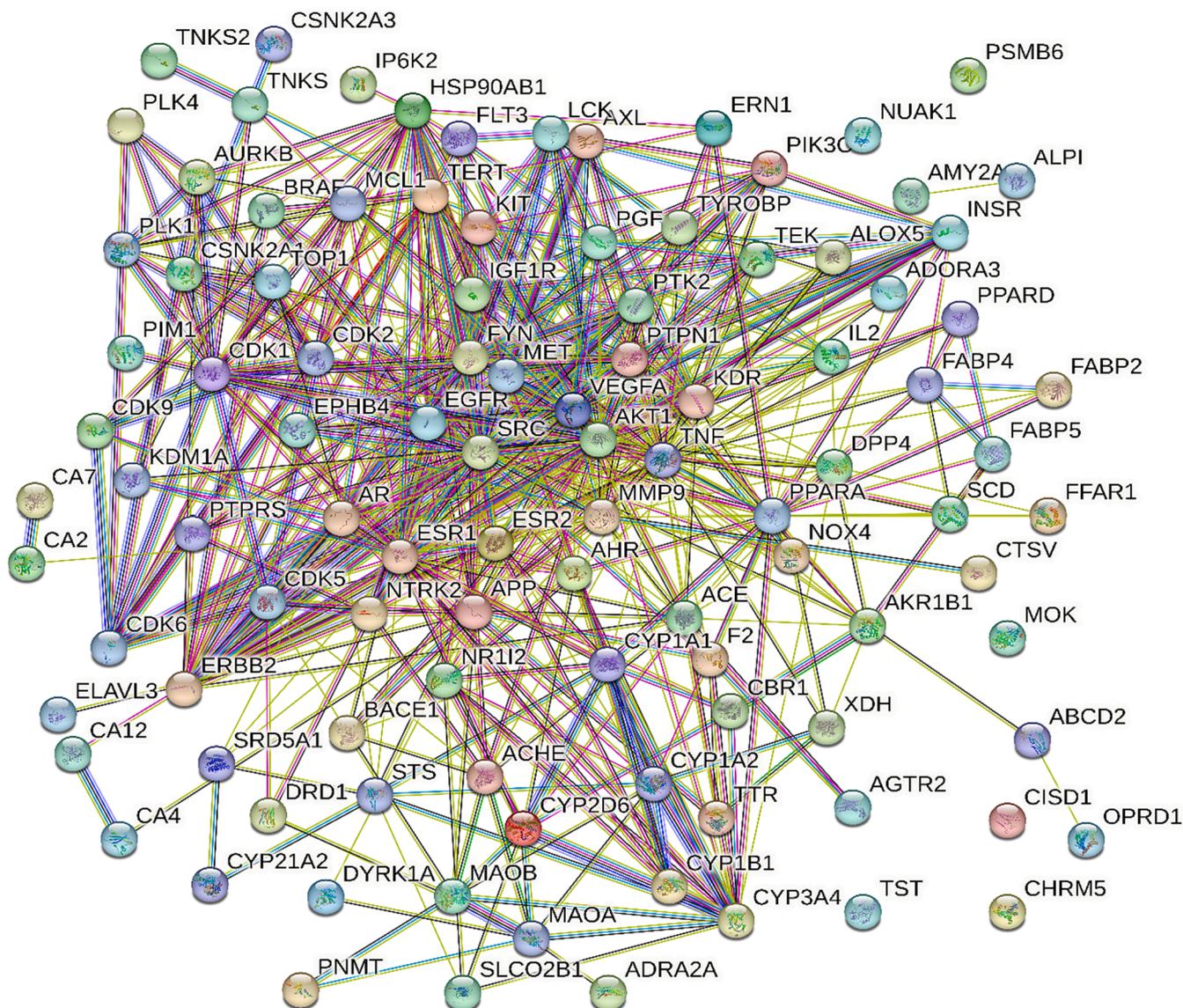


Fig. 3. PPI network, illustrating the interaction between proteins of identified targets.

pharmacology studies would be employed to reveal the potential anti-inflammatory action of the phytochemicals of EMEE.

2. Materials and methods

(1) Chemicals and plant materials

The utilized chemicals and plant were displayed in [supplementary data](#).

(2) LC-ESI-MS/MS profile analysis

Extract of *E. milii* aerial parts was analysed by adopting procedures previously reported by Attallah et al. ([Attallah et al., 2022b](#)).

(3) Network pharmacology study

3.1 Compound targets for *Euphorbia milii* ([supplementary data](#))

3.2 Metabolites-Targets network

The NCBI Gene databas (<https://www.ncbi.nlm.nih.gov/gene/>) ([Boutet et al., 2016](#)), also the Online SwissTargetPrediction database ([Gfeller et al., 2014](#)) were used to identify the related targets.

3.3 Targets-inflammation network

The target genes were specifically identified for the inflammatory disorders related to osteoarthritis, joints, and bone inflammations through the DisGeNET database (<https://www.disgenet.org/search>) ([Piñero et al., 2020](#)). The built in filter option in the database was used. The disease names were specified as “Rheumatoid Arthritis”, “Inflammatory polyarthritis”, “Bone Diseases”, “Inflammation”, “Inflammatory pain”, “inflammatory joint disease”, “Arthritis”, “Degenerative polyarthritis”, “Arthritis, Adjuvant-Induced”, “Osteoarthritis, Knee”, “Arthritis, Reactive”, “Inflammation”.

3.4 Protein-protein interaction (PPI) data ([supplementary data](#))

3.5 Network construction

Using the network analysis programme Cytoscape, the resultant networks were visualised and reorganised ([Kohl et al., 2011](#)), version 3.9.0. The difference was considered statistically significant at $p < 0.05$.

