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# Investigation of phytochemical contents, *in vitro* antioxidant and antibacterial behavior and *in vivo* anti-inflammatory potential of *Ecballium elaterium* methanol fruits extract

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## Abstract

*Ecballium elaterium* species are mostly used as therapeutic agents and food ingredient. The current work was designed to investigate phytochemical contents, antioxidant, antibacterial, and anti-inflammatory properties of methanol fruits extract of *Ecballium elaterium*. Good antioxidant activity was observed with IC<sub>50</sub> values of 156 ± 4 and 377 ± 6 µg/mL for DPPH and ABTS, respectively, and EC<sub>50</sub> of 126 ± 4 µg/mL for FRAP assays, which is related with their richness in total phenolic, flavonoid and condensed tannins contents. The results of antibacterial activity showed the effectiveness of methanol extract against *Bacillus cereus* with value of inhibition zone diameter of 15 ± 0 mm and a MIC and MBC values of 6 ± 0 and 12 ± 0 mg/mL, respectively. The *in vivo* anti-inflammatory effects have been also studied by carrageenan induced rat paw edema assay and the results revealed that a dose of 75 mg/kg induced a significant inhibition of 66.4% at 2 h. FT-IR spectral data justified the presence of biological functional groups such as —OH, C—H, C—O, C—C and C=O. These results highlighted the potential using of *Ecballium elaterium* fruits extract as natural antimicrobial, antioxidant and anti-inflammatory agents for food applications and for the pharmaceutical industry.

**Keywords:** *Ecballium elaterium* methanol fruits extract; phytochemical contents; FT-IR analysis; *in vitro* antioxidant and antibacterial behavior; *in vivo* anti-inflammatory potential.

**Practical Application:** *Ecballium elaterium* is a medicinal plant which possess wide spectrum of different biological activities.

## 1 Introduction

Nowadays, increasing in prevalence of multiple drug resistance implies the development of new synthetic antibacterial, antioxidative and anti-inflammatory drugs from alternative sources (Papadoupoulo et al., 2005). Therefore, there is a renewed interest in natural substances containing phytoconstituents that exhibited several biological properties. They are used in health to prevent oxidative deterioration and some chronic diseases (Tel-Çayan et al., 2015). The development of phagocytes and the production of non-free radicals (H<sub>2</sub>O<sub>2</sub>) and radicals of O<sub>2</sub> and OH species induced many inflammatory disorders which caused many chronic diseases (Zeng et al., 2017). Medicinal plant therapy was well known for their richness in anti-inflammatory substances. In fact, herbal extracts and their major phytochemical compounds possess a good anti-inflammatory potential (Lee et al., 2011).

*Ecballium elaterium* (Cucurbitaceae) is known as a squirting cucumber, perennial herb that is largely abundant in Mediterranean region. Traditionally, this plant has been used for the treatment of fever, cancer, sinusitis, jaundice, constipation, hypertension and rhinosinusitis (Mazokopakis et al., 2009). The fruit juice was known for its antipyretic and analgesic potential. The roots

and fruits extracts of this plant has been used as a curative. In Tunisia, the plant is widely consumed as infusion and used for the treatment of sinusitis and rheumatic (Bizid et al., 2014).

This work was designed to evaluate the total phenolic, flavonoid, and condensed tannin of methanol extract of *E. elaterium* fruits as well as the identification by FT-IR of the main active functional groups, and to evaluate its *in vitro* antioxidant and antibacterial properties. It also aimed to determine for the first time the *in vivo* anti-inflammatory activity of methanol extract by carrageenan induced rat paw edema assay with histopathological and biochemical analysis.

## 2 Materials and methods

### 2.1 Plant collection and preparation of extract

The fruits of *E. elaterium* (L.) were collected in May 2015 from plants grown in the Sidi Bouzid province of Tunisia (latitude 35°02'17", longitude 9°29'05"; elevation: 332 m). After collection, the preparation of extract followed the same method as done by Yilmaz et al. (2017).

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## 2.2 Total phenolic, total flavonoid, and tannin contents

The phytochemical contents of methanol fruits extract were investigated according to the same protocol done by Mrabti et al. (2017). Tannin contents of extract were determined by the same test as used by Sun et al. (1998). Each test was performed thrice.

## 2.3 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of methanol fruits extract were recorded with at room temperature on a Universal ATR Sampling Accessory infrared spectrophotometer. Dried paste of extract was loaded on the sample chamber of FT-IR spectrophotometer and scanned with a scan range from 550 to 4000  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ .

