Pharmacogn J. 2020; 12(6): 1412-1420
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Phytochemical Screening and *In Vivo* Immunosuppressive, Antioxidant and Anti-hemolytic Activities of Zea *mays* Silk Aqueous Extract

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ABSTRACT

Introduction: The use of plants in traditional medicine goes back to antiquity and still represents an essential part of the Moroccan health care system due to their effectiveness. Although *Zea mays* (ZM) silks are considered as waste products, they are consumed for their medicinal properties. They are rich in bioactive components, giving them a wide range of uses as remedies. The aim of this study was to evaluate after a phytochemical screening, the effect of ZM silk aqueous extract on humoral immune response, on Neutrophil bactericidal, antioxidants and hemolytic activities. **Methods:** The antioxidant activity was assessed using DPPH. Hemagglutination titer assay was used to evaluate the effect on humoral immunity. Hemolytic effect of ZM was evaluated by quantifying hemoglobin rates. The effect on Neutrophil bactericidal activity was assessed using MTT colorimetric assay. **Results:** The extract exhibited high quantity of saponins and flavones; a high antioxidant activity (IC50: 247,15 vs. control 0,152 mg/ml), a significant ($p<0, 05$) immunosuppressive effect *in vivo* on titer values of antibodies (80 times**)** as well as an antibacterial effect on *Staphylococcus aureus* and *Salmonella enteritidis*. A significant suppression of Neutrophil bactericidal activity of cells treated with 0, 5 and 1g/ml of ZM extract was observed. It also exhibited a significant dose-dependent anti-hemolytic activity with the lowest hemolytic activity was found with the lowest concentrations of the extract. **Conclusion:** Our results indicate that aqueous extract of ZM silk possess antioxidant, anti-hemolytic activity as well as an immunosuppressive activity by decreasing humoral immune and Neutrophil bactericidal responses.

Key words: Anti-hemolytic, Antioxidant, Inhibition of antibody production, Neutrophil bactericidal activity, *Zea mays* silk.

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History

- Submission Date: 06-06-2020;
- Review completed: 20-07-2020;
- Accepted Date: 03-08-2020.

DOI : 10.5530/pj.2020.12.195

Article Available online

http://www.phcogj.com/v12/i6

INTRODUCTION

Medicinal plants have, for a very long time, played an important role in Moroccan culture. Their use is continually evolving as they remain available by their proximity and low prices.[1] *Zea mays* (ZM) or corn is a member of the family of Graminaea. Originating in Mesoamerica and domesticated in Mexico since at least 600 years. Maize is widely cultivated all around the world. Almost all parts of the plant are used, whether as food for animals and humans, as a form of fuel or as a traditional remedy.[2]

Maize silk are yellow strands, called stigmas and can be found inside corn's Husk. They are considered as a waste product of maize culture but are consumed in many countries, mainly China, Korea, Turkey and France as a traditional remedy for cystitis, edemas, kidney stones, prostate disorders as well as obesity. In Morocco, ZM silks are consumed as a decoction or infusion to treat urinary tract disorders, nocturnal incontinence as well as obesity.[1,3-5]

Consumption of ZM silk extract has been shown to be safe. Indeed, Wang *et al.*[5] showed that a concentration of 9,3 g/kg/day showed no histopathological effects in rats.

In traditional medicine, the properties of plant extracts depend on the part of the plant used as well as the extraction solvent. Methanolic and aqueous extracts of ZM silk have been shown to be rich in alkaloids, flavonoids, tannins and saponins.[6,7] Nawaz *et al.*[8] highlighted that Maize grain extracts had the same composition as the ZM silk extracts.

The methanol, the ethanol and the aqueous extracts of ZM silk showed a strong antibacterial activity. Feng *et al.*[9] showed that ethanol extracts of ZM silk were more active against Gram-positive microorganisms. While investigating antimicrobial activity of different organic extracts of ZM silk, Fazilatun *et al.*[10] revealed that petroleum ether and methanol extracts were more active than the chloroform one.

ZM silk are also usually used to treat urinary tract affections. Meiouet *et al.*[11] showed that aqueous extract of ZM silk were efficient for dissolving cysteine stones in vitro. Oral administration of aqueous extract of ZM at a concentration of 500 mg/Kg body weight (bw) produced a diuretic and kaliuretic effect in rats.[12]

ZM silk is known for its antioxidant activities. Ren *et al.*[13] as well as Liu *et al.*[14] highlighted the antioxidant activity of different flavone glycosides in vitro. Hu *et al.*[15] showed that oral consumption of flavonoids

Cite this article: Bendiar S, El Faqer O, Chennaoui S, Benjelloun N, Mtairag El M, Oudghiri M. Phytochemical Screening and *In Vivo* Immunosuppressive, Antioxidant and Anti-hemolytic Activities of Zea *mays* Silk Aqueous Extract. Pharmacogn J. 2020;12(6):1412-20.

extracted from ZM silk provided protection against oxidative stress in mice.

