**Egyptian Journal of Chemistry**

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

# Potent Therapeutic Effects of Polysaccharides Isolated From Some Edible Plant Wastes: Characterization and Bioactivities

**Salma A. Elsawi [1], Hanan F. Ali [2], Amany A. Sleem [3], Amal M. El-Feky [1*], Marwa M. Elbatanony [1]**

[1] *Pharmacognosy Department, National Research Centre, 33El Buhouth St., Dokki, Cairo, P.O.12622 (ID: 60014618), Egypt.*

[2] *Therapeutic Chemistry Department, National Research Centre, 33El Buhouth St., Dokki, Cairo, P.O.12622 (ID: 60014618), Egypt.*

[3] *Pharmacology Department, National Research Centre, 33El Buhouth St., Dokki, Cairo, P.O.12622 (ID: 60014618), Egypt*

**Abstract**

Polysaccharides have various effects and exhibit several bioactivities that possess an enormous influence on healthcare due to their therapeutic beneficial and comparatively low toxicity. The study is focusing in investigation of the chemical structure of the isolated polysaccharides from wastes of *Punica granatum*, *Musa acuminate*, and *Brassica oleracea*, beside to validation of the analgesic, anti-inflammatory, antipyretic and antiulcer activities. Moreover, very expressive biological activities were obviously presented by all the isolated polysaccharides. The highest analgesic and anti-inflammatory activities exhibited by polysaccharides of *B. oleracea* outer leaves (62.98 and 81.69 %), followed by that of *P. granatum* peels (53.31 and 78.17%), and that of *M. acuminate* peels (50.50 and 76.05%), while the antipyretic activity was showed nearly the same results for both *M. acuminate* and *B. oleracea* polysaccharides (6.59 and 6.35%), then that of *P. granatum* peels (5.30%). On the other, the antiulcer activities of polysaccharides of different wastes showed corrective action of inflammatory markers including tumor necrosis factor -α (TNF-α), interleukin -6,8 (IL-6 &8), myeloperoxidase (MPO) as well as lowering oxidative stress, improved lesion count which lead to ameliorations in histopathological picture of stomach. In conclusion, the polysaccharide isolated from Musa *acuminate* showed the most potent therapeutic effect in the majority of the biomarkers under investigation.

**Key words:** *Punica granatum*, *Musa acuminate*, *Brassica oleracea*, polysaccharides , antiulcer, antipyretic

## 1. Introduction

Peptic ulcer is a medical codition encompassing ulcers in the digestive system in both stomach and/or duodenum. Stress and spicy food developed these types of ulcers. some of its most common causes are *H. pylori* and some drug reactions especially non steroidal anti-inflammatory drugs are considered as the major responsible factors [1].

Losing weight, dysappetite, bloating, pain and vomiting in addition to gastrointestinal bleeding are considered as the main diagnosis for peptic ulcers [2].

The mechanism of ethanol-causes stomach damage is differed , including the depletion mucus content in gastric tissue, perturbations in blood flow of mucosal layer as well as disruption of mucosal cell. It has been reported that alcohol induced great gastrointestinal damage begins with injury of microvascular cells leads to increase cell membranes permeability and formation of edema. Al Rashdi et al. [3] stated that post-gastric ethanol administration leads to production of endothelin-1 in the systemic bloodstream. Moreover, a decrease in the secretion of biocarbonate ($HCO3^-$) levels and the production of mucus, ethanol causes necrotic lesions of the gastric mucosa and leads to activation of TNFα and mitogen-activated protein kinases (MAPKs) [4]. Furthermore, an initiation of apoptosis leading to cell death, and increment in lipid peroxidation [5]. The elevation in

*Corresponding author e-mail: (Amal M. El-Feky) ammelfeky@hotmail.com

**Receive Date:** 02 August 2022, **Revise Date:** 17 August 2022, **Accept Date:** 21 August 2022,
DOI: 10.21608/EJCHEM.2022.153901.6660

oxygenated free radicals causes significant alteration at the cellular level, leading to membrane damage and cell death [6].

Cytokines have a vital effect in the mucosal immune system regulation and hence they are crucial for mucosal defense. Various proinflammatory cytokines such as: IL-1β, IL-2, IL-6, IL-8 and TNF-α have dominant pathogenetic markers in peptic ulcer. Upon inflammation of the gastric mucosa, neutrophils and mononuclear cells infiltration occurs resulting in production of various proinflammatory cytokines [7]. IL-1 plays an important role in reduction of the gastroduodenal damage severity [8].

Nitic oxide (NO) proved to have an essential role in gastrointestinal mucosal defense and the mucosal injury pathogenesis. Also, NO may affect the tone of muscles as well as endocrine and exocrine secretions. On the other hand, it decreases the gastric secretion through prohibition of histamine release [9].

Prostaglandins (PGs) are considered as one of the fatty acid precursors originated from arachidonic acid through cylooxygenase. Konturek et al. [10] reported that prostaglandins have their cytoprotective effects via enhancing both mucus and bicarbonate secretions leading to stabilizing the mucosal blood flow. Nevertheless, they cause augmention of the epithelial cells resistance to damage resulted from cytotoxins. Moreover, (PGs) suppress the production of the leukocytes which proved their significance in protection of the inflamed mucosa. Prostaglandin E2 (PGE2) has a powerful inhibitor of histamine release and of tumor necrosis factor-α from mast cells of both intestinal and peritoneal mucosa which in turn suppress the production of reactive oxygen species by neutrophils [11].

Polysaccharides belong to a structurally various class of macro-molecules which exist in polymers form attached together by glycosidic bonds [12]. They have attracted important consideration in pharmacology and natural chemistry fields due to their significant bioactivities being effective as antioxidants [13], glucosidase inhibitors, anticoagulants [14; 15], prebiotics, anticaners, immunobiological agents [16], immuno-inflammatory and antiviral activities[17]. Most plant based polysaccharides are quite safe comparing to synthetic drugs [18]. In the field of pharmaceutical industries, polysaccharides are exceedingly used as thickener, water retention agent, suspending agent, binders and film former.

Pomegranate peels (*Punica granatum*) belongs to family Punicaceae. It is one of the oldest famous plants worldwide [19]. It is a nutritious food source rich in phytochemical compounds. The peel is wealthy of phenolics, flavonoids and other phytoconstituents [20] with antioxidant and antimicrobial capacities, and could be utilized as bio-preservative in food and neutraceutical industries [21]. Banana peels (*Musa acuminate*) belongs to family Musaceae, phytochemical characterization of banana peel refers to the existence of reducing sugars and carbohydrates, therefore it has been utilized as binding, thickening and stabilizing agent [22]. Also, cauliflower (*Brassica oleracea*), family Brassicaceae, is one of the dominant food crops which contain several phytoconstituents which are essential to human health such as vitamins (C, B and K), soluble fibers and carbohydrates [23].

The current study targeted to isolate, and structurally elucidation of the water-soluble polysaccharide fractions from peels of *P. granatum*, *M. acuminate*, and outer leaves of *B. oleracea* as analgesic, anti-inflammatory, antipyretic and antiulcer agents. The obtained results can be preliminary used for detailed studies of the structure-activity correlation and further applications of the polysaccharides.

## 2. MATERIALS AND METHODS

### 2.1.Plant material

Fresh peels of *P. granatum* and *M. acuminate*, outer leaves of *Brassica oleracea* were collected in September and October 2020, from local markets, Egypt. Specimen of the plants was identified by Prof. Dr. Gamal Farag. The collected plant wastes were dried in shades, separately powdered and kept in tightly-closed containers.

### 2.2.Chemicals

All solvents used in the study are of pure analytical grade and distilled water. The anti-inflammatory and analgesic slandered drug for ulcer induction in this study was indomethacin (EPICO, Egypt). For acute inflammation induction in rats, carrageenan (Sigma Co.) was used while, acetic acid was used for pain induction. Absolute ethanol (99%) was purchased from Multipharma, Egypt, indomethacin (IND) was purchased from Nile Co. for pharmaceuticals. Enzyme linked immunosorbent assay (ELISA kit, Elabscience, USA) was used for measuring of tumor necrosis factor-alpha (TNF-α) and IL-1β. All other chemicals were of analytical grade and bought from commercial sources.

### 2.3.Phytochemical study

#### 2.3.1. Extraction, isolation, and purification of polysaccharides (cold and hot extraction method)

The powdered plant peels of *P. granatum* and *M. acuminate*, in addition to outer leaves of *Brassica oleracea* (250g) were extracted with cold and boiling distilled water three times (2 L X 3) and filtered. The aqueous extract was evaporated at 45°C on a rota-vapor under reduced pressure, and then acidified with

HCl to pH 3.5. The polysaccharides were obtained by precipitation with four volumes of absolute ethanol overnight. The precipitate was collected and washed with absolute ethanol, and acetone till free from chloride ions **[24]**. The polysaccharides were then vigorously stirred in absolute ethanol, filtered and dried in vacuum desiccators over anhydrous calcium chloride **[25]**. The attained polysaccharides gave negative result to both potassium hydroxide reaction and gel formation test which assures absence of pectin **[26&27].**

### 2.3.2. Analysis of chemical composition of the isolated polysaccharides

#### 2.3.2. 1. UV–Visible Spectroscopy

UV–Visible Spectrophotometer double beam UVD–3500 spectrophotometer, Labomed, Inc., Visible Spectrophotometer, Shimadzu UV 240 (PIN 204-58000) (Japane) was used to measure the UV absorption of the isolated polysaccharides under 190–400 nm **[28].**

#### 2.3.2. 2. Fourier transform infrared (FT-IR) spectroscopy

Room temperature Fourier transform infrared, FTIR spectra of the samples in KBr pellets were measured using MB154S Bomem spectrometer, Quebec, Canada equipment, in the range 4000–500 $cm^{-1}$ by averaging 20 scans at a maximum resolution of 4 $cm^{-1}$ is used for the qualitative analysis of the functional groups in the isolated polysaccharides.