3.6 Gene ontology and pathway analysis

ShinyGo database ([Ge et al., 2020](#)) (last accessed on November 25,

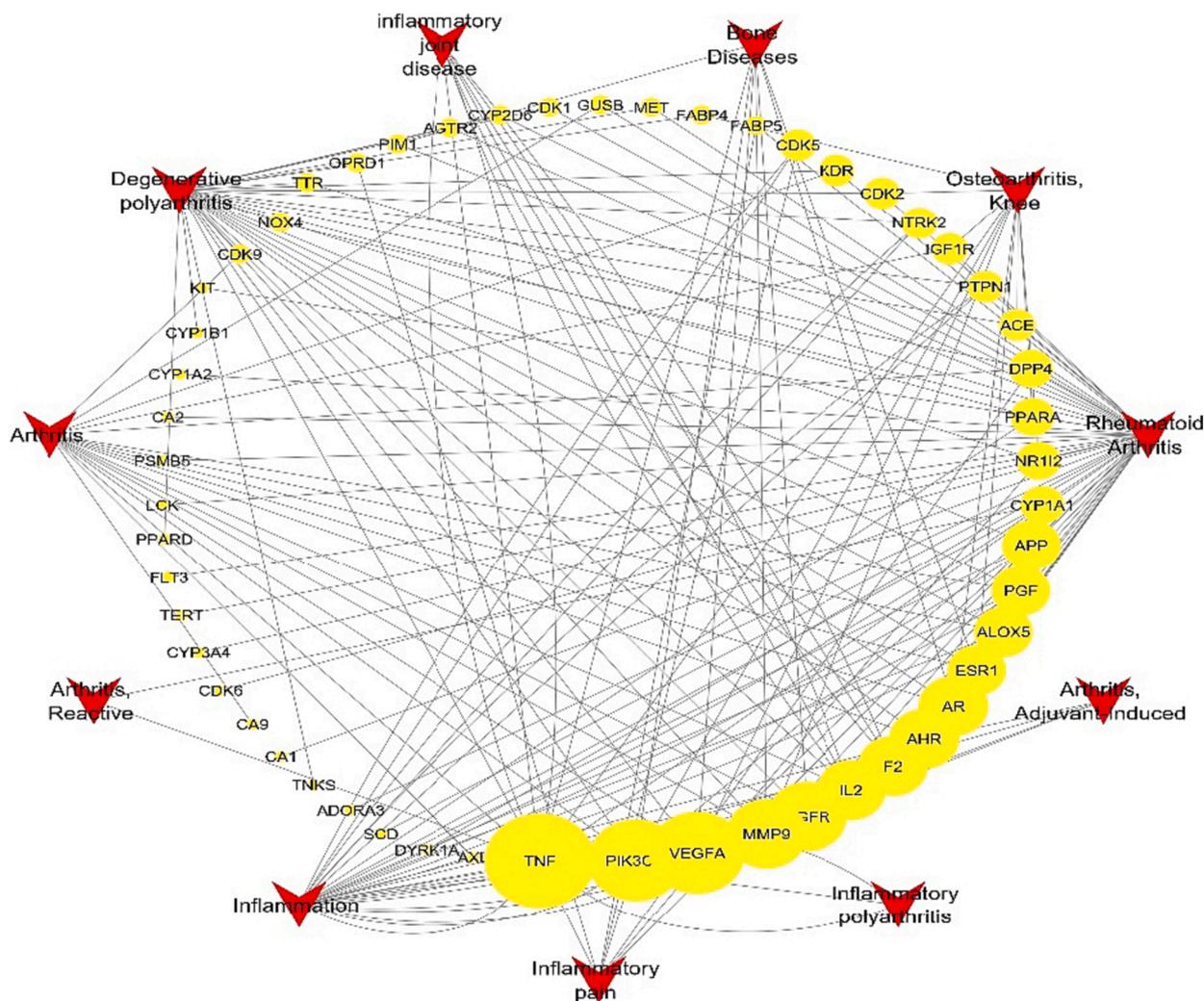


Fig. 4. Targets-inflammation network, a network illustrating the relations between identified targets and inflammatory disorders, yellow circles represent the targets (higher size indicate higher representation), the red triangles represent the types of inflammatory disorders. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

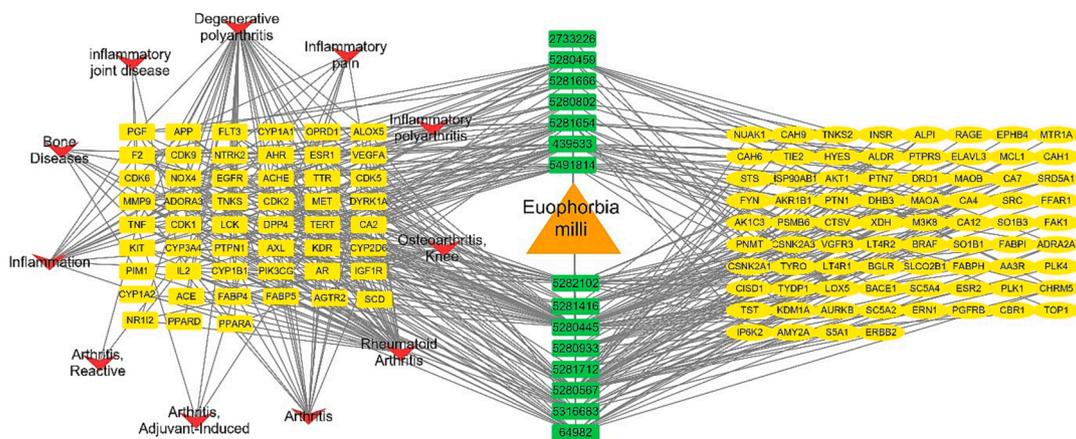


Fig. 5. Complete pharmacology network analysis (Metabolites-targets-inflammation), orange triangle is the plant names, green rectangles represent compounds PCIDs, yellow oval shapes represent targets not involved in inflammation, yellow rectangles represent targets involved in inflammations, red arrow heads represent types of inflammation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2022) and FunRich software (Pathan et al., 2017) were used to research the involved gene pathways, cellular components, molecular activities, and biological processes.