## 2.4 Antioxidant activities

### DPPH and FRAP assays

Measurements followed the same method as done by Amessis-Ouchemoukh et al. (2017).

### ABTS test

In this assay we followed the same protocol as described by Re et al. (1999). Trolox was used as a reference.

## 2.5 Screening of antibacterial activity

The antibacterial activity of extract was tested against Gram positive bacteria *Bacillus subtilis* JN 934392, *Bacillus cereus* JN 934390, *Staphylococcus aureus* ATCC 6538 and Gram negative bacteria *Escherichia coli* ATCC 25922, *Salmonella enteritidis*, and *Klebsiella pneumoniae*. The determination of the inhibition zone diameter, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were based on the same method used by Mzid et al. (2017).

## 2.6 In vivo anti-inflammatory activity

### Carrageenan-induced acute inflammatory model

Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay as ascribed by Olajide et al. (2000) and Owoyele et al. (2004).

### Biochemical evaluation and histopathological analysis

The biochemical and histopathological evaluation has been done using the same protocol of Saoudi & El Feki (2012) and (Sadeghi et al., 2011), respectively.

## 2.7 Statistical analysis

The results were analyzed by SPSS, ANOVA variance and Tukey test for multiple comparisons. Differences were considered statistically significant at  $p < 0.05$ .

# 3 Results and discussion

## 3.1 Phytochemical contents

The results indicated that phenolic, flavonoid and condensed tannin contents in methanol extract were found to be  $93 \pm 2$  mg GAE/g,  $7 \pm 1$  mg QE/g and  $1 \pm 0$  mg CE/g,

respectively. These phytochemicals play a versatile role in curing many diseases. Therefore, we attempted to estimate the biological active functional groups in methanol *E. elaterium* extract of by FT-IR method.

## 3.2 FT-IR spectral analysis of methanol extract

FT-IR spectral analysis data for the methanol extract (Figure 1) revealed the existence of multiple biological active functional groups. For the higher wavenumbers, the absorption band at 3350.4  $\text{cm}^{-1}$  was attributed to —OH stretching vibration specific of phenolic compounds which indicate hydroxyl groups exist in extract, while the peaks at 2924.3 and 2850.7  $\text{cm}^{-1}$  were due to  $\text{CH}_2$  anti-symmetric stretch of methyl groups mainly from lipids. The absorption peaks around 1746.1 and 1635.1  $\text{cm}^{-1}$  are assigned to the stretching vibration of carbonyl group and to ring C—C stretch of phenyl, respectively. The presence of peak at 1372.66  $\text{cm}^{-1}$  respectively, is due to the in-plane C—O stretching vibration combined with the ring stretch of phenyl. Between 1200 and 1050  $\text{cm}^{-1}$ , one can observe a stretching absorption band of C—O for phenols. The “shoulder” peak at 1160.0  $\text{cm}^{-1}$  is from carbohydrate, whereas the absorptions bands at 1055.4 and 984.0  $\text{cm}^{-1}$  are derived from the vibrational frequency of the  $\text{CH}_2\text{OH}$  groups of carbohydrates.

## 3.3 Antioxidant activity

DPPH radical scavenging assay: The antioxidant potential of methanol fruits extract of *E. elaterium* was estimated in terms of  $\text{IC}_{50}$  (i.e., the concentration required to scavenge 50% of the initial DPPH radicals). Five concentrations (50, 100, 200, 400 and 500  $\mu\text{g/mL}$ ) were used and the % inhibition and  $\text{IC}_{50}$  values were presented in Table 1. We observed that scavenging capacity strongly enhanced with concentration in the range of 50–500  $\mu\text{g/mL}$  and the results were statistically significant ( $p < 0.05$ ).

In this assay, methanol fruit extract maybe participate as hydrogen-donating. Our results revealed significant correlations between phenolic compounds and antioxidant properties in methanol fruit extract and DPPH scavenging, in agreement with Daffodil et al. (2012) because of their redox properties and to