#### 2.3.2. 3. The $^{1}$HNMR spectroscopy

$^{1}$HNMR data were recorded on an FT-NMR spectrometer using DMSO as a solvent at 400 MHz (BRUKER. Germany).

#### 2.3.2.4. GLC Analysis

Derivatization of sugar Hydrolyzate was performed according to **[29].** One $\mu$l was injected in GLC for analysis using column **(**ZB-170, 30mx 0.25x025$\mu$m, 14% cyanopropyl phenyl methyl**).** Helium was carrier gas at a flow rate 1.2ml/min under pressure10.6psi with velocity and the following temperature program Initial temperature: 150ºC and initial time: 2 min with rate: 7c/min. Final temperature: 200ºCfinal time: 20 min using detector FID, 270ºC with air flow 450mL/min and $H_2$ flow 40mL/min.

### 2.4.Biological study

#### 2.4.1. Animals

Male Wistar albino rats (140–150 g) were obtained from Animal House Lab, National Research Centre (NRC). They were acclimatized for one week before the starting of the experiment (adaptation period).Then, housed with a well-ventilation (20°C) for twelve hours cycle. The standard diet consisted of commercial rat chow (El-Nile Company). Animals were cared according to the Ethical Committee guide lines of NRC, Giza, Egypt for animal experiments ( ethics number approval no:16/193). Prior to the study, rats were fasted for 24 hours and to avoid variations due to diurnal rhythms of putative regulators of gastric functions, all experiments were performed at the same time of the day.

#### 2.4.2. Acute toxicity

To determine the oral $LD_{50}$, the different extracts dissolved or suspended in water were administered to Waster rats (4 rats /group) as a single oral dose of 100- 500 mg /kg body weight via gavage. The control group was treated with water vehicle. The animals were observed during 24 h investigation for signs of morbidity or mortality **[30].**

#### 2.4.3. Analgesic activity (Writhing test)

The analgesic activity was measured using acetic acid induced writhing method on Swiss male albino mice (20-25g). Adult male albino mice (30) were divided to five groups, each of six animals, as follows: First group; mice received 1 ml saline (control). The second, third, and fourth groups; mice had 100 mg/kg b.wt. of the isolated polysaccharides of peels of *P. granatum*, *M. acuminate*, and outer leaves of *B. oleracea*, respectively. Fifth group; mice received 4 mg/kg b.wt. of indomethacin as a standard drug. After 30 min. acetic acid (0.6%) was intraperitoneally injected (0.2 ml/mice). Then, each mouse was kept separately in an individual clear plastic observed chamber and the total number of writhes/ 30 min. was counted **[31].**

The number of writhing and stretching was determined in comparing against the standard drug. The percentage of protection was calculated using the following ratio:

% of protection = Control mean- treated mean ×100/Control mean.

**2.4.4. Acute anti-inflammatory activity**

Rat paw edema method is used for evaluation of acute anti-inflammatory activity **[32].** Carrageenan is used as to cause paw edema irritation. Typically, the isolated polysaccharides are tested for acute anti-inflammatory by measuring their capacity for decreasing and/or preventing the carrageenan-induced paw swelling. Indomethacin alleviates the paw swelling in a dose-dependent manner. Adult male albino rats (42) were divided into seven groups, each of six, as follows: First group; animals received 1 ml saline (control). Second, third, fourth, fifth and sixth groups; rats received 100 mg/kg b.wt. of the isolated polysaccharides of peels of *P. granatum*, *M. acuminate*, and outer leaves of *B. oleracea*, respectively. Seventh group received 20 mg/kg b.wt. of indomethacin. One hour later to oral administration, all groups were sub-plantary injected with 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. After 4 hours of drug administration, the animals were sacrificed. Both hind paws were, separately, excised and weighed. The oedema percentage was calculated according to the following equation:

% Oedema = (Wt. of right paw –Wt. of left paw) x 100/ Wt. of left paw

% Oedema inhibition = (Mc-Mt) x 100/ Mc

Where**:** Mc**=** the mean edema in control group, Mt **=** the mean edema in the drug- treated group.

**2.4.5. Antipyretic activity[33]**

Male albino rats (30) were divided into 5 groups each of 6. The normal rectal temperature was measured before beginning of the experiment. Yeast suspension (44%) was intramuscularly injected (1ml /100 g b.wt.) to induce pyrexia. After 18 hours, the rectal temperature was remeasured for all groups to act as the base line of elevated body temperature, to which the antipyretic activity will be attributed. A single oral dose of each tested polysaccharide, paracetamol or saline has been administrated.

**2.4.6. Antiulcer activity**

**2.4.6. 1. Biochemical analysis**

ELISA methods were applied for determination of TNF-α ,IL-6 ,IL-8 and MPO. Optical densities were measured at 450 nm. The samples concentration was calculated from the standard curve, multiplied by the dilution factor and was expressed as mean standard deviation. Certain enzyme markers; succinate dehydrogenase (SDH) (mitochondria marker) **[34],** lactate dehydrogenase (LDH) (cytoplasm marker) **[35]** and acid phosphatase (AP) (lysosome marker) **[36]** have been in the gastric tissue. All groups were subjected for stomach oxidative stress markers; glutathione (GSH) **[37]**, malondialdehyde (MDA) **[38]** and glutathione peroxidase (GPX) assays**[39]**. Gastric total protein was determined as well**[38].**

**2.4.6. 2. Experimental design**

Fifty four male Wister rats were divided into nine groups of six each as follows: Groups 1 Control male rats were daily orally administered 1 ml of 0.9% normal saline solution. Groups 2-4: rate were administered 200 mg /kg b.wt. of different extracts. Group 5: Gastric ulcer was induced in rats (ulcerogenic group) with 1 mL of absolute ethanol orally **[40]**. One hour after administration of ethanol animals were intraperitoneally anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg). The Dissection of stomachs were performed, then they were opened along greater curvature for evaluation of the number of gastric lesions **[41].** A piece of the stomach was dissected and homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was stored at −80 °C until measurement of antioxidant, oxidative stress and inflammatory markers. Group 6-8,ulcerated rats were treated with 200 mg /kg b.wt. of different polysaccharides for consecutive 14 days . Group 9 ulcerated rats were treated with Ranitidine (50 mg/kg) as a standard drug for 14 days .At the end of 14 days rats were anesthetized and the stomach was dissected.

**2.4.6.3. Analysis of gastric juice**

The gastric contents were collected, measured and centrifuged at 5000 rpm to remove solid debris. The supernatant of the gastric juice (1 ml) was diluted in 10 ml of distilled water. Two drops of phenolphthalein were added and titrated with 0.1M sodium hydroxide until a permanent pink color appeared. The alkali amount was recorded and the acidity was calculated using the formula below **[42].**

Acidity = volum of NaOH x normality of NaOH X 100 /0.1 mEq/l

**2.4.6.4. Evaluation of gastric mucosal lesion**

The collected stomachs were washed with normal saline and examined using microscope for

measuring perforation and ulcer lesions number. The lesions were expressed in the terms of ulcer index (U.I) **[43**; **44].**

**2.5. Histopathological Examination**

Histopathological stomach variation were recorded and scored as following, no changes (0), mild (1), moderate (2) and severe (3) changes, the grading was determined by percentage as follows: <30% changes (mild change), <30% – 50% (moderate change), and >50% (severe change) **[45].** The U.I for each group was calculated by finding the mean score for all rats in the group. Therapeutic index (T.I) was calculated using this equation **[46].**

T.I = UI of ethanol group – UI of treatment group /UI of ethanol group X 100.

On the other hand, stomach tissues were fixed in 10% buffered formalin solution and were embedded in paraffin. Sections were deparaffinized and stained with hematoxylin and eosin (H&E) for further histopathological assessment **[47].**

**2.6.Statistical analysis**

one way analysis of variance, ANOVA (SPSS) was performed using computer program combined with co state computer program, whereas different letters are significant at $p \leq 0.05$. The quantitative data were expressed as mean±SD. All statistical comparisons were carried out using one-way ANOVA (analysis of variance) test followed by Tukey's test. P-values of 0.05 were considered to be statistically significant.

**3.Results**

3.1. Monosaccharide analysis and characterization of polysaccharides

The isolated polysaccharides were pale grey in color, odorless, soluble in water and not soluble in ethanol, ether and chloroform. The yields of the total polysaccharides of the studied plant wastes (peels of *P. granatum* and *M. acuminate*, in addition to outer leaves of *B. oleracea*) by the cold and hot methods are listed in table (1).

3.1.1. UV-visible spectra analysis

The UV-visible spectra analysis illustrated the presence of six polysaccharides having an absorption peak at 190 nm, which is attributed to the polysaccharides. on the other hand, no absorption bands were observed at 260 and 280 nm signifying that the polysaccharides not contain nucleic acids, proteins, or polypeptides.