The immune system is a vast network of cells, organs, tissues and proteins that are spread throughout the body. This system constitutes the body's defense against foreign pathogens. Immune deficiency can be described by an impaired immune system and can lead to recurrent affections. An over expression of the immune system, on the other hand, can lead to allergies and autoimmune diseases. Few studies have investigated the effect of ZM silk on the immune system both *in vivo* and *in vitro*. Maysin, extracted from corn silk was shown to activate macrophages to secrete TNF- α, highlighting the Immunomodulatory effect of ZM silk extracts on innate immunity.[16] Namba *et al.*[17] showed that glycoprotein extracted by hot water from ZM silk inhibited production of IgE in mice.

The objective of the present study was to evaluate the effect *in vivo*, of aqueous extract of ZM silk on immune humoral response as well as its effects on Neutrophil bactericidal, antioxidant and hemolytic activities.

MATERIALS AND METHODS

Preparation of Zea mays aqueous extract

The silks of *Zea mays* (ZM), authenticated by Professor Khyati Najat, a plant taxonomist at the Department of Biology, Faculty of Sciences Ain Chock, Hassan II University of Casablanca, was dried on shade, grounded into powder and stored in a dry place prior to use. Extraction was carried out by decoction of 100 g of ZM powder in 1000 ml of distilled water and heated under reflux at 60°C for 1h. The mix was then centrifuged, filtered and evaporated at 40°C under reduced pressure using rotary vacuum evaporator. The extract was then stored at -20°C until use. On the day of the experiments, the crude extract was dissolved in distilled water at the described concentrations.

Qualitative phytochemical screening

The aqueous extract was subjected to phytochemical analysis, using the methods described below:

- For the detection of phenols, 1 ml of 20% of Na_2CO_3 and 1 ml of Folin Ciocalteu were added to 3 ml of aqueous extract. The appearance of blue color indicated the presence of Phenols.

- For the detections of flavonoids, 1 ml of a solution of 10 % of NaOH was added to 3ml of aqueous extract. The presence of flavonoids is defined by the apparition of a yellow color.

- For the detection of tannins, 2ml of a 5% solution of $FeCl_3$ was added to 5ml of aqueous extract. The appearance of a dark blue color indicated the presence of Gallic tannins. A dark green color defined the presence of catechic tannins.

- For the detection of saponins, 0,5 g of powder of ZM was added to distilled water in a test tube. The test tube was shaken horizontally for 10 seconds. The stable presence of foam defined the presence of saponins.

Antioxidant activities assay

The 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) free radical scavenging method was used for the determination of the antioxidant activities of the aqueous extract of *Zea mays* silk. The DPPH assay was performed according to the method of Roy *et al.*[18] with some modifications. To 50 µl of aqueous extract at different concentrations (100, 200, 300, 400 and 500 mg/ml) or ascorbic acid (positive standard) at different concentrations (0,075; 0,09; 0,2; 0,4; 0,6; 0,8 a 1 mg/ml) were added to 1,950 ml of methanol DPPH solution and incubated at room temperature, in the dark for 30 minutes. Absorbance was then measured at 517 nm. All the samples were done in triplicates. The blank solution consisted of methanol DPPH solution. Antiradical DPPH activity was expressed as IC50 in mg/ml; which denoted the concentration of sample required to scavenge 50 % DPPH free radicals. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

$$\%\ Inhibition = \frac{(Ad - As)}{Ad} x100$$

Where: Ad = Absorbance of DPPH and As = absorbance of sample (aqueous extract / ascorbic acid)

Assessment of Zea mays's silk aqueous extract on humoral immunity: Hemagglutination antibody titer assay

The mice were immunized by intra-peritoneal injection of 200 µl of Rat Red Blood Cells (RRBCs) diluted in PBS (30% v/v) on day 0. Two groups of 5 mice each were used. The first received, orally, the aqueous extract at a concentration of 5g/kg body weight starting 3 days before immunization and continued 7 days after. The second group received the vehicle only. On day 7, the mice were scarified and blood samples were collected and the serum recuperated after overnight incubation at 4°C, then centrifuged, incubated for 30 min at 56°C to inactivate the complement, and stored at 20°C until use. The antibody levels were determined by Hemagglutination using the method described by Bin Hafeez *et al.*[19] Briefly, 25 µl of 1% (v/v) RRBCs in physiological solution (0.9% NaCl) were added to serum serially diluted in 25 µl PBS two-folded in 96 well micro plates. The mixture was incubated at room temperature for 2h. The reciprocal of the highest dilution of the positive test serum agglutination was taken as the antibody titer. This experiment was repeated three times.