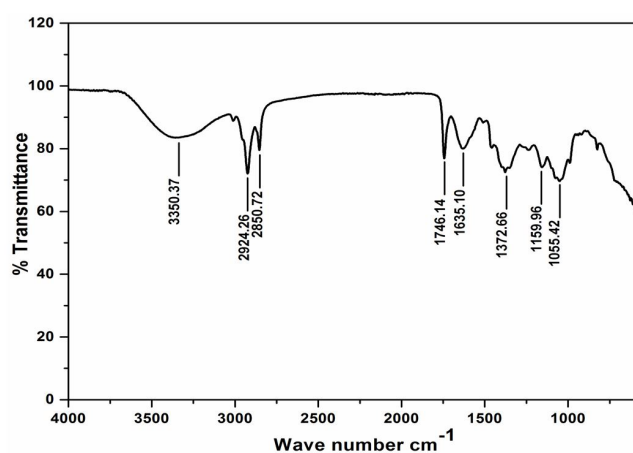


Figure 1. FT-IR spectra of methanol fruits extract of *E. elaterium*.

the existence of free OH groups that act as donating hydrogen and therefore to remove the extra electron (Lobo et al., 2010).

**ABTS radical scavenging assay:** ABTS method is widely used to estimate antioxidant activity in foods and biological systems. The scavenging ability of methanol fruits extract of *E. elaterium* was expressed in terms of percentage of inhibition and  $IC_{50}$  values (Table 1). The results were dose dependent manner and statistically significant ( $p < 0.05$ ).

The scavenging ability of  $ABTS^{+}$  could presumably related to the richness of extract in phenolics compounds, which can act as electron donors by reacting with free radicals. Hagerman et al. (1998) reported that tannins are powerful compounds for soaking free radicals ( $ABTS^{+}$ ). Higher scavenging antiradical effect with ABTS method may be due to the higher contents of polyphenolics in this extract.

**Ferric reducing power:** As depicted in Table 1, reducing capacity of the same extract significantly ( $p < 0.05$ ) increased with increasing in concentration (50–400  $\mu\text{g/mL}$ ). The obtained results suggest that *E. elaterium* may have important applications in the

future as a good source of natural antioxidants for plant-based food products.

### 3.4 Antibacterial activity

The results depicted in Table 2 showed that methanol fruit extract were more effective against *Bacillus cereus* with an inhibition zone diameter of  $15 \pm 0$  mm, MIC of  $6 \pm 0$  mg/mL, and MBC of  $12 \pm 0$  mg/mL. In the same way, the extract showed an interesting activity against *S. enteritidis* with inhibition zone diameter, MIC, and MBC values of  $12 \pm 0$  mm,  $12 \pm 0$  and  $25 \pm 0$  mg/mL, respectively. While the other bacteria showed moderate sensitivity toward the tested extract with inhibition zone diameter ranging from  $8 \pm 1$  to  $9 \pm 1$  mm.

Sasmakov et al. (2012) study justified that methanol extract of *E. elaterium* had weaker activity against *S. aureus* (ATCC 29213) with inhibition zone diameter of  $8 \pm 0$  mm, and negative activity against *B. subtilis* (ATCC 6059) and *E. coli* (ATCC 25922), these results are different to our study. In agreement with the report of Chamandi et al. (2015), an extract is bacteriostatic

**Table 1.** The inhibitory effects of methanol fruits extract of *E. elaterium* on DPPH, ABTS scavenging activities and ferric reducing power assay.

DPPH scavenging activity (percentage inhibition %)						
Test samples	Concentrations (μg/mL)					
	50	100	200	400	500	IC <sub>50</sub> (μg/mL)
Methanol extract	21 ± 1 <sup>e</sup>	36 ± 1 <sup>d</sup>	56 ± 1 <sup>c</sup>	61 ± 2 <sup>b</sup>	63 ± 0 <sup>a</sup>	156 ± 4
BHT	76 ± 2 <sup>d</sup>	87 ± 0 <sup>c</sup>	89 ± 1 <sup>b</sup>	90 ± 1 <sup>b</sup>	92 ± 1 <sup>a</sup>	30 ± 1
Ascorbic acid	61 ± 1 <sup>e</sup>	74 ± 2 <sup>d</sup>	81 ± 1 <sup>c</sup>	85 ± 1 <sup>b</sup>	88 ± 1 <sup>a</sup>	39 ± 1
ABTS scavenging activity (percentage inhibition %)						
Test samples	Concentrations (μg/mL)					
	50	100	200	400	500	IC <sub>50</sub> (μg/mL)
Methanol extract	11 ± 1 <sup>e</sup>	19 ± 1 <sup>d</sup>	31 ± 1 <sup>c</sup>	52 ± 1 <sup>b</sup>	59 ± 1 <sup>a</sup>	377 ± 6
Trolox	17 ± 1 <sup>e</sup>	28 ± 1 <sup>d</sup>	47 ± 1 <sup>c</sup>	81 ± 1 <sup>b</sup>	95 ± 0 <sup>a</sup>	218 ± 4
Ferric reducing power assay (absorbance value at 700 nm)						
Test samples	Concentrations (μg/mL)					
	50	100	200	400	EC <sub>50</sub> (μg/mL)	
Methanol extract	0.2 ± 0.0 <sup>d</sup>	0.4 ± 0.0 <sup>c</sup>	0.6 ± 0.02 <sup>b</sup>	0.8 ± 0.1 <sup>a</sup>	126 ± 4	
Ascorbic acid	0.4 ± 0.0 <sup>d</sup>	0.7 ± 0.0 <sup>c</sup>	1.2 ± 0.0 <sup>b</sup>	1.8 ± 0.0 <sup>a</sup>	67 ± 3	