**Table (1): polysaccharide yields of the studied plant wastes**

| Powdered plants | % of the polysaccharide | |
|---|---|---|
| | Cold method | Hot method |
| *P. granatum* peels | 7.13 | 2.50 |
| *M.* acuminate peels | 8.70 | 4.60 |
| *B. oleracea* outer leaves | 8.40 | 3.76 |

3.1.2. FT-IR analysis

FT-IR data for the cold and hot polysaccharide hydrolyzates of the studied plants are illustrated in Figs. (1-3).The spectra obtained between 400-4000 $cm^{-1}$ can be used to analyze the structural features of polysaccharides, including glycosidic bonds and functional groups **Kóňa and Tvaroška, 2009 [48]** discussed the absorptions of polysaccharides' functional groups from many previous studies. The spectra of the isolated polysaccharides indicate the possible functional groups. The absorption peaks range [I] (3400 - 3500 $cm^{-1}$) are characteristic for –OH stretching in the sugar residues. Peaks at [II] (2863-2940 $cm^{-1}$) are attributed to C-H. Moreover, the absorbance at (1650-1750 $cm^{-1}$) are associated with the carboxyl group (C=O stretch), the bands developed at [III] (1640-1655 $cm^{-1}$) were due to the bending vibrations of HOH, absorption peaks: (1315-1420 $cm^{-1}$) could be represented to carboxylic acid (C-O stretch) and [IV] (1100-1250 $cm^{-1}$) for double bond conjugation. Peaks at (1025-1100 $cm^{-1}$) represented the bending vibration of hydroxyl groups; [V] (710-790 $cm^{-1}$ may be associated with the glycosidic linkage**.**

Comparative investigation of the isolated polysaccharides of both *P. granatum*, and *M. acuminate* peels beside the outer leaves of *B. oleracea* were performed using GLC analysis. Results are illustrated in Tables (2& 3).

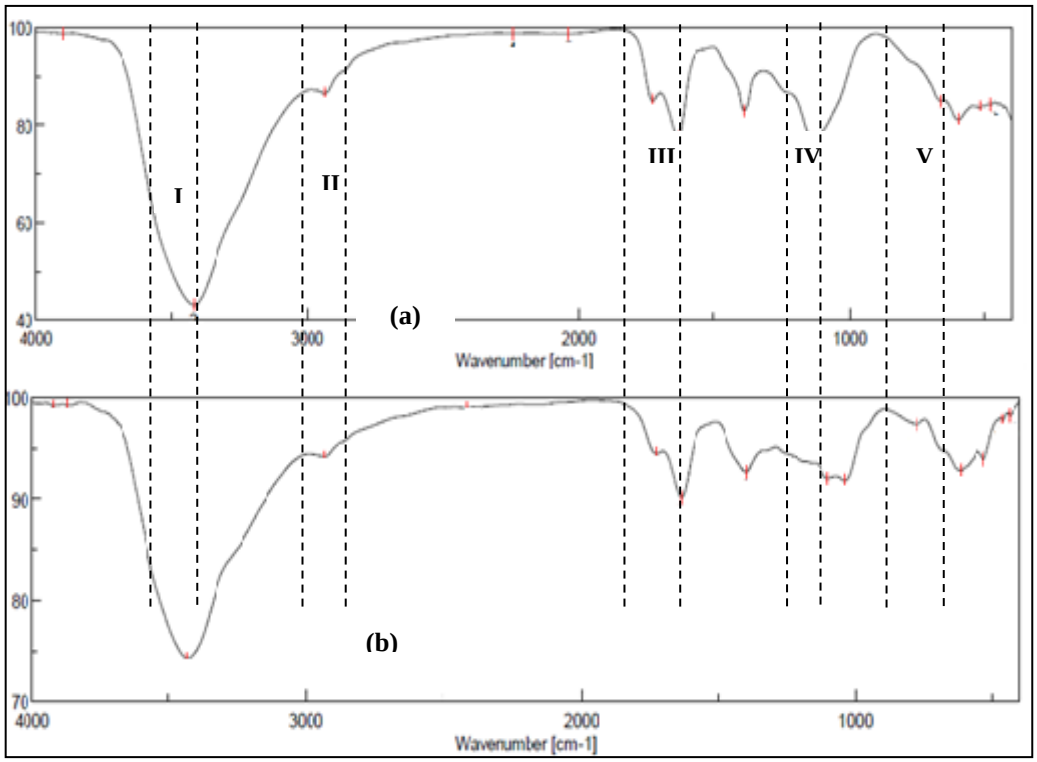


**Fig.1: FT-IR spectra of cold (a) and hot (b) Polysaccharide hydrolyzates of *P. granatum***

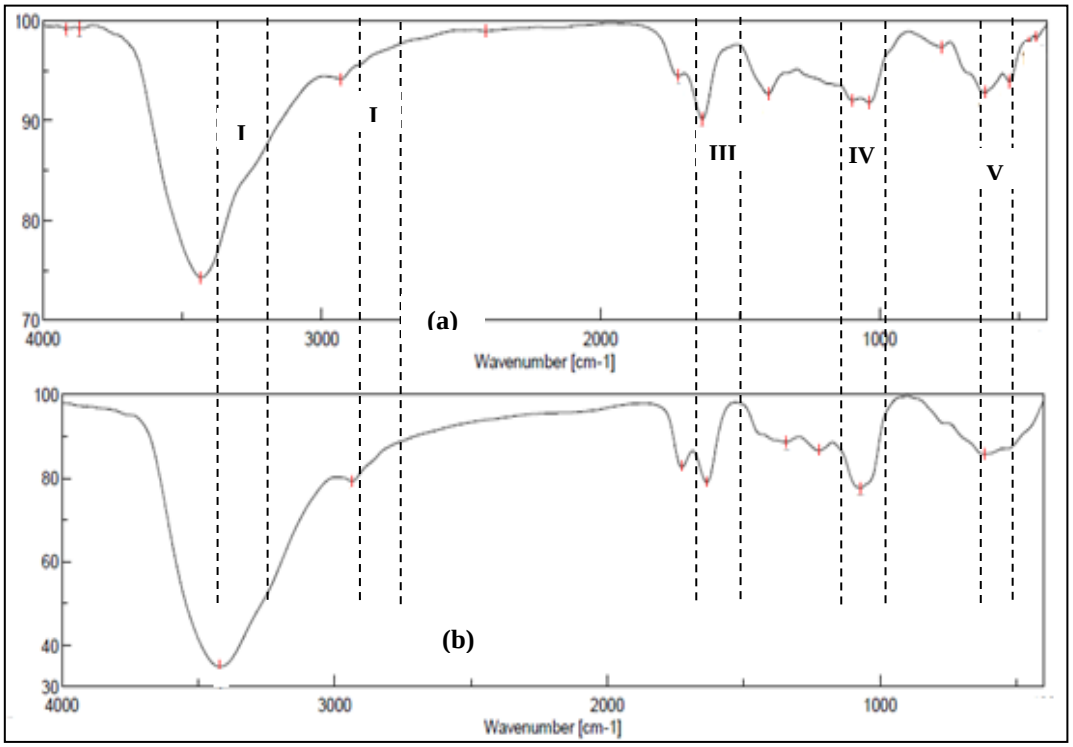


Fig.2: FT-IR spectra of cold (a) and hot (b) Polysaccharide hydrolyzates *M. accuminate*