In vitro hemolytic activity assay

Hemolytic assay was carried out by adopting the method of Bulmus *et al.*[20] Whole human blood was collected in EDTA vacutainers after informed consent was obtained from all volunteers. The plasma was collected after centrifugation (15 min at 2500 rpm) and the human red blood cells (HRBCs) were washed three times with a solution of NaCl (150 mM). The HRBCs were re-suspended in 100 mM phosphate buffer solution (PBS), and diluted to 10% of their initial concentration to have a final concentration of 10^8 HRBCs per 200 µl. Then, 800 µl of PBS was mixed to 200 µl of HRBCs and to 50 µl of different concentrations (10, 100, 500 and 1000 mg/ml) of aqueous extract of *Zea mays* silk. After 1 h of incubation at 37°C, the tubes were centrifuged (13500 g for 5 minutes). The supernatant was collected and the absorbance measured at 540 nm. HRBCs incubated with Triton-X 100 (1wt %) were used as the positive control, and PBS alone was used as negative control.

Each experiment was performed in triplicate and inhibitory activity of the extract was calculated using the following equation and expressed as percent of hemolytic activity:

$$\%\ Hemolytic\ activity = \frac{(Abs\ sample - Abs\ Cn)}{(Abs\ Cp - Abs\ Cn)} x\ 100$$

Cn: negative control, Cp: Positive control and Abs: Absorbance

Neutrophil bactericidal activity assay

Antibacterial activity

Zea mays was investigated to evaluate its antibacterial activity against 4 bacterial strains *Staphylococcus aureus* ATCC43300 strain, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* and *Salmonella enteritidis*, using the disk diffusion method.

The bacteria were cultured overnight at 37°C in Nutritive broth. It was then diluted at a final concentration of 10^7 CFU/ml. Plates containing Mueller Hinton agar were inoculated using a sterile cotton swab. A

sterile paper disc embedded with 20 µl of different concentrations of the plant extract (2; 1 and 0, 5 g/ml) was then placed on the agar surface. A sterile disc embedded with sterile water was used as negative control. Amoxicillin at 0, 05 % was used as positive control. The plates were then incubated at 37°C for 24 h after which the diameter of the inhibition zones was measured and the sensitivity if the plant was classified according to the size of the diameter: <8: non sensitive: sensitive for a diameter between 9 and 14 mm; very sensitive for diameters above 15 mm.

Isolation of human neutrophil

Human Neutrophil cells used in this study were isolated from peripheral blood from healthy volunteers using the original method of Boyum[21] modified by Kobayashi *et al.*[22] Human heparinized blood was obtained from healthy donors in accordance with a protocol approved by the Institutional Review Board for Human Subjects of NIAID (Bethesda, MD, USA). Written informed consent was obtained from each participant. Fresh Human heparinized blood mixed 1:1 with 0.9% sodium chloride containing 3.0% Dextran T-500 (Pharmacia) was incubated for 20 min at room temperature to sediment erythrocytes. The supernatant (leukocyte-rich) was then centrifuged at 550 × g for 10 min, then cells were suspended in 35 ml of 0.9% sodium chloride and underlayed with 10 ml of Ficoll-Hipaque (1.077 g/liter, Pharmacia) and centrifuged for 30 min to separate PMNs from peripheral blood mononuclear cells (PBMCs). The interface layer was aspirated and the remaining red blood cells were eliminated by standard hypotonic lyses. Purified PMNs were suspended in RPMI 1640 medium (GIBCO), buffered with 10 mM Hepes, and incubated on ice until used. PMNs in each preparation were enumerated visually on a hemacymeter in 2% acetic acid, and slides were routinely prepared and stained with a modified Wright-Gigemsa (Sigma). Each PMN preparation contained 95–98% Neutrophils, with the remaining cells being predominantly eosinophils.

In vitro treatment of Neutrophils with Zea mays aqueous extract

Fifty µl of 1 x 10^7 Neutrophils /ml in RPMI containing 5% Fetal Bovine Serum and 50 µl of ZM extract at the following final concentrations: 1g/ml; 0,5 g/ml and 0,1 g/ml were incubated 30 min at 37°C in wells of a flat bottom 96 well plates. All tests were done in triplicate.