- (4) Anti-inflammatory activity of EMEE (supplementary data)
- (5) Statistics

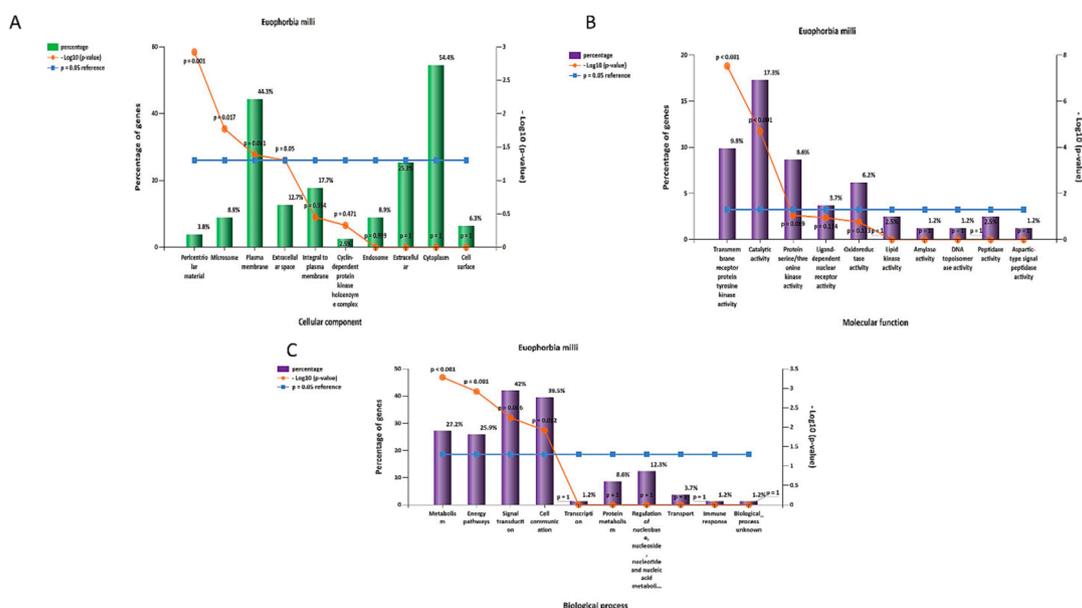


Fig. 6. Gene enrichment analysis of the targets of *Euphorbia milli*; (A) cellular components, (B) molecular functions, (C) biological process.

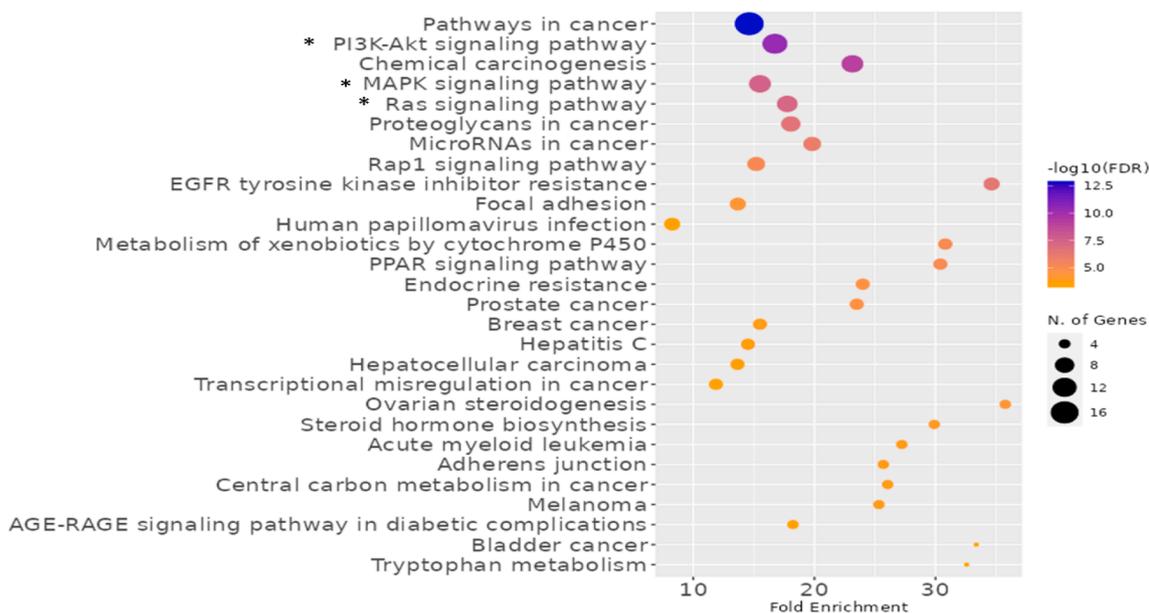


Fig. 7. Biological pathways of the genes of *Euphorbia milli* related to inflammation disorders, the top signalling pathways marked with (*), the more intense colour the more no. of genes represented in the pathway.

The experimental studies were performed in triplicates and the obtained results are revealed as the mean \pm standard deviation (SD). The utilized software was Graph-Pad prism version 8 and ANOVA test followed by post-hoc test was utilized. The significance level was recognized at $p < 0.05$.

3. Results

3.1. LC-ESI-MS/MS analysis

A total of 16 compounds were tentatively identified in *E. milli* using LC-ESI-MS/MS in a negative mode. The main substances were several types of flavonoids and their glycosidic derivatives, phenolics, and carboxylic acids. The comprehensive profile is listed in Table 1. While Fig. S1 shows the total ion chromatogram of detected metabolites in

negative ion mode.

3.2. Network analysis

3.2.1. Plant-metabolite network analysis

Using LC/MS/MS analysis technique (negative mode), the identification of the active metabolites (16 metabolite) from *Euphorbia milli* was established. A simple illustrating network was constructed and visualized by Cytoscape software (Fig. 1).

3.2.2. Metabolite-target network analysis

The identified compounds of *Euphorbia milli* by LC/MS/MS analysis were introduced in PubChem database and Swiss Target Prediction in order to extract the target hits. Interestingly, a total of 127 target hits were obtained. A network of metabolites-target was established. The