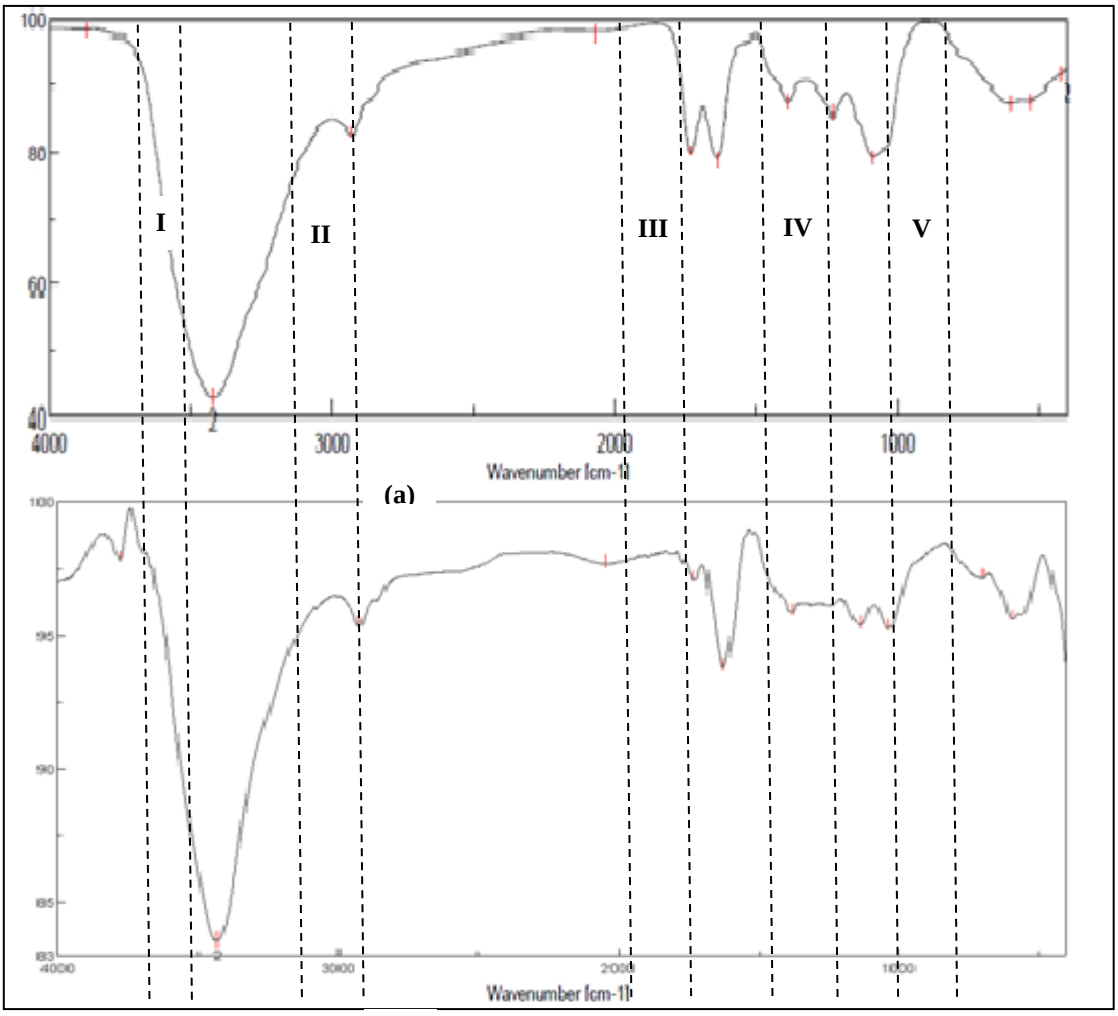


**Fig. 3: FT-IR spectra of ( (b) ) and hot (b) Polysaccharide hydrolyzates *B. oleracea***

3.1.3. [1]HNMR spectral analysis

[1]HNMR results showed a set of wide signals between δ 3.40 and 4.95 ppm was characteristic of typical polysaccharide signals **[49].** Upon literature, the signal peaks in the range of 3.40 and 4.55 ppm were recognized to be $CH_2$-O and CH-O groups of the sugar rings **[50].** Moreover, the signals between 4.55 and 5.57 suggested that the three polysaccharide samples contained α-configuration of saccharide residues **[51]**which is in accordance with the results of the FT-IR spectra.

**3.2.GLC Analysis**

Tables (2 &3) showed that the cold polysaccharide hydrolyzate of *P. granatum* contains six sugars representing (76.81%) of the total sugar content in which arabinose was the major identified sugar (22.59%). While, the polysaccharide hydrolyzate of M. acuminate powder contains eight sugars representing (71.89%) of which glucose was the major sugar (45.29%) of the total sugar content. Finally, *B. oleracea* total sugar content yielded 57.43% (six sugars) of which glucose was the major sugar (29.25%). Concerning the sugars identified from the hot polysaccharide hydrolyzate of investigated plants; *P. granatum* contains eight sugars representing (71.30%) of the total sugar content in which glucose was the major identified sugar (40.18%). While, the polysaccharide hydrolyzate of M. acuminate contains six sugars representing (64.96%) of which glucose was the major sugar (30.72%) of the total sugar content. Finally, *B. oleracea* total sugar content yielded 76.34% (six sugars) of which mixture of Mannose + galactose was the major sugar (26.43%).

Table (2): Carbohydrates identified by GLC analysis of the cold polysaccharide hydrolyzates of the studied plants

| Authentic sugars | $R_t$ (min.) | Relative percentage (%) of total cold polysaccharide hydrolyzate | | |
|---|---|---|---|---|
| | | ***P. granatum*** | ***M. acuminate*** | ***B. oleracea*** |
| Arabinose | 5.59 | 22.59 | 02.80 | ---- |
| Xylose | 6.032 | 03.14 | 0.72 | 1.74 |
| Ribose | 6.190 | 00.59 | 0.218 | 0.52 |
| Rhamnose | 7.436 | 09.12 | 01.12 | 4.50 |
| Mannitol | 10.10 | --- | 16.30 | 7.28 |
| Fructose | 11.45 | ---- | 01.50 | ---- |
| Mannose +galactose | 13.66 | 22.18 | 03.94 | 14.14 |
| Glucose | 14.31 | 19.19 | 45.29 | 29.25 |
| **Total identified sugars** | | **76.81%** | **71.89%** | **57.43%** |

$R_t$: Retention time

Table (3): Carbohydrates identified by GLC analysis of the hot polysaccharide hydrolyzates of the studied plants

| Authentic sugars | Rt (min.) | Relative percentage (%) f total cold polysaccharide hydrolyz | | |
|---|---|---|---|---|
| | | ***P. granatum*** | ***M. acuminate*** | ***B. oleracea*** |
| Arabinose | 5.98 | 05.10 | 6.90 | 21.93 |
| Xylose | 6.20 | 01.20 | 2.54 | 2.54 |
| Ribose | 6.68 | 0.235 | 0.90 | 0.28 |
| Rhamnose | 7.42 | 2.51 | 4.19 | 11.34 |
| Mannitol | 9.94 | 11.73 | --- | ---- |
| Sorbitol | 10.41 | 1.049 | ---- | ----- |
| Fructose | 11.45 | ---- | ---- | ----- |
| Mannose + galactose | 13.65 | 09.31 | 19.71 | 26.43 |
| Glucose | 14.54 | 40.18 | 30.72 | 13.82 |
| **Total identified sugars** | | **71.30%** | **64.96%** | **76.34%** |

## 4. Bioactivities of the isolated polysaccharides

A significant analgesic, anti-inflammatory, antipyretic and antiulcer activities were exhibited by all isolated polysaccharides as indicated by number of abdominal constrictions, inhibition of rat paw edema weight caused by carrageenan and body temperature change. The highest analgesic and anti-inflammatory activities exhibited by polysaccharides of *B. oleracea* outer leaves (62.98 and 81.69 %), followed by that of *P. granatum* peels (53.31 and 78.17%), and that of *M. acuminate* peels (50.50 and 76.05%), while the antipyretic activity was showed nearly the same results for polysaccharides of *M. acuminate* and *B. oleracea* peels (6.59 and 6.35%), then that of *P. granatum* peels (5.30%). On the other hand, the severity of the lesions observed in the antiulcer study decreased in the order of polysaccharides of *B. oleracea*, and *P. granatum* peels recording the same percentage 96.74%, then *M. acuminate* peels 95.12% (Tables 4, 5, 6 and 7).

Table (4): Analgesic effect of the isolated polysaccharides of P. granatum, M. acuminate, and B. oleracea on number of abdominal constrictions and acetic acid in mice (n=6)

| Group | Dose mg/kg b.wt. | Number of abd. Constriction | % inhibition |
|---|---|---|---|
| Control | 1 ml saline | 49.7 ± 1.1 | ----- |
| *P. granatum* peels | 100 | 23.2 ± 0.4* | 53.31 |
| *M.* acuminate peels | 100 | 24.6 ± 0.3* | 50.50 |
| *B. oleracea* outer leaves | 100 | 18.4 ± 0.1* | 62.98 |
| Indomethacin | 20 | 16.2 ± 0.2* | 67.40 |

* Statistically significant from control group at P< 0.01

### 4.1. Acute toxicity study

Acute toxicity study revealed the polysaccharides of different plants are safe up to 500 mg/kg.body weight and no signs of toxicity or abnormality in food behavior for 48 h.

### 4.2. Anti-ulcer activity

Reduction in score lesion was noticed in all treated groups compared to ulerogenic rats. *P. granatum*, *M. acuminate* and *B. oleracea*polysaccharidesshowed lower score lesion than standard drug (Table 7).

Table (5):Anti-inflammatory activity of the isolated polysaccharides of *P.* granatum, *M. acuminate*, and *B. oleracea* and indomethacin drug in male albino rats (n=6)

| | **Zero** | **1 h** | | **2 h** | | **3 h** | | **4 h** | | % of inhibition after 4h |
|---|---|---|---|---|---|---|---|---|---|---|
| | Paw diameter (mm) | Paw diameter (mm) | Oedema thickness (mm) | Paw diameter (mm) | Oed. Thickness (mm) | Paw diameter (mm) | Oed. thickness (mm) | Paw diameter (mm) | Oed. thickness (mm) | |
| **Control** | 3.49 ±0.04 | 4.51 ±0.06* | 1.02 | 4.62 ±0.03* | 1.13 | 4.72±0.04* | 1.23 | 4.91±1.42* | 1.42 | ---- |
| *P. granatum* peels | 3.48 ±0.02 | 4.24 ±0.04* | 0.76 | 4.06 ±0.04* | 0.58 | 3.92±0.04* | 0.44 | 3.82±0.02* | 0.34 | 76.05 |
| *M.* acuminate peels | 3.51 ±0.01 | 4.22 ±0.05* | 0.71 | 4.02 ±0.06* | 0.51 | 3.97±0.03* | 0.46 | 3.82±0.03* | 0.31 | 78.17 |
| *B. oleracea* outer leaves | 3.46 ±0.03 | 4.09 ±0.03* | 0.63 | 3.91 ±0.5* | 0.45 | 3.84±0.02* | 0.38 | 3.72±0.01* | 0.26 | 81.69 |
| **Indomethacin** | 3.52±0.08 | 4.06±0.02* | 0.54 | 3.88 ±0.06* | 0.36 | 3.82 ±0.01* | 0.30 | 3.73±0.01* | 0.21 | 85.21 |

* Significantly different from zero time at $p < 0.05$.