Colorimetric bactericidal assay

Staphylococcus aureus (pre-cultured for 18h at 37°C in Nutrient Broth; 1 x 10^8 Bacteria /ml) was opsonized with autologous inactivated human serum in RPMI 1640 (Dubelco) for 20 min at 37°C. Fifty µl /well of opsonized *S. aureus* were then added to the Neutrophils treated or not treated with ZM extract. Plates were then incubated for 1h at 37°C under agitation to allow killing of bacteria by Neutrophils. Opsonized bacteria (diluted In RPMI to 0, 30, 60 and 90%) were co-incubated in the plates to enable the construction of a standard curve of bactericidal activity. Neutrophils were lyses by adding 50 µl of 0, 2% Triton X-100 in PBS for 5 min on ice. The bacteria and Neutrophils were triturated in the well. Then 50 µl/well of 2mg/ml of MTT were added and plates were incubated for 10 min at room temperature and then centrifuged at 1600 g for 5 min. Supernatant was removed and 150 µl of DMSO was added to the wells. After 10 min at room temperature, the plates were vigorously shaken to dissolve the formazan and 50 µl of PBS was finally added to solubilize the remaining formazan. Quantification of formazan produced by bacteria was performed by measuring optical density (OD) at 560 nm. OD corresponding to 0 - 90 % killing bacteria was established by linear regression analysis using standard curve. Positive control consisted of incubating Neutrophils with opsonized bacteria alone. Percentage of killed bacteria was determined using the following formula:

$$\% \text{ of killed bacteria} = 1 - \frac{(\text{OD sample}) - (\text{OD 90\% killing})}{(\text{OD 0\% killing}) - (\text{OD 90 \% killing})} \times 90\%$$

Statistical analysis

All experiments were repeated 3 times and results are represented as mean ± SEM (n ≥3). The significance of the difference between all values was determined using ANOVA, the significance level chosen being $p < 0, 05$.

RESULTS

Qualitative phytochemical screening

Preliminary qualitative phytochemical screening of aqueous extract of *Zea mays* silk showed the presence of high quantity of saponins and flavones and some phenols and catechic tannin. Results are presented in Table 1.

Antioxidant activity of Zea mays silks extract

In the current study, aqueous extract of *Zea mays* silk showed and IC50 value of 247,15 mg/ml which was approximately 200 times higher ($p<0,05$) than IC50 of ascorbic acid which was 0,152 mg/ml. ZM extract thus shows a high antioxidant activity.

Effect of Zea mays silks extract on humoral activity

The effect of the aqueous extract of ZM on the level of the production of antibodies in mice was carried out. Results are shown in Figure 1. A decrease of 80 times in titer values of antibodies of mice treated with ZM silks extract (683) was observed, as compared to the valued of the untreated mice (54 613). The aqueous extract of ZM silks has thus shown a significant ($p<0,05$) immunosuppressive effect on humoral immune response evaluated by the level of the production of antibodies *in vivo*.

The mice were immunized by an intra-peritoneal injection of Rat Red Blood Cells (RRBC). Two groups of 5 mice each were used. The first received orally, the aqueous extract at 5g/kg/body weight starting 3 days before immunization and continued 7 days after. The second group received the vehicle only. On day 7, the serum was recovered and the antibody levels were determined by Hemagglutination technique. Each value is represented as mean ± SEM (n=5).

In vitro hemolytic activity test

This experiment was aimed to assess whether ZM extract had a hemolytic effect on erythrocytes *in vitro*. Results are presented in Figure 2. The extract seems to have an anti-hemolytic effect at low concentration 10mg/ml (0.8%). Anti-hemolytic effect increases (1; 2 and 4%) with increases of concentrations (100; 500 and 1000mg/ml).

HRBCs were re-suspended in 100 mM phosphate buffer solution (PBS) at a final concentration of 5x10^8 RBCs/ml. Then, 800 µl of PBS was mixed to 200 µl of RBC and to 50 µl of different concentrations (10; 100; 500 and 1000 mg/ml) of aqueous extract of ZM. After 1h at 37°C of incubation the tubes were centrifuged. The supernatant was collected, and the absorbance measured at 540 nm. HRBC incubated with Triton-X100 (1wt %) were used as the positive control, and PBS alone

Table 1: Results of phytochemical screening of aqueous extract of *Zea mays* silks.

	Aqueous extract of ZM silks
Phenols	++
Flavones	+++
Saponines	++++
Catechic tannins	+