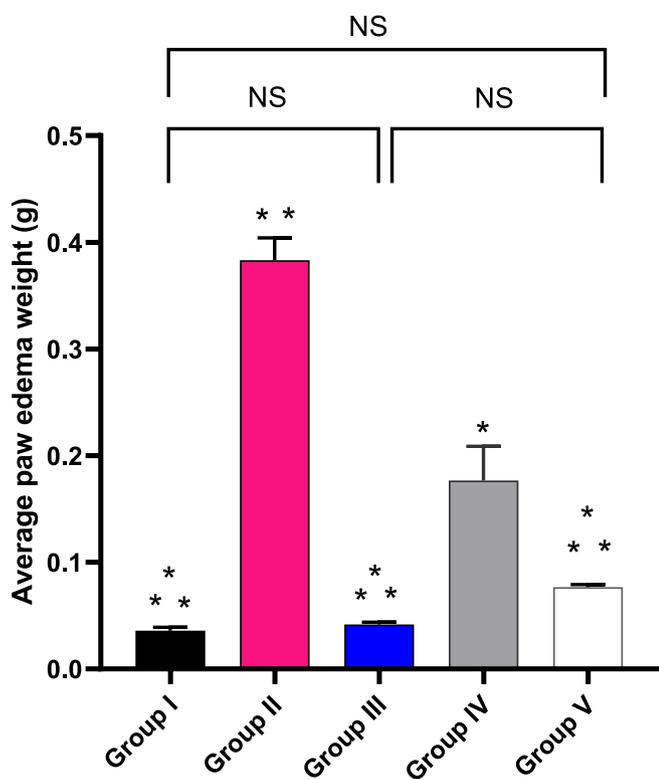


Fig. 8. Average weight of the paw edema of the studied groups. The symbol (*) denotes a significant difference ($p < 0.05$) as regards to group II. The symbol (**) denotes a significant difference ($p < 0.05$) as regards to group IV. The abbreviation (NS) denotes non-significant difference among the groups ($p > 0.05$).

nodes of the network were 142 node for the metabolites and 127 node for the targets, with network heterogenicity of 1.682 (Fig. 2).

3.2.3. Protein-protein interaction (PPI) data

The PPI network was constructed with 76 nodes, 501 edges, and an average node degree of 9.19. The interactions between the target proteins were performed and visualized using the online STRING database (Fig. 3). With clustering coefficient of 2.095 and network centralization of 0.384 (Alqahtani et al., 2022).

3.2.4. Common targets-inflammation network analysis

A network assessment was performed to establish the relations between the identified targets and the different kinds of inflammations. A sum of 55 genes was connected to 11 types of inflammation (Fig. 4) and the top revealed genes were TNF, VEGFA, PIK3CG, EGFR, and MMP9 with number of edges of 16, 8, 8,7, and 7, in the same order.

3.2.5. Complete pharmacology network (Metabolites- targets-inflammation)

The whole pharmacology network was created by combining and integrating the networks (plant-metabolites, metabolite-targets, targets-inflammation) to analyse the interactive connections between the identified compounds from *Euphorbia milli* to the target proteins and to the inflammatory disorders (Fig. 5). This network showed the genes related to inflammation in this gene set and other genes not involved in inflammatory disorders.

3.2.6. Target genes-pathways analysis

The top cellular components identified with this gene through the FunRich software were: cytoplasm followed by plasma membrane, the top molecular functions were the catalytic activity followed by

transmembrane receptor protein tyrosine kinase activity, while the top biological processes were signal transduction and cell communication (Fig. 6).

We used the KEGG and ShinyGO databases to create the network of the hit genes targeted by the detected compounds interacting with the defined types of inflammatory illnesses in order to develop target genes-pathways networking. The PI3K-Akt signalling route, MAPK signalling pathway, and the Ras signalling pathways were the top signalling pathways identified by the databases (Fig. 7 & Table S2).

3.3. In vivo study

The *in vivo* study was employed to reveal the potential anti-inflammatory action of EMEE in an animal model.

3.3.1. Effect of EMEE on the paw edema weight

The influence of the EMEE on the paw edema average weight was inspected as revealed in Fig. 8. Compared to groups II and IV, group V's average paw edema weight significantly decreased ($p < 0.05$). Regarding group II (carrageenan group), there was a significant increase in the edema weight by 925.64 % in comparison with group I. EMEE, with concentrations of 100 and 200 mg/kg, resulted in a marked decrease in the paw edema weight by 65 % and 80.75 %, respectively, in comparison with group II.

3.3.2. Histological studies

Tissue sections of the paws from the different studied groups were stained with H&E as exhibited in Fig. 9.

3.3.3. Immunohistochemical studies

The COX-2 and TNF- α immunostaining of the paw tissues of the different studied groups is exhibited in Figs. 10 and 11.

3.3.4. qRT-PCR

3.3.4.1. Gene expression of GM-CSF and MCP-1. The impact of EMEE on the relative gene expression of GM-CSF and MCP-1 was examined as revealed in Fig. 12. In comparison with group I, there was a significant overexpression of the GM-CSF and MCP-1 genes in group II by 380 % and 445 %, respectively. In addition, there was a significant downregulation in the gene expression of GM-CSF in groups IV and V by 58.13 % and 71.88 %, respectively, and the gene expression of MCP-1 in groups IV and V by 61.28 % and 73.03 %, respectively, in comparison with group II.

3.3.4.2. Gene expression of IL-5, IL-10, and IL-12. The influence of EMEE on the relative gene expression of IL-5, IL-10, and IL-12 was studied as revealed in Fig. 13. Group II revealed a significant increase in the gene expression of IL-5 by 455 % and a marked decrease in the gene expression of IL-10 and IL-12 by 84.26 % and 83.7 %, respectively, in comparison with group I. Regarding IL-5, its expression significantly decreased in groups IV and V by 60.36 % and 75.86 %, respectively, relative to group II.

Concerning IL-10 and IL-12, there was a significant increase in their expression in group V by 370.5 % and 354.5 %, respectively, relative to group II.

3.3.4.3. Gene expression of iNOS. The effect of EMEE on the relative gene expression of iNOS was elucidated and it is shown in Fig. 14. Group II revealed a significant increase in the gene expression of iNOS by 400 % in comparison with group I. Regarding group IV and V, they exhibited a significant downregulation of the gene expression of iNOS by 59.2 % and 73.4 %, respectively, in comparison with group II.