Table (6): Antipyretic effect of the isolated polysaccharides of P. granatum, M. acuminate, and B. oleracea and paracetamol drug in male albino rats (n=6)

| **Group** | **Dose in mg/ kg b.wt.** | **Body temperature change** | | | | |
|---|---|---|---|---|---|---|
| | | **Induced rise in temperature** | **One hour** | | **Two hours** | |
| | | | **Mean ± S.E.** | **% of change** | **Mean ± S.E.** | **% of change** |
| **Control** | 1 ml saline | 38.9 ± 0.03 | 39.2 ± 0.04* | --- | 39.4 ± 0.04* | ---- |
| *P. granatum* peels | 100 | 39.4 ± 0.04 | 38.1 ± 0.03* | 2.80 | 37.3 ± 0.02* | 5.30 |
| *M.* acuminate peels | 100 | 39.5 ± 0.03 | 37.9 ± 0.01* | 3.31 | 36.8 ± 0.03* | 6.59 |
| *B. oleracea* outer leaves | 100 | 39.3 ± 0.04 | 37.8 ± 0.04* | 3.57 | 36.9 ± 0.03* | 6.35 |
| **Paracetamol** | 20 | 39.6 ± 0.05 | 37.4 ± 0.02* | 4.59 | 36.5 ± 0.02* | 7.36 |

* P< 0.01 corresponding induced rise in temperature of the tested group

Table (7): Scoring of histopathological alterations in stomach of ulcerogenic and all treated groups by *P. granatum*, *M. acuminate* and *B. oleracea*polysaccharides

| **Lesions** | **G1-G5** | **Ulcerative rats** | **Ulcergenic rats + *P. granatum*** | **Ulcergenic rats + *M. acuminate*** | **Ulcergenic rats + *B. oleracea*** | **Ulcergenic rats +Standard drug** |
|---|---|---|---|---|---|---|
| Edema in submucosa | 0 | 3 | 1 | 1 | 1 | 2 |
| Mononuclear inflammatory cells infiltration in submucosa. | 0 | 2 | 1 | 1 | 1 | 2 |
| Congestion of submucosal blood vessels | 0 | 2 | 1 | 1 | 1 | 1 |
| Necrosis of mucosa | 0 | 3 | 0 | 0 | 0 | 0 |
| Mononuclear inflammatory cells infiltration in mucosa | 0 | 3 | 1 | 0 | 0 | 2 |
| Mucosal hemorrhage | 0 | 2 | 0 | 0 | 0 | 0 |
| Hyperplasia of goblet cells | 0 | 3 | 1 | 1 | 1 | 2 |

The score system was designed as: score 0 = absence of the lesion in all rats of the group (n= 6), score 1= (<30%), score 2= (<30% – 50%), score 3= (>50%).

**4.3. Acidity of gastric juice**

Table 8 revealed significantly reduction in acidity post treatment of ulcerated rats with different extracts compared to untreated ulcerated group. *M. acuminate* polysaccharides showed the most promising treated group, where it showed insignificant difference compared to control and standard drug group , followed by *P. granatum* and finally *B. oleracea*. Referring to ulcer lesion count , significant reduction in ulcer lesion was noticed post treatment of ulcerogenic rats with different extracts compared to untreated ulcerated rats accompanied with reduction in the grade of lesion score . *M. acuminate* showed the highest reduction followed by *M. acuminate* and then *B. oleracea* compared to standard drug .

Table (9)revealed significant highest U.I. for ulcerated rats compared to control group. Treated rats with different polysaccharides of wastes showed significantly lowered U.I, where *M. acuminate* and *B. oleracea* showed the significant lowest U.I, then *P. granatum*. Also, *M. acuminate* and *B. oleracea* showed the same higher T.I (78.33%) , followed by *P. granatum* which recorded T.I 7 2.00%

Table 8: Effect of different *P. granatum*, *M. acuminate* and *B. oleracea*, on average acidity of gastric juice and ulcer lesion counts of ulcerogenic rats

| **Groups** | **Acidity mEq/l** | **Ulcer lesion counts** |
|---|---|---|
| **Control** | **9.12±0.23a** | **-** |
| ***P. granatum*** | **9.00±0.11a** | **-** |
| ***M. acuminate*** | **10.00±0.11a** | **-** |
| ***B. oleracea*** | **9.62±0.41a** | **-** |
| **Ethanol group(ulcerated)** | **55.00 ±4.13b** | **7.65a ± 0.87a** |
| **Treatment *P. granatum*** | **17.12±2.13c** | **3.23**± 0.65b |
| **Treatment *M. acuminate*** | **12.10±1.00a** | **1.55**± 0.11 c |
| **Treatment *B. oleracea*** | **19.00±1.45c** | **2.32**± 0.23 d |
| **Treatment standard drug** | **11.56±1.00a** | **1.54**± 0.31 c |

Data represent the mean ± S.D of observation from 6 rats. Statistical analysis is carried out using SPSS (one way analysis of variance, ANOVA) , computer program combined with co state computer program, where different letter are significant at p ≤0.05**.**

**Table 9: Effect of *P. granatum*, *M. acuminate* and *B. oleracea* polysaccharides on U.I and T.I in ethanol induced peptic ulcer in rats.**

| Groups | U.I | T.I % |
|---|---|---|
| Control | 0.00 ±0.00$^{a}$ | -- |
| *P. granatum* | 0.00±0.00$^{a}$ | - |
| *M. acuminate* | 0.00±0.00$^{a}$ | - |
| *B. oleracea* | 0.00±0.00$^{a}$ | - |
| Ethanol group(ulcerated)group | 3.00 ±0.21$^{b}$ | - |
| Treatment *P. granatum* | 0.82±0.03$^{c}$ | 72.00 |
| Treatment *M. acuminate* | 0.65±0.06$^{d}$ | 78.33 |
| Treatment *B. oleracea* | 0.65±0.05$^{e}$ | 78.33 |
| Treatment standard drug | 1.50±0.20$^{d}$ | 50 |

Data represent the mean ± S.D of observation from 6 rats. Different letters are significant at p ≤0.05.

With respect to inflammatory markers (Table 10), TNF-α increased only in the ulcerated group significantly upon comparing to the control group (P < 0.05) while the rats treated with different extracts recorded lesser amount of TNF-α comparing to the ulcerated group (Table 4). another inflammatory marker, IL-6 levels in the ulcer groups were higher than that in the control group. While, treated groups with different extracts showed significant lower level. Another inflammatory marker IL-8 level in the gastric tissue significantly elevated in the ulcerated group as compared to control group but the treated rats with different polysaccharides preserved the IL-8 amount in the levels of control tissues (Table 10). In all the detected inflammatory markers, *M. acuminate* polysacchardies showed the most potent effects. Myeloperoxidase enzyme (MPO) activity, that recognized as a significant indicator for the oxidative stress, was extensively increased in the gastric tissues of ulcer group untreated and treated with extracts in comparison to the control group (P < 0.05). The elevation value in the untreated ulcer group was four times greater than that recognized in the treated ulcer group.

measuring the LPO enzyme level in the gastric tissue. Consequently, the ulcerogenic rats showed a considerable increase by comparing with the control group (P < 0.05). Polysaccharides treatment ameliorated ulcer-induced elevation in gastric LPO levels (Table 11). Ulcerogenesis produced remarkable decrease in gastric GSH levels by comparing with the

control group ($P < 0.05$) while the ulcer group administered with different polysaccharides had dramatically ameliorated levels of GSH ($P < 0.05$), approximately twice –three times higher than the ulcerated group (Table 11). Nevertheless, the amount of an antioxidant enzyme GPx was remarkably reduced in the ulcer group compared to the control group ($P < 0.05$). In addition, GPx amount in ulcer-induced rats treated with different polysaccharides was twice to three times higher than the amount of ulcerated rats (Table 11). From all manipulated biomarkers *M. acuminate* polysaccharides showed the most potent results.

Concerning to the mucosal enzymes, insignificant changes were observed in SDH, LDH and AP after administration of normal rats with polysaccharides extracts. Ulcerogenic rats showed significant decrease in LDH and SDH by64.27and 57.98 %, respectively, while a significant increase in AP levels by329.60 % was observed as compared to control group. Treatment with different polysaccharides or standard drug showed significant increase in LDH level and improved by 21.46, 30.47and 26.82%, respectively for *P. granatum*, *M. acuminate* and *B. oleracea*compared with reference drug (36.05%). An increase in SDH was noticed in therapeutic -ulcerogenic rats with amelioration percentage reached to 9.96, 18.13 and 12.73%, respectively for *P. granatum*, *M. acuminate* and *B. oleracea*compared to standard drug(25.93%) Contradictory, the reduction in AP level was observed for different treated groups with improving values amounted 160.98, 171.64 and122.93%, respectively for *P. granatum*, *M. acuminate* and *B. oleracea* compared to standard drug (224.89%) (Table 12 ).