Values are averages  $\pm$  standard deviation of triplicate analysis. Means with different superscript letters (a–e) in the same row indicate significant ( $p < 0.05$ ) difference among concentrations tested. Results are ranked in ascending order;  $a > b > c > d > e$ .

**Table 2.** Antibacterial activity, MIC and MBC values of methanol fruits extract of *E. elaterium* and chloramphenicol.

Bacterial strains	Methanol extract				Antibiotic
	Inhibition zone diameter (mm)	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Chloramphenicol (15 $\mu\text{g/well}$ )
<b>Gram-positive</b>					
<i>Staphylococcus aureus</i>	—	—	—	—	$16 \pm 0$
<i>Bacillus cereus</i>	$15 \pm 0$	$6 \pm 0$	$12 \pm 0$	4	$26 \pm 1$
<i>Bacillus subtilis</i>	$9 \pm 1$	$6 \pm 0$	$12 \pm 0$	4	$24 \pm 0$
<b>Gram-negative</b>					
<i>Klebsiella pneumonia</i>	$8 \pm 1$	$12 \pm 0$	$25 \pm 0$	2	$22 \pm 1$
<i>Salmonella enteritidis</i>	$12 \pm 0$	$12 \pm 0$	$25 \pm 0$	2	$16 \pm 0$
<i>Escherichia coli</i>	—	—	—	—	$23 \pm 0$

Data are mean  $\pm$  standard deviation of three individual determinations. (—): Not detected.

when  $MBC/MIC \geq 4$  and bactericidal if  $MBC/MIC \leq 4$ . Based on this study, it can be concluded that methanol extract of *E. elaterium* have a bacteriostatic and bactericidal effect on Gram-positive and Gram-negative, respectively. The differential sensitivity of Gram-positive and Gram-negative bacteria to plant extract may be explained by the morphological differences between the microorganisms. In this study, the Gram-negative isolates were less susceptible to the plant extracts than the Gram-positive bacterial isolates. In fact, the strongly resistance of Gram-negative bacteria was accredited to the complexity of the double membrane including cell envelope, that be expressed by lipoprotein and lipopolysaccharide, and which plays the role of a barrier to the antibacterial substances, contrasted to the single membrane structure of Gram-positive bacteria. A shown, antibacterial behavior may be related to the richness of this extract in bioactive components. This superior antibacterial property may be related to the higher contents of polyphenolics in this extract. Our results clearly demonstrate the possibility to use *E. elaterium* fruits as a source of new antibacterial agents of multiple-barrier food preservation systems.

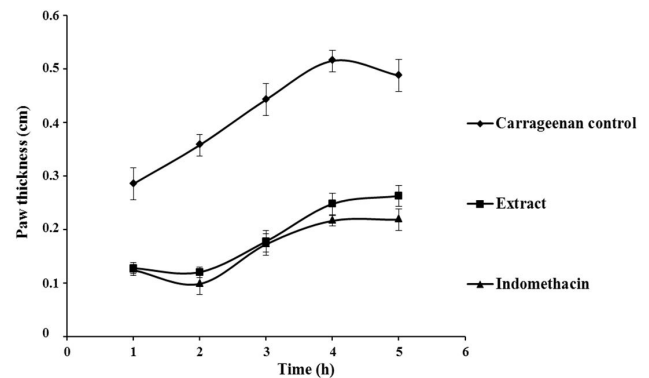
### 3.5 Anti-inflammatory studies

The results showed that pre-treatment of rats with the extract (75 mg/kg) showed significantly reduced paw edema from 1 h until 5 h (Figure 2 and Table 3). The highest inhibition of edema obtained by the extract with 75 mg/kg dose was 66.4% at 2 h. The extract compared effectively with standard drug indomethacin (10 mg/kg) used in this study, which produced a peak inhibition of edema (72.6%) at 2 h.