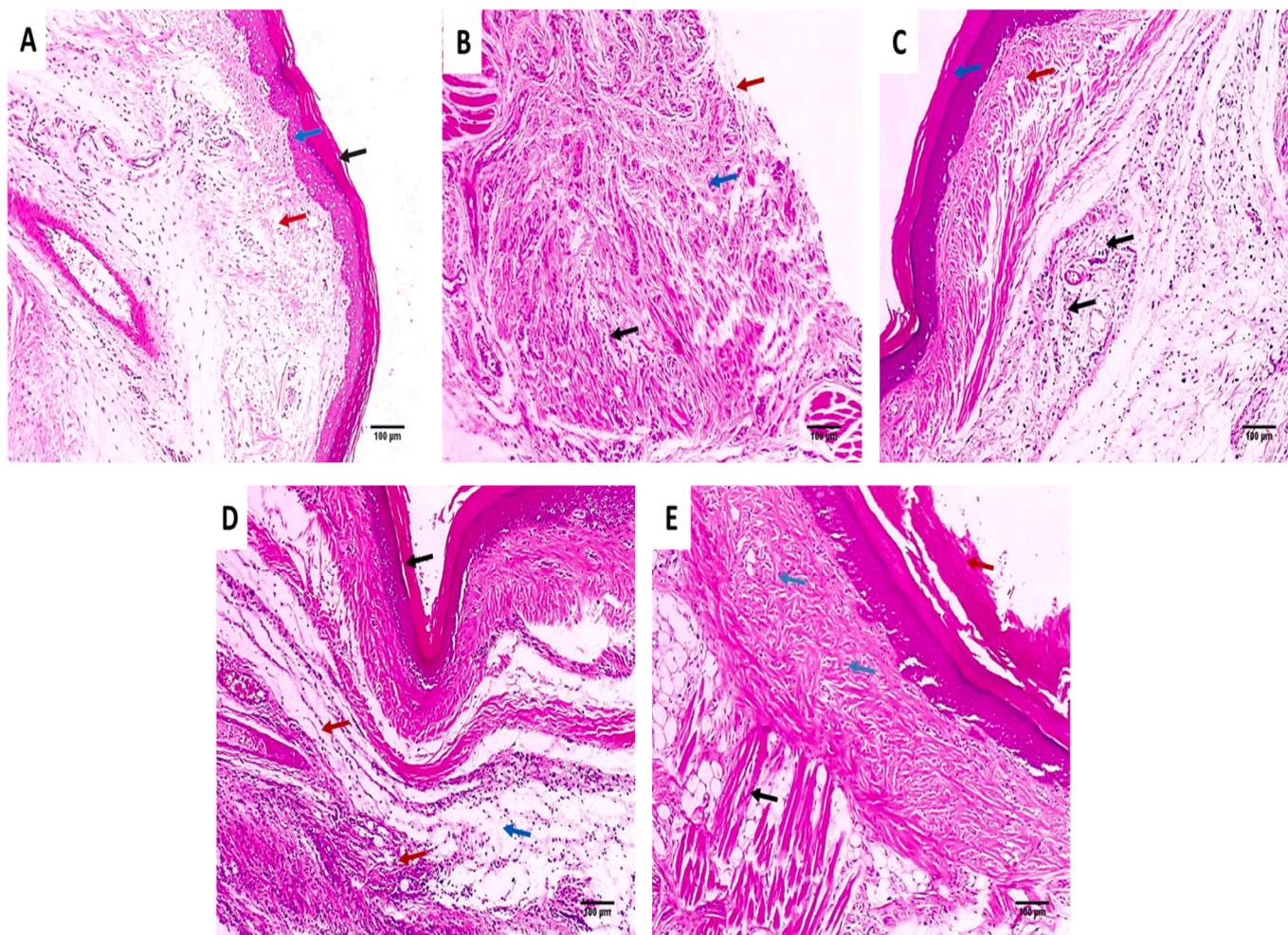


Fig. 9. H&E stained paw tissue sections of: A) Group I presenting normal skin consisting of epidermis of average thickness (blue arrow) lined with thick keratin (black arrow) and underlying normal dermis (red arrow) ($\times 100$). B) Group II presenting surface ulceration (red arrow) with underlying granulation tissue (blue arrow) with infiltration by chronic inflammatory cells (black arrow) ($\times 100$). C) Group III presenting very mild dermal inflammation (black arrows). The epidermis is thickened and covered with excessive keratosis (blue arrow) with underlying mild collagenosis (red arrow) ($\times 100$). D) Group IV presenting epidermal healing with superficial dermal mild chronic inflammation (red arrows) and mild edema (blue arrows) ($\times 100$). E) Group V presenting absence of inflammation and the muscles are normal (black arrow). The epidermis is thickened and covered with excessive keratosis (red arrow) with underlying mild collagenosis (blue arrows) ($\times 100$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Inflammation usually leads to a progress of different pathophysiological proceedings that can provoke disease development (Chen et al., 2018, El-Banna et al., 2019). In the literature, there are many animal models that were used to elucidate the anti-inflammatory potential of various tested compounds. Carrageenan-induced paw edema is regarded as one of the most used models to assess the anti-inflammatory activity of different natural and synthetic materials. Carrageenan is a chemical agent which is capable of stimulating the release of several inflammatory and pro-inflammatory mediators (Patil et al., 2019, Alherz et al., 2022).

Here, EMEE (200 mg/kg) treated group (group V) presented a significant decline ($p < 0.05$) in the paw edema weight in regard to groups II (positive control) and IV (treated with EMEE, 100 mg/kg). Interestingly, there wasn't a significant difference ($p > 0.05$) between groups I (normal control), III (treated with celecoxib), and V. Edema is an important character for the local inflammation and it is formed owing to the fluid accumulation in the interstitial fluid of the tissues (Zamorano et al., 2021). Thus, it negatively affects tissue functions because the accumulated fluid hinders the diffusion of nutrients and oxygen to the cells of the affected tissue and compromises their metabolism (Wiig,

2011, Almukainzi et al., 2022).