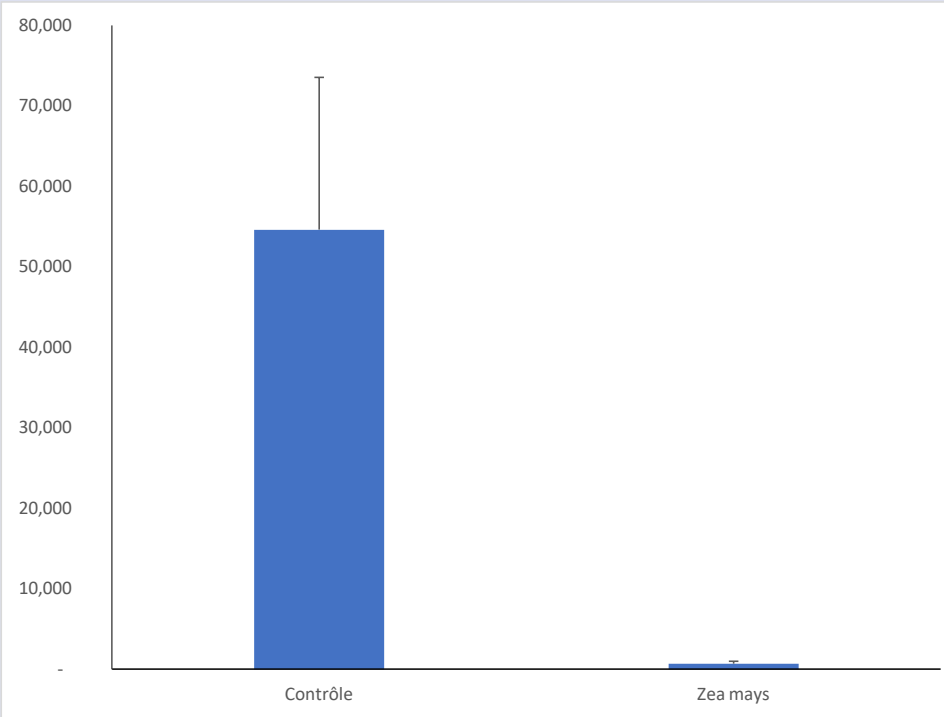

Figure 1: Immunomodulatory effect of *Zea mays* silk's aqueous extract on humoral immune response in mice.

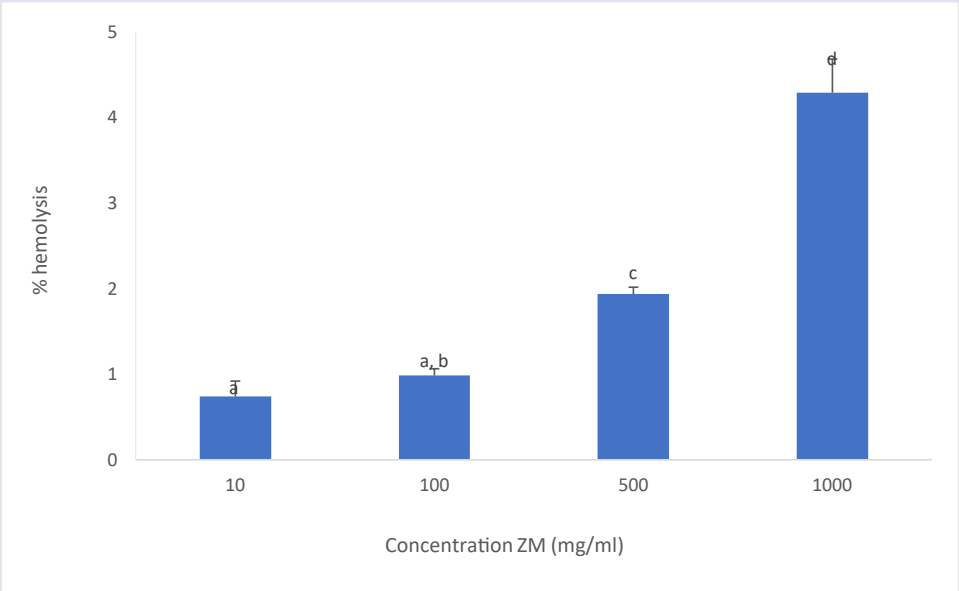

Figure 2: Hemolytic activity of *Zea mays* silks (ZM) aqueous extract on Human Red Blood Cells (HRBC) *in vitro*.

was used as negative control. Each value is represented as mean ± SEM (n=3). Values followed by different letters (a, b, c, d) are significantly different (p<0, 05).

Antibacterial activity

At a concentration of 2g/ml, ZM aqueous extract showed an antibacterial effect on *Staphylococcus aureus* and *Salmonella enteritidis* with respective inhibition diameters of 19,67 0,6 mm and 20,67 ± 1,5 mm (Figure 3). This antibacterial activity is stronger than the one showed by amoxicillin with inhibition diameters of 18,33 1,5 mm and 15,83 ± 0,8 mm respectively for SA and SE (difference is not statistically significant p>0,05). ZM silk aqueous extract did not show any bactericidal activity on SA and SE at concentrations of 1 and 0,5 g/ml nor on E-coli and PA at all concentrations used.

Antibacterial effect was investigated on 4 strains using the disk diffusion method.