Our results showed that the methanol fruits extract of *E. elaterium* exerted higher anti-inflammatory potential. The extract also inhibited the inflammatory processes of intraperitoneal injection administration at low dose. Herbals can to synthesize a large variety of chemical compounds with beneficial effects on health. It has been reported that certain flavonoids have an inhibitory effect against histamine release in mast cells (Weng et al., 2012).

By their anti-inflammatory potential, flavonoids can interact directly with the prostaglandins system like the non-steroidal anti-inflammatory drugs (Aro et al., 2016). So, this activity was correlated to the richness of this extract in flavonoids and tannins and therefore, justified clearly that *E. elaterium* fruits may be used for the treatment of chronic inflammation.

**Biochemical evaluation:** The administration of carrageenan in rats induced a significantly increase in AST, ALT, ALP, Ferritine and CK levels as compared to control vehicle rats. The methanol fruits extract of *E. elaterium* and indomethacin reduced significantly the elevated levels of serum AST, ALT, ALP, Ferritine and CK as compared to untreated rats and carrageenan rats alone (Table 4). The above results are consistent with the reports of Sengar et al. (2015) which verified that AST, ALT and ALP enzymes were enhanced in both acute and sub-chronic inflammation. Sengar et al. (2015) confirmed that extract of *Jasminum sambac* at 400 mg/kg (p.o) considerably restored the AST and ALT level but without alteration in ALP level.



**Figure 2.** Effects of methanol fruits extract of *E. elaterium* on carrageenan-induced rat paw edema model. Values are mean  $\pm$  standard deviation.

**Table 3.** Effects of methanol fruits extract of *E. elaterium* and indomethacin of on carrageenan-induced hind paw edema in rats.

Group	Dose (mg/kg)	Time after carrageenan injection				
		1 h	2 h	3 h	4 h	5 h
		Mean paw diameter (% inhibition)				
Control	—	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Indomethacin	10	0.1 ± 0.0*** (56.5)	0.1 ± 0.0*** (72.6)	0.2 ± 0.0*** (61.1)	0.2 ± 0.0*** (58.0)	0.2 ± 0.0*** (55.3)
Extract	75	0.1 ± 0.0*** (55.3)	0.1 ± 0.0*** (66.4)	0.2 ± 0.0 *** (59.9)	0.2 ± 0.0*** (51.9)	0.3 ± 0.1*** (46.1)

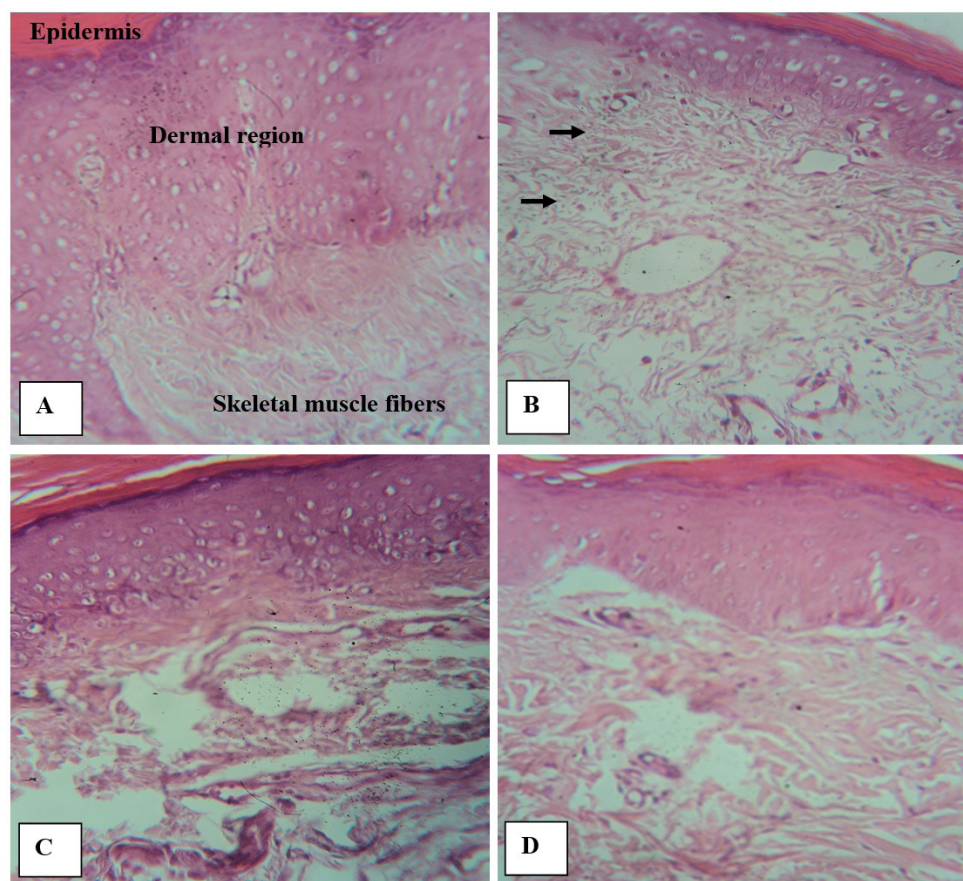
Significant differences: Values are mean  $\pm$  standard error of mean (n = 6). \*\*\*  $p < 0.001$  vs. vehicle control.