During the inflammatory process, different cells like neutrophils and macrophages migrate to the site of the inflammation and they develop large amounts of inflammatory mediators such as TNF- α and interleukins. Such mediators induce a prolonged inflammatory reaction (Apostolova et al., 2020, Elekhawy and Negm, 2022). Also, during inflammation, the stimulation of the COX-2 pathway is usually triggered and this leads to an increase in the release of prostaglandins like PGE2 which augment the inflammatory process (Guay et al., 2004, Attallah et al., 2022a). Herein, the carrageenan group (group II) revealed a strong positive COX-2 and TNF- α immunostaining (score 3) and this is due to the inflammation which is triggered by carrageenan. Remarkably, this was mitigated by EMEE treatment as the low dose (100 mg/kg) resulted in a moderate positive COX-2 and TNF- α immunostaining (score 2) and the high dose (200 mg/kg) resulted in a mild positive COX-2 and TNF- α immunostaining (score 1). Decreasing the positive COX-2 and TNF- α immunostaining is an important indication as it means that both COX-2 and TNF- α are decreased which results in a decrease in the inflammatory reaction (Mercogliano et al., 2020, Alotaibi et al., 2021, Negm et al., 2021). Besides, the histological examination of the paw skin tissues of the different studied groups showed that treatment with EMEE (200 mg/kg) resulted in absence of inflammation and appearance of

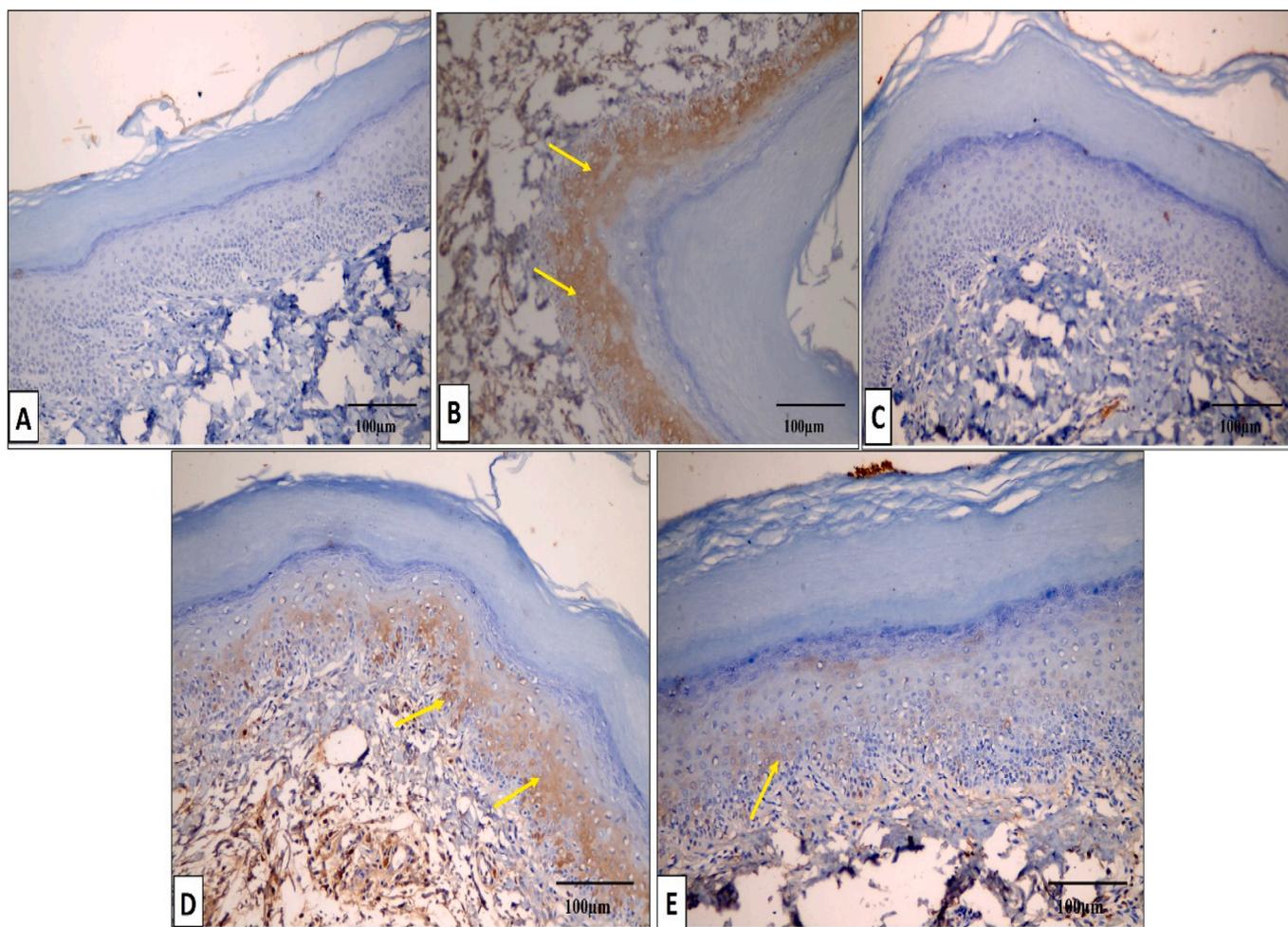


Fig. 10. COX-2 immunostained paw tissue sections of: A) Group I presenting a negative COX-2 immunostaining (score 0). B) Group II presenting a strong positive COX-2 immunostaining (score 3). C) Group III presenting a negative COX-2 immunostaining (score 0). D) Group IV presenting a moderate positive COX-2 immunostaining (score 2). E) Group V presenting a mild positive COX-2 immunostaining (score 1). ($\times 200$, scale bar = 100 μm).

normal muscles are normal (black arrow).

EMEE (particularly at concentration of 200 mg/kg) significantly enhanced ($p < 0.05$) the levels of the anti-inflammatory cytokines (IL-12 and IL-10). It also significantly decreased ($p < 0.05$) the release of the pro-inflammatory mediators (IL-5, GM-CSF, iNOS, and MCP-1). These results imply that EMEE, at least in carrageenan-stimulated paw edema, acts to reduce inflammation by preventing the release of pro-inflammatory cytokines and mediators. Carrageenan solution into the paws produces an inflammatory reaction that is characterized by an infiltration of the inflammatory cells, an increase in exudate, and a marked production of pro-inflammatory mediators, such as prostaglandins, leukotrienes, and cytokines, as well as components of the oxidative stress response (Duarte et al., 2016, Attallah et al., 2022c).

It is commonly acknowledged that controlling inflammation involves a number of signalling channels and it is a difficult process. For instance, through controlling the expression of several pro-inflammatory mediators, the NF- κ B signalling pathway contributes significantly to inflammation. The MAPK signalling route, in addition to the NF- κ B signalling pathway, influences the release of several inflammatory mediators. There is a vast range of biological effects caused by the MAPK family, which comprises the enzymes ERK1/2, JNK, and p38 kinase. These enzymes are crucial in controlling the activity of several proteins, enzymes, and transcription factors (Thouvery and Caverzasio, 2015).