SE: *Salmonella enteritidis* ; PA: *Pseudomonas aeroginusa*; E-coli: *Escherichia coli*; SA: *Staphylococcus aureus*

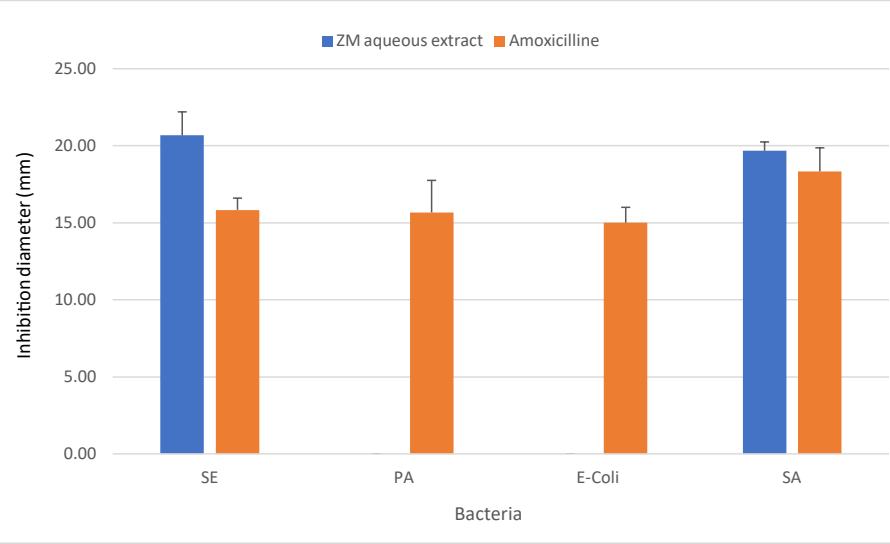

Figure 3: Inhibition diameters of ZM aqueous extract.

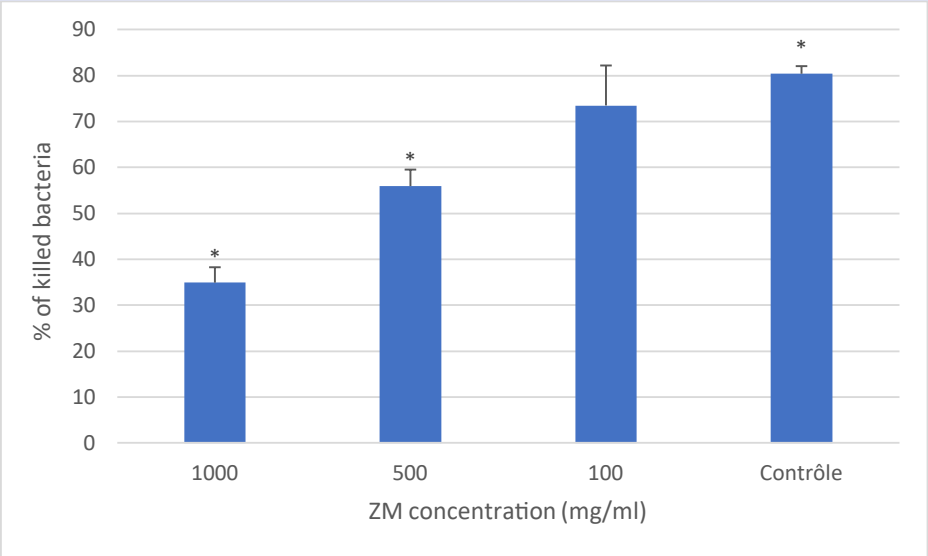

Figure 4: *In vitro* effect of aqueous extract of ZM silk on human Neutrophil bactericidal activity.

Neutrophil bactericidal activity assay

Incubation of Neutrophils for 30 minutes with 0,1; 0,5 and 1 g/ml of aqueous extract of *Zea mays* silk showed an inhibition of Neutrophil bactericidal activity. Results are presented in Figure 4. This inhibition seems dose dependent as it increases with increase of ZM concentration of the extract. Indeed, Neutrophil treated with 0,5 g /ml were able to kill 55,94 ± 3,6 % of bacteria, and those treated with 1 g/ml showed a killing rate of 34,93 ± 3,35 %. Treatment with 0,1 g/ml (73,57 ± 8;7 % of killed bacteria) showed no significant effect compared to untreated Neutrophil bactericidal activity (80,40 ± 1,6 % of killed bacteria). Significant suppression (p<0, 05) of bactericidal activity compared to untreated Neutrophils, resulted with concentrations of 0,5 g/ml and 1g/ml of the aqueous extract.

Neutrophils were incubated with ZM aqueous extract for 30 min before adding *S. aureus*. Neutrophils were lysed using Triton X 100 and MTT was added to evaluate the number of surviving bacteria. Results were expressed as mean SEM (n=3). Values followed * were significantly different (P<0, 05)

DISCUSSION

The present study was undertaken to evaluate the effect of ZM silk aqueous extract on different functions of the immune responses *in vivo* and *in vitro*.