**Table (10) : Effect of *P. granatum*, *M. acuminate* and *B. oleracea* polysaccharides on inflammatory markers in ulcerated rats**

| | Inflammatory markers | | | oxidative stress |
|---|---|---|---|---|
| **Groups/ Markers** | TNF-a (pg/ml) | IL-6(pg/ml) | IL-8(pg/ml) | MPO(ng/ml) |
| **Control** | 109.80 ± 7.30$^{a}$ | 129.2 ± 6.82$^{a}$ | 4.10 ± 0.25$^{a}$ | 5.80 ±0.40$^{a}$ |
| **Normal +** *P. granatum* | 102.00 ± 9.20$^{a}$ | 119.00±10.00$^{a}$ | 4.90 ± 0.98$^{a}$ | 5.00 ±0.20$^{a}$ |
| **Normal +** *M. acuminate* | 100.48 ± 6.19$^{a}$ | 120.00±7.30$^{a}$ | 5.18 ± 0.87$^{a}$ | 4.70 ±0.60$^{a}$ |
| **Normal +** *B. oleracea* | 100.00±6.99$^{a}$ | 133.00±8.90$^{a}$ | 4.09 ± 0.45$^{a}$ | 5.00 ±0.88$^{a}$ |
| **Ulceration** | 1200.00 ± 90.40$^{b}$ | 219.00±10.00$^{b}$ | 80.00 ± 6.25$^{b}$ | 47.89 ±3.60$^{b}$ |
| **Ulcerated ++** *P. granatum* | 210.00±10.11$^{c}$ | 156.00 ±8.87$^{c}$ | 7.00 ± 0.50$^{a}$ | 12.80 ±1.50$^{c}$ |
| **Ulcerated +** *M. acuminate* | 187.00±8.90$^{d}$ | 145.00±9.90$^{c}$ | 6.00 ± 0.55$^{a}$ | 11.00 ±1.00$^{c}$ |
| **Ulcerated +** *B. oleracea* | 265.00±9.00$^{e}$ | 155.00±7.94$^{c}$ | 12.00 ± 1.05$^{c}$ | 12.00 ±1.00$^{c}$ |
| **Ulcerated +standard drug** | 108.00±9.00$^{a}$ | 150.00±10.11$^{c}$ | 6.i0 ± 0.20a | 8.00 ±0.95$^{a}$ |

Data represent the mean ± S.D of observation from 6 rats. Different letters are significant at $p \leq 0.05$. TNF-a: Tumor necrosis factor a, IL-6: Interleukin 6, IL-8: Interleukin 8, MPO: Myeloperoxidase,

**Table (11 ): Effect of *P. granatum*, *M. acuminate* and *B. oleracea*polysaccharides on oxidative stress and antioxidant biomarkers in ulcerogenic rats**

| **Groups / Markers** | **MDA (umole/mg protein )** | **GSH ((umole/mg protein )** | **GPX (umole/ug protein)** |
|---|---|---|---|
| **Control** | 4.89± 0.40$^{a}$ | 20.50 ± 1.12$^{a}$ | 11.10 ± 1.05$^{a}$ |
| **Normal + *P. granatum*** | 4.00 ±**0**.20$^{a}$ | 22.00±1.00$^{a}$ | 14.00 ± 1.18$^{a}$ |
| **Normal + *M. acuminate*** | 4.40 ± 0.60 $^{a}$ | 23.00±1.30$^{a}$ | 15.00 ± 1.11$^{a}$ |
| **Normal + *B. oleracea*** | 4.30±0.99$^{a}$ | 20.00±1.90$^{a}$ | 14.10 ± 1.40$^{a}$ |
| **Ulceration** | 16.00 ± 1.40$^{b}$ | 4.00±0.20$^{b}$ | 3.00 ± 0.20$^{b}$ |
| **Ulcerated + *P. granatum*** | 10.00±0.91$^{c}$ | 11.00 ±1.07$^{c}$ | **7.00** ± 0.10$^{c}$ |
| **Ulcerated ++ *M. acuminate*** | 8.00±0.90$^{d}$ | 14.00±1.00$^{c}$ | **9.00** ± 0.95$^{d}$ |
| **Ulcerated + *B. oleracea*** | 10.00±0.20$^{c}$ | 9.00±1.04$^{d}$ | 7.00 ± 0.15$^{c}$ |
| **Ulcerated +standard drug** | 8.00±0.67$^{d}$ | 15.00±1.10$^{c}$ | 9.i0 ± 0.50$^{d}$ |

Data represent the mean ± S.D of observation from 6 rats. Different letters are significant at $p \leq 0.05$.

Table 12: Effect of *P. granatum*, *M. acuminate* and *B. oleracea*polysaccharides on some mucosal of cell organelles marker enzymes of ulcerogenic rats

| Groups / Parameters | Control | Controls -Treated | | | Ulcerative rats | ulcerative-Treated rats | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ***P. granatum*** | ***M. acuminate*** | ***B. oleracea*** | | ***P. granatum*** | ***M. acuminate*** | ***B. oleracea*** | **Standard drug** |
| LDH Mean± SD | 9.32±0.45[a] | 8.50±2.42[b] | 8.55±0.87[b] | 8.56±0.87[b] | 3.33±0.88[e] | 5.33±1.75[d] | 6.17±1.47[c] | 5.83±1.47[d] | 6.69±0.43[c] |
| % [a] | | 8.80 | 8.26 | 8.15 | 64.27 | 42.81 | 33.80 | 37.45 | 28.22 |
| % [b] | | | | | | 21.46 | 30.47 | 26.82 | 36.05 |
| SDH Mean± SD | 8.33±0.32[a] | 7.51±0.30[b] | 7.33±1.88[b] | 7.42±1.69[b] | 3.50±0.33[e] | 4.33±0.7[d] | 5.01±0.53[c] | 4.56±1.90d | 5.66±0.98[c] |
| %[a] | | 9.84 | 12.00 | 10.92 | 57.98 | 48.01 | 39.86 | 45.26 | 32.05 |
| %[b] | | | | | | 9.96 | 18.13 | 12.73 | 25.93 |
| AP Mean± SD | 11.25±0.41[d] | 11.89±1.34[d] | 11.67±1.16[d] | 11.98±0.83[d] | 48.33±1.16[a] | 30.22±1.22[b] | 29.02±1.75[c] | 34.50±1.87[b] | 23.03±1.72[c] |
| % [a] | | 5.69 | 3.73 | 6.49 | 329.6 | 168.62 | 157.96 | 206.67 | 104.71 |
| % [b] | - | - | - | - | - | 160.98 | 171.64 | 122.93 | 224.89 |

a: % change as compared to normal control rats, % b: % of improvement. Data are means ± SD of ten rats in each group. Data are expressed as Unit/mg protein. Statistical analysis is carried out using SPSS computer program (version,8) coupled with Co-Stat computer program , where unshared letters between groups are the significance value at $P \leq 0.05$.

### 4.4. Stomach histopathological picture

In this study, severe lesions, edema and infiltration of mononuclear inflammatory cells in the sub mucosa, diffuse necrosis in the mucosa (yellow arrow), and edema and infiltration of mononuclear inflammatory cells in the submucosa with obstruction of the submucosal blood vessels (yellow and black arrows) were observed (H and EX100). The *P. granatum* treatment group showed a normal mucosal layer with mild mucosal blood vessel obstruction (arrow) and mild submucosal edema. While treatment of ulcerogenic rats with *M. acuminate* showed normal histological structure of the mucosal layer with slight edema in the submucosa. Furthermore, treatment of ulcerogenic rats with *B. oleracea* revealed normal histological structure of the mucosal layer with mild edema and little mononuclear infiltration in the submucosa. In addition, the standard drug showed a normal mucosal layer with edema in the submucosa (yellow arrow) and a slight congestion of the submucosal blood vessels (Figures 1 and 2).

## 5. Discussion

In the current study, the isolated polysaccharides promising anti-inflammatory activity of the rat paw oedema weight induced by carrageenan. This effect due to reduction of TNF-α and IL1-β secretion induced by a pro-inflammatory agent (LPS) as mentioned by **[52].** No previous studies has directly handled the analgesic and antipyretic effects of the isolated polysaccharides from plant origin so far.

Ethanol is normally used to induce stomach lesions in rodents **[53]**. It is characterized by state of inflammatory cascade, free radicals generation and cell death **[53& 54].**Lesion counts and acidity level are considered two indicativefactors in gastric ulcer post toxins exposure **[55]**. The acidity and the gastric juice levelsare principally dependenton the value of pH, therefore the decrease in the concentration of hydrogen ion in gastric secretion resulting in gastric injury **[56].** These results are in accordance with our results showed significant reduction in PH of gastrictissue and increment in lesion counts which may be attributed to the equilibrium between the formation of free radicals and scavenging ability **[53].** Recently, numerous researche aretargeted to discover novel safe and powerful antiulcer drugs or dietary supplements of herbal sources **[53 & 57]**, **.T**he therapeutic effects of differentpolysaccharides under investigation succeeded to improvethe PH level and lower lesions counts in stomach tissue of ulcerative rats. **[53 & 58]** illustrated that, the materials whichrelieve acidity have the efficacy totreat the gastric lesions and protect the stomach mucosa layer from injury. With respect to the inflammatory mediators, the present results are run in parallel with the study of **[59 & 60]** who revealed that ethanol ingestion up-regulated the inflammatory response as documented by a suppress in the IL-10anti-

inflammatory marker. Moreover, ulcer is surrounded by inflammatory regions which induce the migration of macrophagesand polymorphonuclear cells, resulting in enhance inproduction of pro-inflammatory cytokines from these cells. From these mediators, TNF-a and IL-6 and -8 are known to elevate particularly in ulcer patients with *H. pylori* **[61].**

The present results on oxidative stress, inflammatory mediators and mucosal enzymes clearly indicated severe disturbance and damage which are in accordance with the findings of **[53, 57& 58]**who observed that ethanol enhances gastric juice that leads to increment in oxidative stress associated with reduction in the concentration of GSH ,inhibition in GPX activity , damage in mucosal layer pathological conditions and cellular dysfunctions (Figs1 and 2).