According to several research, inflammatory activation and oxidative stress-activated the MAPK signalling pathway, which led to the phosphorylation of ERK1/2, JNK, and p38 (Li et al., 2018). It is well

known that the generation of the pro-inflammatory cytokines depends on the activation of MAPKs (Kang et al., 2016). In the current work, the phosphorylation of ERK1/2, JNK, and p38 by carrageenan-induced proteins promoted the release of TNF- α , which then induced the secretion of IL-6 and boosted the creation of COX-2. It is hypothesized that the MAPK signalling pathway contributes to the release of the inflammatory cytokines and the dysregulation of the production of COX-2 (Kang et al., 2016). The network pharmacology study determined the TNF gene to be the gene incorporated to most inflammatory disorders. The gene enrichment analysis of target genes identified by the compounds of EMEE determined the top signalling pathways; PI3K-Akt signalling pathway, MAPK signalling pathway, Ras signalling pathway, these results confirmed the hypothesis built in this study towards the signalling pathway contributed to the inflammation and proved that EMEE activity could be referred to a direct activation potential on MAPK signalling pathway and this anti-inflammatory potential attributed to TNF gene in addition to other identified 54 genes.

5. Conclusion

Our study suggested that EMEE had anti-inflammatory activity in a carrageenan-induced paw edema model in rats. This was revealed via improving the histological findings as well as the mild positive TNF- α and COX-2 immunostaining of the EMEE-treated group (200 mg/kg). Our hypothesized mechanism is that EMEE inhibits the MAPK-mediated COX-2 signalling pathway, which dampens inflammation in

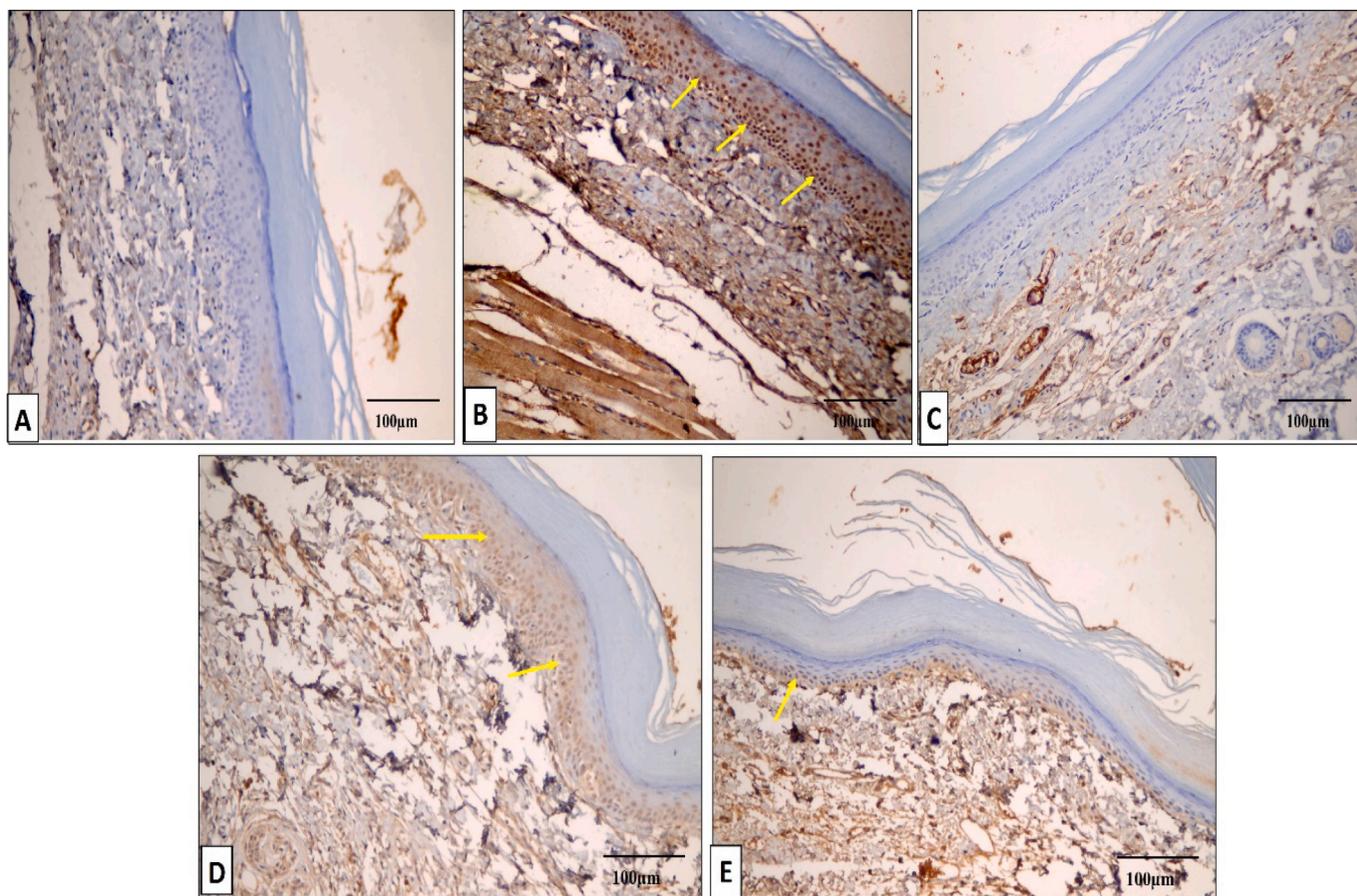


Fig. 11. TNF- α immunostained paw tissue sections of: A) Group I presenting a negative TNF- α immunostaining (score 0). B) Group II presenting a strong positive TNF- α immunostaining (score 3). C) Group III presenting a negative TNF- α immunostaining (score 0). D) Group IV presenting a moderate positive TNF- α immunostaining (score 2). E) Group V presenting a mild positive TNF- α immunostaining (score 1). ($\times 200$, scale bar = 100 μm).