**Table 4.** Serum biochemistry changes of control and rats treated with carrageenan, carrageenan + extract and carrageenan + Indomethacin.

Treatment and parameters	Experimental groups			
	Vehicle control	Carrageenan control	Carr+extract	Carr+Ind
AST (UI/L)	241 $\pm$ 3	306 $\pm$ 52*	259 $\pm$ 24 <sup>#</sup>	299 $\pm$ 41*
ALT (UI/L)	36 $\pm$ 2	47 $\pm$ 9*	38 $\pm$ 5 <sup>#</sup>	43 $\pm$ 2*
ALP (UI/L)	216 $\pm$ 6	233 $\pm$ 32	238 $\pm$ 8*	227 $\pm$ 21
Ferritine (ng/ml)	1.5 $\pm$ 0.1	1.7 $\pm$ 0.1**	1.6 $\pm$ 0.1 <sup>#</sup>	1.5 $\pm$ 0.0 <sup>#</sup>
CK (UI/L)	10527 $\pm$ 754	13032 $\pm$ 3448*	10907 $\pm$ 1486	11421 $\pm$ 1263

Significant differences: Values are mean  $\pm$  standard error of mean (n = 6). \*  $p < 0.05$ ; \*\*  $p < 0.01$  vs. vehicle control; Carrageenan group vs Carr+extract group and Carr+Ind; <sup>#</sup>  $p < 0.05$ .





**Figure 3.** Histopathological examinations on carrageenan induced edema paw tissues after treatments. Histological evaluation was performed by hematoxylin and eosin staining. A: normal rats showed the normal appearance of epidermis and dermis without any lesion; B: carrageenan injected paw skin in control group (vehicle-treated) showed edema and accumulation of infiltrating inflammatory cells (arrow); C: carrageenan injected paw skin of rats treated with methanol fruits extract of *E. elaterium* showed that edema and inflammatory cells accumulation reduced; D: carrageenan injected paw skin of rats treated with indomethacin showed that the migration of inflammatory cells reduced.

**Histopathological findings:** Histological investigation was examined by hematoxylin and eosin staining. High infiltration damage due to accumulation of infiltrating inflammatory cells was observed for Group B. However, the vehicle control Group A was devoid in inflammation and the infiltration damage was moderated in the methanol fruits extract of *E. elaterium* and indomethacin drug treatment groups compared to high level of infiltrating inflammatory cells in Group B (Figure 3). Similar studies were conducted previously by Maleki et al. (2001) which demonstrated the protective effects of the hydroalcoholic extract of *Stachys inflata* aerial parts of against carrageenan induced histopathological paw tissue and the results found in this study also support the facts. These findings provide the potential use of *E. elaterium* as a good source of anti-inflammatory agents, or food supplement for prevention of chronic diseases.

#### 4 Conclusions

The results suggest that *E. elaterium* fruits extract contain a wide range of phenolics, flavonoids and condensed tannins contents, associated with potent pharmacological activities. From this study it can be suggested that the tested *E. elaterium* fruits extract have potential antioxidant behavior. The results

show also that the extract exhibited a higher antibacterial activity which can be used as a potent antibacterial agent against *Bacillus cereus*. Besides, the extract was found to possess considerable anti-inflammatory properties at dose tested, and could have significant effect against chronic inflammation. We confirmed in this study the useful of *E. elaterium* as a promising plant for food industry. The methanol fruits extract of *E. elaterium* needed more investigation in order to identify the major active compounds and to demonstrate their mode of action.

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