Free radicals and reactive oxygen species (ROS) are highly reactive molecules due to their unpaired electron. ROS are generated to serve different actions by participating to the defense against foreign bodies; during phagocytosis by macrophages and Neutrophils for example as well as by modulating processes like cell proliferation and apoptosis.[23] Live organisms possess systems to maintain ROS levels by keeping their production and elimination well balanced. However, this balance can be disrupted mainly due to an increase of the production of ROS and/or a decrease or inactivation of antioxidants. The detrimental effect caused by excessive levels of ROS results in alteration of cell signaling pathways. This alteration is called oxidative stress. Oxidative Stress induces damages of nucleic acids, of proteins and lipids leading to pathologies ranging from cardio-myopathies to auto-immune diseases.[24,25]

Antioxidants are molecules that can be produced to regulate levels of ROS by interacting and neutralizing these species. A wide range of antioxidant can be found in foods and medicinal plants, among which, flavonoids and phenol molecules showed the most powerful antioxidant priorities. Flavonoids are usually composed of one or more hydroxyl groups, which are capable of scavenging free radicals, giving them their antioxidant activity. Phenols are widely distributed in plants and are generally involved in the defense against aggression by pathogens as well as ultraviolet radiation. The phenols antioxidant activity is based on their redox activity.[26]

Due to its stability and reproducibility, DPPH free radical scavenging test is one of the most used procedures to assess antioxidant activity of plant extracts. This test is based on the ability of the extracts compound to give a hydrogen atom, reducing the purplish color of DPPH to yellow. In the present study, this test enabled us to highlight that aqueous extract of ZM silk exerted an antioxidant activity 200 times higher than ascorbic acid. These results can be attributed to the presence of flavonoids and phenol compounds in the aqueous extract of ZM silk which we highlighted by preliminary phytochemical screening and were reported by other scientific studies.[7, 27] Liu *et al.*[14] have shown similar results evaluating the antioxidant activity of various extracts (aqueous and organic) with aqueous extract showing the best antioxidant activity. Ren *et al.*[13] have shown the antioxidant activity *in vitro* of 5 different flavones Glycosides isolated from ZM silk; antioxidant activity which was dose dependent. Maize grains extracts were also shown to have strong antioxidant activities.[8]

It has also shown in the present study, that ZM silk aqueous extracts exerted an anti-hemolytic activity. Erythrocytes membrane is primarily composed of lipids and proteins, arranged in a configuration that enables red blood cells to undergo reversible deformation while maintaining their integrity.[28] One of the mechanisms that could lead to erythrocytes hemolysis is lipids and proteins oxidation, and specifically, lipids peroxidation. Lipid peroxidation is a free radical chain reaction, leading to a damage of the erythrocytes membrane and thus, hemolysis. Costa *et al.*[29] showed that the presence of phenols and flavonoids may inactivate free radicals, thus decreasing oxidative hemolytic activity, which is in accordance with the anti-hemolytic activity of ZM extract showed in our study.

Another mechanism, by which phenols and flavonoids might have an anti-hemolytic effect, is by changing the conformity of erythrocytes membrane. Indeed, many studies showed that certain types of polyphenols and flavonoids decrease the fluidity of the erythrocytes membrane by interacting with its belayed membrane, which decreases the diffusion of free radicals into the membrane.[28, 30]

Neutrophils represent the first line of defense in response to invading microorganisms. There are many different processes used by Neutrophils to protect the body against invading pathogens including production of reactive oxygen species (ROS). Together with bacteria phagocytosis, Neutrophils release by catalyzation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, high levels of bactericidal reactive chemical species: it is the oxidative burst. This oxidative burst results in generation of H_20_2, which together with myeloperoxidase and Cl^-, are highly bactericidal agents[31-32]. Inhibition or decrease of Neutrophils bactericidal activity observed in our study can be explained by the presence of antioxidants in the aqueous extract of ZM silk. These results are in accordance with those of Ciz *et al.*[33] who showed similar results by demonstrating that flavonoids inhibit respiratory burst of Neutrophils.

The effects of ZM extract *in vivo* have rarely been tested on the humoral immune response. Very few studies have been carried out on the effect of ZM silk aqueous extract on immune system. In the present study, we highlighted that aqueous extract of ZM silk decreased the production of antibodies in mice when they were challenged by Rat Red Blood Cells as antigen. These results are in accordance with those of Namba *et al.*[17] who showed that glycoprotein extracted from hot water extract of ZM silk inhibited production of IgE in mice.

However, Lee *et al.*[16] showed that Maysin, a flavonoids isolated by methanol extract from ZM corn silk, possesses an immuno-stimulating activity *in vivo*. These results proved the presence, in the ZM silks aqueous extract, of an immunosuppressive molecule.

CONCLUSION

ZM silk aqueous extract showed to possess antioxidant, anti-hemolytic and immunosuppressive activities by decreasing humoral immune response and Neutrophil bactericidal activity. Further clinical studies are essential to investigate the therapeutic potential of *Zea mays* aqueous silk extract. Indeed, it is important to identify and to isolate the molecules as well as to define the mechanisms responsible for the immunosuppressive activity of the extract and then to explore the possibility of its use in the case of autoimmune diseases.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGEMENTS

The authors thank the Moroccan Ministry of High Education and Hassan II University of Casablanca for their financial contribution.