Regarding the mucosal enzymes, SDH is a mitochondria marker enzyme, ethanol induced a decrease in the mitochondrialmembrane potential of stomach that might be due to the cumulative productsof lipid peroxidation and protein oxidation, allowing the release of its enzymes into the cytosol **[62].**These observation ascertained the inhibition activity of SDH enzyme in ulcerogenicgastric tissue **[53].** A positive correlation between ethanol administration and destruction of mucosal cell membranes was observed **[62],** resulting in enzyme leakage**.** This additionally supported the reduction of the enzyme LDH due to its release from the cytoplasmic membrane**.** Additionally, **[58]** found that ethanol ingestion caused severe gastropathy, gastroduodenal ulceration, and damage to lysosomal membranes and leakage of their enzymes. This was consistent with the observed release of AP enzyme. Previously, **Amirov and Trubitsyna (1982) [63]** observed the increase in AP accompanied with an increase in proteolytic activity**,** leading to the release of lysosomal proteases by disrupting the lysosomal membranes.

Regarding to the histopathological examinations and parallel to **Park et al. (2008)[64],** ethanol causes damage in mucosal layer of stomach , rupture of mucosal cell membranes and gastric micro vessels injuries which in turn cause bleeding and necrosis, submucosal edema and epithelial cell damage **[59].** In a good agreement with our results, **Beserra *et al.*(2016) [65],** declared that the antiulcer efficacy by suppressing the formation of gastric lesion. The possible mechanisms involved in the gastroprotective effect of polysaccharides can be explained by their ability to bind to the gastric surface. Mucosa, thus acting as a protective layer, antisecretory activity against gastric juice, as well as increased mucus synthesis to improve and protect the mucosal layer

## Conclusions

The isolated polysaccharides from wastes of *P. granatum*, *M. acuminate*, and *B. oleracea* exhibited significant effects as analgesic, anti-inflammatory, antipyretic and antiulcer activities through suppress inflammatory mediators, improve antioxidant status and raises acidity as well as alleviates lesions and ameliorate histopathological pictures of mucosal layer of stomach. Thus, they could be utilized as dietary supplement in different dosage forms due to their broad margin of safety. This requires the development of phytopharmaceuticals containing the standardized bioactive ingredients after applying clinical studies.

## Acknowledgment

The authors appreciate the funding support of National Research Centre for implementation of this study**.**

## Conflicts of interest

There are no conflicts to declare.

## References

1. Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK. Gastric toxicity and mucosal ulceration induced by oxygen-derived reactive species, protection by melatonin. CurrMol Med,1:501-513 (2001).
2. Kaur Amandeep K , Robin S, Sharma R , Sunil K..Peptic Ulcer: A Review On Etiology And Pathogenesis International Research Journal Of Pharmacy 3(6):34-38 (2012) .
3. Al Rashdi AS , Salama SM , Alkiyumi SS , Abdulla AM , Hadi HA, Abdelwahab S , Taha M M , Hussiani J and Asykin N. Mechanisms of Gastroprotective Effects of Ethanolic Leaf Extract of *Jasminum sambac* against HCl/Ethanol-Induced Gastric Mucosal Injury in Rats.Evid Based Complement Alternat Med. 2012: 786426.doi: 10.1155/2012/786426 .
4. Vidya S, Ramesh A, Alekhya N, Lohitha I. Antiulcer activity of Eugenia Jambolana leaves against Ethanol-induced gastric ulcers in albino rats. IIJPRD:3:106-112 (2018).
5. Mahmood AA, Al-Bayaty FH, Salmah I, Nor Syuhada AB, Harita H, Mughrabi FF.

Enhacement of gastric ulcer of gastric ulcer by areca catechu nut in ethanol induced gastric mucosal injuries in rats. Journal of medicinal plant Research5:2562-2569 (2011).

6. Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK. Antiulcer and in vitro antioxidant activities of Jasminumgrandiflorum L. J. Ethnopharmacol, 110: 464- 470 (2007).
7. Lajoie J , Boily-Larouche G, Juno JA , Kirwan SE and Fowke KR . Mucosal Immune Regulation: Does it Help Thwart HIV? J Clin Cell Immunol 2012, S7 DOI: 10.4172/2155-9899.S7-006 .
8. Wallace Jl and Ma L . Inflammatory Mediators In Gastrointestinal Defense And Injury Experimental Biology And Medicine , 226 (11) :1003-15 (2002). Doi: 10.1177/153537020122601107.
9. Freitas CS, Baggio CH, Da Silva-Santos JE, Rieck L, de Moraes Santos CA, et al. Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of Pfaffiaglomerata Pedersen, Amaranthaceae, in rats. Life Sci., 74:1167-1179 (2004).
10. KonturekS J, KonturekP C, Brzozowski T. Prostaglandins and ulcer heating .Journal of physiology and pharmacology: an official journal of the Polish Physiological Society , 56 Suppl 5(Suppl 5):5-31, (2005).
11. Ricciotti E and FitzGerald GA. Prostaglandins and Inflammation. Arteriosclerosis, Thrombosis, and Vascular BiologyVol. 31, No. 5,2011 31:986–1000.
12. Shi L.. Bioactivities, isolation and purification methods of polysaccharides from natural products: A review. International Journal of Biological Macromolecules 92 (2016) 37–48.
13. Hoang, M.H.; Kim, J.Y.; Ji, H.L.; You, S.G.; Lee, S.J. Antioxidative, hypolipidemic, and anti-inflammatory activities of sulfated polysaccharides from Monostromanitidum. Food Sci. Biotechnol., 24, 199–205 (2015).
14. Li, N.; Mao, W.; Yan, M.; Liu, X.; Xia, Z.; Wang, S.; Xiao, B.; Chen, C.; Zhang, L.; Cao, S. Structural characterization and anticoagulant activity of a sulfated polysaccharide from the green alga Codiumdivaricatum. Carbohydr. Polym., 121, 175–182 (2015).
15. Cai,W.; Xu, H.; Xie, L.; Sun, J.; Sun, T.;Wu, X.; Fu, Q. Purification, characterization and in vitro anticoagulant activity of polysaccharides from Gentianascabra Bunge roots. Carbohydr. Polym., 140, 308–313 (2016).
16. Heiss, W.D. Antioxidant and immunostimulatory activities in vitro of polysaccharides from pomegranate peels. J. Chem. Soc. Pak., 37, 86–91 (2015).
17. Laurienzo, P., Marine polysaccharides in pharmaceutical applications: An overview. Marine Drugs, 8, 2435-2465 (2010) .
18. Zhang, D.; Li, S.; Xiong, Q.; Jiang, C.; Lai, X. Extraction, characterization and biological activities of polysaccharides from Amomumvillosum. Carbohydr. Polym., 95, 114–122 (2013).
19. Elbatanony M.M., Amal M. El-Feky ,Bahaa A. Hemdan , M. Azab El-Liethy. Assessment of the antimicrobial activity of the lipoidal and pigment extracts of *Punicagranatum* L. leaves,*Acta Ecologica Sinica*, 39, 89–94 (2019) .
20. Li, Y., C. Guo, J. Yang, J. Wei, J. Xu and S. Cheng. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry, 96: 254-260 (2006).
21. Rowayshed G., Salama A., Abul-Fadl M., Akila-Hamza S. and Emad A. M. Nutritional and Chemical Evaluation for Pomegranate (*Punicagranatum L.*) Fruit Peel and Seeds Powders by Products Middle East Journal of Applied Sciences, 3(4): 169-179 (2013).
22. Reddy V. R. and Reddy M. R**.** Evaluation of *Musa paradisiaca*(Banana peel) mucilage as pharmaceutical excipient. International journal of pharmaceutical and chemical sciences. 2 (4), (2013).
23. Suganya D, F. Hussain A.M and Kanimozh K. Antibacterial and phtyochemical analysis on *Brassica oleracea*var. *botrytis* Linn.International Journal of Applied and Pure Science and Agriculture 2, (3), (2016).
24. El-Feky A. M., Elbatanony M. M., AboulNaser A. F. and Hamed M. A.A therapeutic insight of carbohydrate and fixed oil from Plantagoovata L. seeds against ketoprofen-induced hepatorenal toxicity in rats. Bulletin of the National Research Centre, 42:28 (2018 a).
25. Karawya M. S., Wassel G. M., Baghdadi H. and Ammar N. M. Polysaccharides and pectins of *Opuntia*, *Tamarindus* and *Cydonia*. PlantaMedica, (40): 68–75(1980).