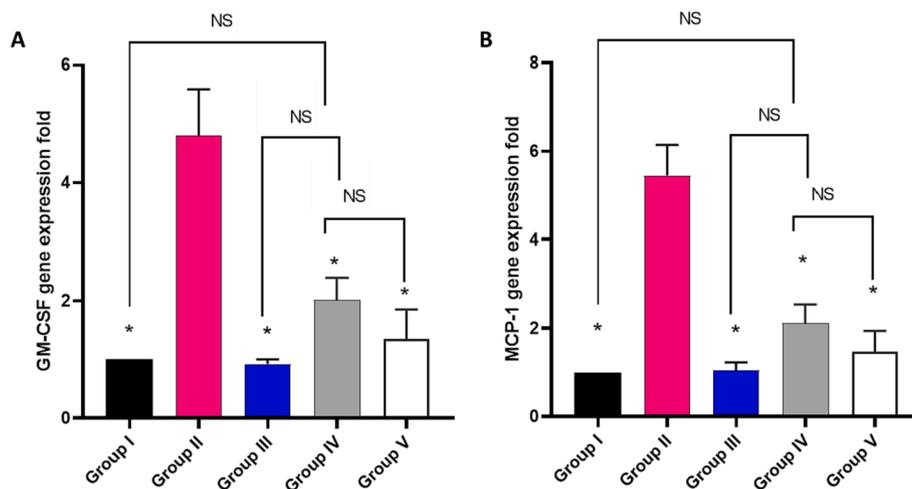


Fig. 12. Impact of treatment with EMME on the gene expression of A) GM-CSF and B) MCP-1. The single asterisk represents a significant difference ($p < 0.05$) in comparison with group II. The abbreviation NS means non-significant difference ($p > 0.05$) in comparison with group IV.

carrageenan-induced paw edema. This was supported by the network pharmacology studies.

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CRediT authorship contribution statement

Walaa A. Negm: Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing. **Engy Elekhawwy:**

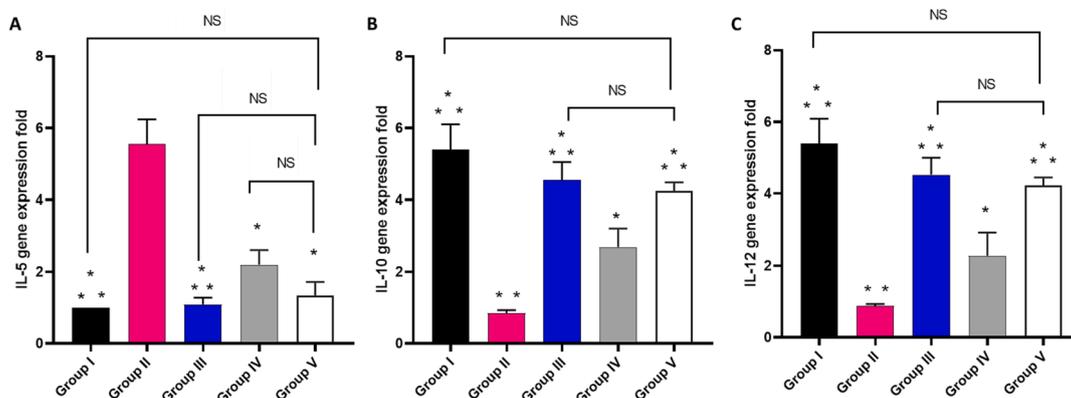


Fig. 13. Impact of treatment with EMME on the gene expression of A) IL-5, B) IL-10, and C) IL-12. The single asterisk represents a significant difference ($p < 0.05$) in comparison with group II. The double asterisks represent a significant difference ($p < 0.05$) in comparison with group IV. NS means non-significant difference ($p > 0.05$) in comparison with group V.

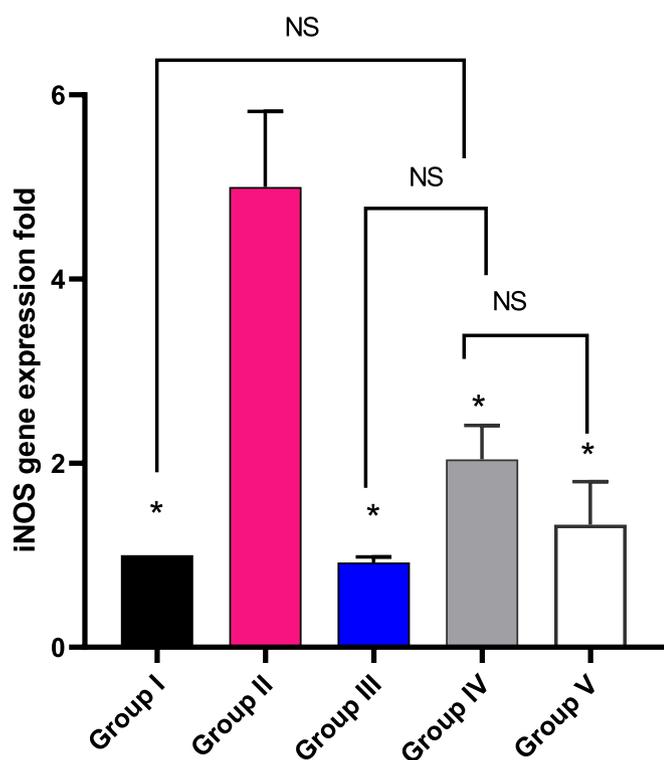


Fig. 14. Impact of treatment with EMME on the gene expression of iNOS. The single asterisk represents a significant difference ($p < 0.05$) in comparison with group II. The abbreviation NS means non-significant difference ($p > 0.05$) in comparison with group IV.

Conceptualization, Investigation, Visualization, Methodology, Data curation, Writing – original draft. **Fatma A. Mokhtar**: Investigation, Methodology, Data curation, Writing – original draft. **Reem Binsuwaidan**: Methodology, Resources, Funding acquisition. **Nashwah G.M. Attallah**: Resources, Investigation, Data curation. **Sally Abdallah Mostafa**: Methodology, Resources, Writing – original draft. **Ehssan Moglad**: Methodology, Resources, Writing – review & editing. **Sarah Ibrahim**: Methodology, Investigation, Data curation. **Omnia Momtaz Al-Fakhrany**: Methodology, Investigation, Data curation, Writing – original draft. **Duaa Eliwa**: Methodology, Resources, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Table S1. Sequences of the primers, Table S2. Top 20 biological KEGG pathways of genes related to inflammations among the studied data set, Figure S1. The total ion chromatogram of detected metabolites of *E. millii* ethanol extract in negative ion mode.

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