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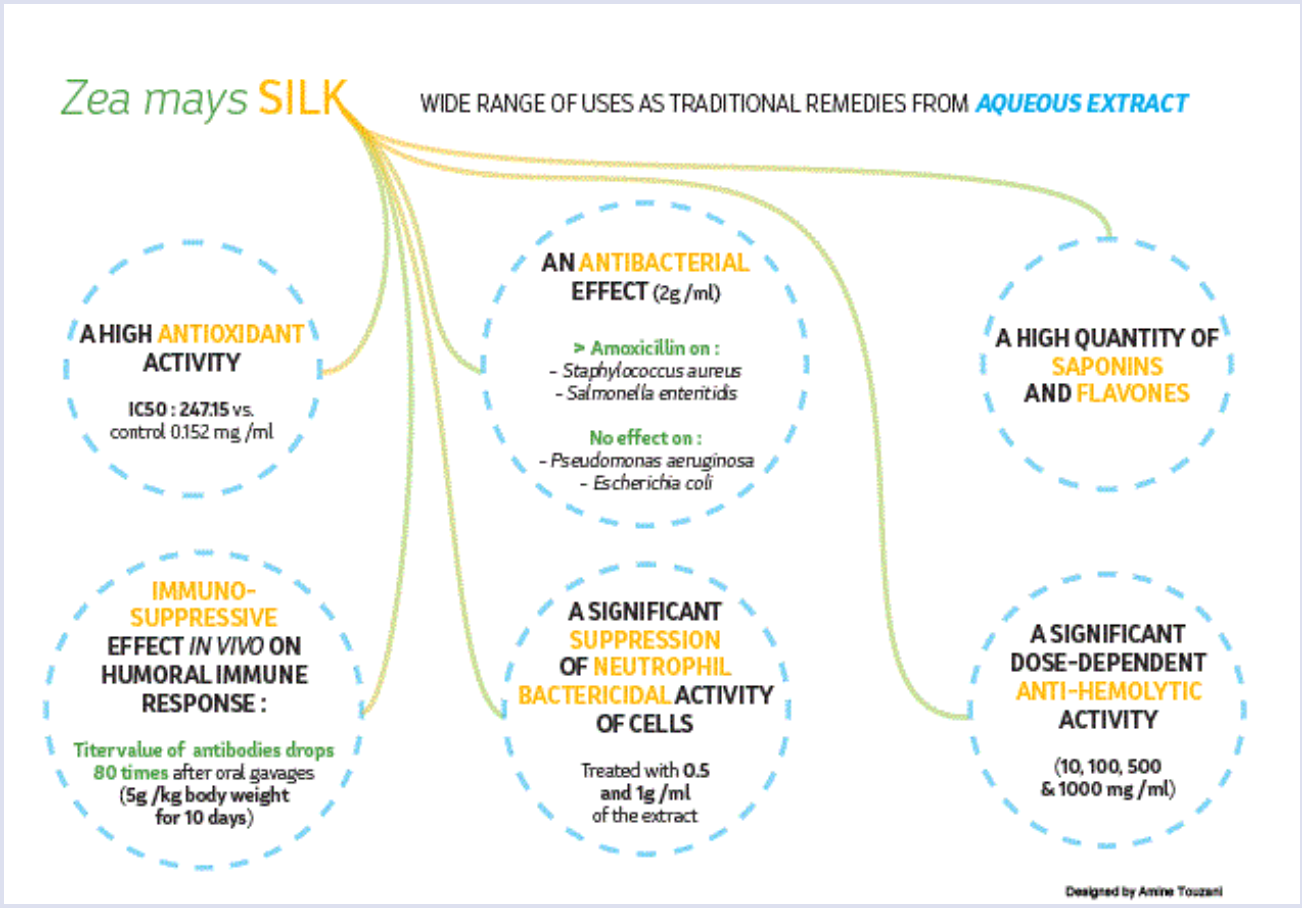
GRAPHICAL ABSTRACT



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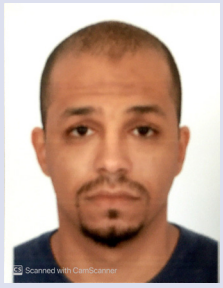

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Cite this article: Bendiar S, El Faqer O, Chennaoui S, Benjelloun N, Mtairag El M, Oudghiri M. Phytochemical Screening and *In Vivo* Immunosuppressive, Antioxidant and Anti-hemolytic Activities of Zea *mays* Silk Aqueous Extract. Pharmacogn J. 2020;12(6):1412-20.