26. Evans WV (1962) Pharmacognosy, 3rd edn. BalillieneTindall, London.
27. Matz SA (1962) Food Texture. The Avi Publishing Company Inc., West Port, Connecticut
28. El-Feky A. M., Elbatanony M. M., Mounier M. M.Anti-cancer potential of the lipoidal and flavonoidal compounds from *Pisum sativum* and *Vicia faba* peels. Egyptian Journal of Basic and Applied Sciences, 5, 258–264 (2018 b).
29. Kiker R. S and Sawer R. Pearson's Composition and Analysis of Foods, 9th ed., Longman scientific and Technical, Harlow, UK. pp182-235(1991).
30. Desoukey, S.Y.; El Kady, W.M.; Salama, A.A.; Hagag, E.G.; El-Shenawy, S.M.; El-Shanawany, M. Hepatoprotection and antioxidant activity of *azanialongiscapa and G. rigens* with the isolation and quantitative analysis of bioactive metabolites. Int. J. Pharmacogn. Phytochem. Res., 8, 1121-1131 (2016).
31. Koster R., Anderson, M., Beer, E.J., Acetic acid for analgesic screening. *Federation Proceeds*. 18, 412–416 (1959).
32. Winter G.A., Risley E.A., Nuss G.W. Carrageenan-induced oedema in hind paw of the rats an assay for anti-inflammatory drugs, *ProcSocExpBiol Med*. 111, 544-7(1962).
33. Saini N K and Singhal M. Anti-inflammatory, analgesic and antipyretic activity of methanolic *Tecomaria capensis* leaves extract. Asian Pac J Trop Biomed. 2(11): 870–874 (2013).
34. Rice, M.E., and Shelton, E. Comparison of the reduction of two tetrazolium salts with succinoxidase activity of tissue homogenates. Journal of the national cancer institute, 18 (1), 117–125 (1957).
35. Babson, A.L., and Babson, S.R. Kinetic colorimetric measurement of serum lactate dehydrogenase activity. Clinical chemistry, 19 (7), 766–769 (1973).
36. De Duve, C., and Wattiaux, R., Tissue fractionation studies. VII. Release of bound hydrolases by means of triton X-100. Biochemical journal, 63 (4), 606–608 (1956).
37. Moron, M.S., Depierre, J.W., and Mannervik, B., Level of glutathione, glutathione reductase and glutathone-S-transferase activities in rat lung and liver. Biochimicaetbiophysicaacta (bba) - general subjects, 582 (1), 67–78 (1979).
38. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical biochemistry, 72, 248–254. Buege, J.A., and Aust, S.D., Microsomal lipid peroxidation. Methods in enzymology, 52, 302–310 (1978).
39. Sedaghatfard F , Razavi2S A , Hedayati M , Razmi N , Rahnama A .Glutathione Peroxidase Activity Assay with Colorimetric Method and Microplate Reading Format and Comparison with Chemiluminescence Method . European Online Journal of Natural and Social Sciences. 5(1) 15-21, (2016).
40. Santos RC, Kushima H, Rodrigues CM, Sannomiya M, Rocha LR, et al. Byrsonimaintermedia A. Juss.: gastric and duodenal anti-ulcer, antimicrobial and antidiarrheal effects in experimental rodent models. J Ethnopharmacol 140(2): 203-212 (2012).
41. Sánchez-Mendoza M.E., Reyes-Trejo B., Sánchez-Gómez P., Rodríguez-Silverio J., Castillo-Henkel C., Cervantes-Cuevas H., Arrieta J. Bioassay-Guided Isolation of an Anti-Ulcer Chromene from Eupatorium Aschenbornianum: Role of Nitric Oxide, Prostaglandins and Sulfydryls. *Fitoterapia.* 81:66–71 (2010). doi: 10.1016/j.fitote.2009.07.009.
42. Mishra A., Arora S., Gupta R., Manvi, Punia R.K. and Sharma A.K. Effect of Feroniaelephantum (Corr) fruit pulp extract on indomathacin-induced gactric ulcer in albino rats. Tropical Journal of Pharmaceutical Research. 8(6): 509 (2009).
43. Balogun M.E., Oji J.O., Besong E.E., Ajah A.A. and Michael E.M. Anti-ulcer activity of aqueous leaf extract of Nauclealatifolia (rubiaceae) on Indomethacin-induced gastric ulcer in rats. African Journal of Biotechnology. 12(32): 5080-5086 (2013).
44. Peskar B.M., Ehrlich K. and Peskar B.A. Role of ATPsensitive potassium channels in prostaglandin-mediated gastroprotection in the rat. Journal of Pharmacology. 301(3): 969-974 (2012).
45. Korany, R. M., Ahmed, K. S., Halawany, H. A. and Ahmed, K. A. Effect of long-term arsenic exposure on female Albino rats with special reference to the protective role of

Spirulinaplatensis.Explor. Anim. Med. Res. 9 (2): 125-136 (2019).
46. Hano, J., Bugajski, J. and Danek, L. Effect of adregenic blockage on gastric secretion altered by catecholamines in rats. Archivum Immunologiae et Therapia Experimentalis. 24(4): 507-524 (1976).
47. Bancroft D., Stevens A. and Turmer R. Theory and practice of histological technique. 4th ed. Edinburgh, London, Melbourne: Churchhill Living Stone; (1996).
48. Kóňa, J., Tvaroška, I. Comparative DFT study on the *α*-glycosidic bond in reactive species of galactosyl diphosphates. *Chem. Pap.* 63, 598–607 (2009).
49. Wang, L., Liu, H., Qin, G., Structure characterization and antioxidant activity of polysaccharides from Chinese quince seed meal. Food Chem. 234, 314–322 (2017).
50. Ktari, N., Feki, A., Trabelsi, I., Triki, M., Maalej, H., Slima, S.B., Nasri, M., Amara, I.B., Salah, R.B., Structure, functional and antioxidant properties in Tunisian beef sausage of a novel polysaccharide from *Trigonellafoenum-graecum* seeds. Int. J. Biol. Macromol. 98, 169–181 (2017).
51. Jiang Y., Zi W., Pei Z., Liu S. Characterization of polysaccharides and their antioxidant properties from *Plumulanelumbinis.* Saudi Pharmaceutical Journal 26, 656–664 (2018).
52. Oliveira A F. D., Nascimento G E. D., Iacomini M, Cordeiro L M C, Cipriani T R. Chemical structure and anti-inflammatory effect of polysaccharidesobtained from infusion of Sedum dendroideum leaves. International Journal of Biological Macromolecules. 105, 940–946 (2017).
53. Aziz RS , Muhammad AS , Shabbir SA , Naseem N. Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-α, NF-κB, and COX-2 levels, and upregulation of TFF-2 levels. Bomedicine & Pharmacotherapy 110, 554–560 (2019)
54. Choi, E.Y., Hye-Jeong H , In-Hye K, Taek-Jeong N. Protective effects of a polysaccharide from Hizikiafusiformis against ethanol toxicity in rats. Food and chemical toxicology : an international journal published for the british industrial biological research association, 47 (1), 134–139 (2009).
55. Adhikary, B., Adhikary B , Yadav S., Roy K., Bandyopadhyay S., Chattopadhyay S. 2011. Black tea and theaflavins assist healing of ndomethacin-induced gastric ulceration in mice by antioxidative action. Evidence-based complementary and alternative medicine, 1–560 (2011).
56. Lullmann, H., Color Atlas of Pharmacology. 2nd ed. New York: Thieme Stuttgart (2000).
57. Michael T., Murray ND, John Nowicki ND. Antiulcer Activity - an overview, in Textbook of Natural Medicine (Fifth Edition), volume 1 (2020).
58. Aditi A, and Graham DY. Vitamin C, Gastritis, and Gastric Disease: a historical review and update. Dig Dis Sci. , 57(10): . doi:10.1007/s10620-012-2203-7 (2012).
59. Li, W., Huang H, Niu X, Fan T, Mu Q, **Li** H. Protective effect of tetrahydrocoptisine against ethanolinduced gastric ulcer in mice. Toxicology and applied pharmacology, 272 (1), 21–29 (2013).
60. Arab, H.H., Salama SA, Omar HA , Arafa E A, Maghrabi I A . Diosmin protects against ethanol-induced gastric injury in rats: novel anti-ulcer actions. Plos one, 10 (3), e0122417. (2015).
61. Haghazali M, Molaei M, Mashayekhi R, et al. Proinflammatory cytokines and thrombomodulin in patients with peptic ulcer disease and gastric cancer, infected with Helicobacter pylori. Indian J PatholMicrobiol. 54(1):103e106 (2011).
62. Mignotte, B., and Vayssiere, J.L., Mitochondria and apoptosis. European journal of biochemistry, 252 (1), 1–15(1998).
63. Amirov, NSh., and Trubitsyna, I.E., Changes of acid phosphatase in the gastric mucosa during ulcerogenesis. Biulleten'eksperimental'noibiologiiimeditsiny, 94 (9), 55–57 (1982).
64. Park, S.W., Park, Oh TY , Kim, Y S , Sim H , Park SJ , Jang EJ ,J Park JS , Wook H , Ki B, Hahm B . *Artemisia asiatica* extracts protect against ethanol induced injury in gastric mucosa of rats. Journal of gastroenterology and hepatology, 23 (6), 976–984 (2008).
65. Beserra F P, Rozza A L, Vieira A J, FernandoL, Gushiken S, Pellizzon C. H. Antiulcerogenic Compounds Isolated From Medicinal Plants Ch.6. Studies in Natural Products Chemistry, 47, 215-234 (2016).