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Evaluation of *in-vitro* Anti-inflammatory Activity of Seed Extract of *Zea mays* Using Albumin Denaturation Method

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A B S T R A C T

The most widely used medications in the world today to treat inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs). However, long-term use of NSAIDs leads to side effects like gastrointestinal irritation. Therefore, there is a renewed interest in finding new anti-inflammatory drugs and medicines from natural sources. Hence, in the current study we aimed for qualitative phytochemical analysis and determination of *in-vitro* anti-inflammatory activity of seed extract of *Zea mays* using albumin denaturation method. Results delineated that there was dose dependent inhibition (%) was observed in standard as well as methanolic seed extract of *Z. mays*. Furthermore, the inhibition (%) of methanolic seed extract of *Z. mays* at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) of methanolic seed extract of *Z. mays* was better than that of standard drug i.e., Aspirin. Furthermore, major phytochemicals found in methanolic seed extract of *Z. mays* were found to be alkaloids, flavonoids, glycosides, proteins & amino acids, steroids, phenolic compounds, tannins and terpenoids. The total phenolic quantity was found to be highest followed by total flavonoids, and quantities of tannins. In conclusion, results of our study clearly demonstrated that methanolic seed extract of *Z. mays* possess anti-inflammatory activity. Hence, it could be recommended that methanolic seed extract of *Z. mays* could be employed for the management of inflammatory conditions and could be considered for development of natural anti-inflammatory drugs.

Keywords

Zea mays, Seeds, Methanolic extract, Anti-inflammatory, Albumin denaturation

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Introduction

Medicinal plants have been known for millennia and are considered as rich source of pharmaceutical agents for the prevention and treatment of diseases and ailments. According to WHO, more than 80% of the population within developing countries uses herbal and other traditional medicines to treat their common ailments (WHO, 1998). Nature has bestowed with an enormous wealth of medicinal plants which are widely used in traditional systems of medicine (Ghazanfar and Al Sabahi, 1993).

Inflammation is a body's immune system response to harmful stimuli associated with immune cells, molecular negotiators, and inflammatory cytokines. The exposure of pathogen, radiation, extremely high or low temperatures and autoimmune processes induce an inflammation (Ferrero-Miliani *et al.,* 2007; Medzhitov, 2010). Chronic inflammatory responses are related to the progression and manifestation of various inflammatory-related diseases, including rheumatoid arthritis, septic syndrome, cardiovascular diseases, cancer and neurodegenerative diseases (Chen *et al.,* 2018).

Synthetic drugs commonly used for the treatment of pain and inflammation like non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide symptomatic and short-lived relief. Also, their long-term uses are associated with several serious adverse effects. Hence, the discovery of new and safe analgesic and antiinflammatory drug is needed (Khan *et al.,* 2005).

Zea mays L. (Family- Poacae) known as maize or corn, is an annual grass plant cultivated for human consumption and rearing of animals (Osagie and Eka, 1998). It is tall with strong erect stalks and a fibrous root system. The plant has long narrow leaves that are spaced alternately on opposite side of the stem and bears ears that are enclosed in modified leaves known as husks as shown in Figure 1 and Figure 2 (Simmonds, 1979).

Besides its nutritive values, maize grains, leaves, cornsilks, stalk, and inflorescence are also used in ethnomedicine for the treatment of several ailments. The corn silk is used as an antidiabetic ordiuretic, and decoction of the silk is consumed for the treatment of urinary troubles and gallstones (Foster and Duke, 1990; Gill, 1992; Abo *et al.,* 2008). The ash of the cob is used for the treatment of cough as well as inflammatory diseases (Gill, 1992). The husks are used in the treatment of pains and arthritis (Owoyele *et al.,* 2010). It is also taken as warm tea for the treatment of malaria in traditional medicine. Biological activities reported on the leaf extract include; anticancer (Balasubramanian and Padma, 2013; Balasubramanian *et al.,* 2014), antioxidant (Balasubramanian and Padma, 2012) and antioxidative stress activities (Balasubramanian *et al.,* 2015). Anti-inflammatory and analgesic activities have been reported on the husk extract (Owoyele *et al.,* 2010).

Phytochemicals are plant chemicals. Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases (Liu *et al.,* 2009). It is estimated that 5000 individual phytochemicals have been identified in fruits, vegetables, and grains. They are otherwise called as the secondary metabolites.

The phytochemicals vary in distribution within the plant parts, as well as in their occurrence within plant species (Bako *et al.,* 2005). Previous studies revealed that eight phenolic compounds *viz.* gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, femlic acid, rutin, resveratrol, and kaempferol have been detected in ethanol husk extract of *Z. mays* (Dong *et al.,* 2014).

Furthermore, search for new pharmacologically active agents obtained by screening natural sources such as medicinal plants or their extracts can lead to potent anti-inflammatory agents. With this background, we designed the current study with the main purpose of qualitative phytochemical analysis and determination of *in-vitro* anti-inflammatory activity of seed extract of *Z. mays* using albumin denaturation method.

Materials and Methods

Collection of Seeds

The seeds of *Zea mays* were collected from the local markets of Chikkaballapura, Karnataka, India and washed several times with running tap water to remove adhered dirt and debris.

The seeds were finally washed once with distilled water, and then shade dried at room temperature. The dried seeds were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered seeds of *Z. mays* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of methanol. Extract was concentrated by distilling the solvent in a rotary flash evaporator. The extract was preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Phytochemical screening was carried out on the methanolic seed extract of *Z. mays* by using standard procedure to detect constituents as described by Sofora (1993); Trease and Evans (1989) and Herborne (1973).

Test for Alkaloids

Approximately 0.2g of methanolic seed extract of *Z. mays* was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins and Phenolic Compounds

The methanolic seed extract of *Z. mays* in small quantity was mixed with water and heated on water bath and filtered.

To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green coloration indicates the presence of tannins and phenolic compounds.

Test for Glycosides

About 0.6g of methanolic seed extract of *Z. mays* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugars

The methanolic seed extract of *Z. mays* was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

Test for Saponins

About 0.2g of methanolic seed extract of *Z. mays* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids

0.2g of methanolic seed extract of *Z. mays* was dissolved in diluted 10% NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for Steroids

2 mL of acetic anhydride was added to 0.5g of methanolic seed extract of *Z. mays* and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for Terpenoids

0.3g of methanolic seed extract of *Z. mays* was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

Test for Proteins and Amino Acids

To the 0.3g of methanolic seed extract of *Z. mays* few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue coloration indicates the presence of proteins and amino acids.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the methanolic seed extract of *Z. mays* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium (Singleton *et al.*, 1999). The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in methanolic seed extract of *Z. mays* (Ordonez *et al.*, 2006). The flavonoid content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/mL) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Tannins

The tannin concentration was determined for methanolic seed extract of *Z. mays* following a modified version of the vanillin-HCl method (Chanwitheesuk *et al.*, 2005).

In-vitro Anti-inflammatory Activity Assay

The *in-vitro* anti-inflammatory activity of methanolic seed extract of *Z. mays* was determined using modified method of Saleem *et al.*, (2011). Control, Standard (Aspirin), and different concentrations of methanolic seed extract of *Z. mays* (i.e., 100-1000 µg/mL) were prepared as follows;

Control

2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and final volume was made up to 50 ml with double distilled water.

Standard (Aspirin)

2 ml of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations (100-1000 µg/mL) of standard drug (Asprin) were taken and final volume was made up to 50 ml.

Extract

2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations of methanolic seed extract of *Z. mays* (i.e., 100-1000 µg/mL) were taken and final volume was

made up to 50 ml. The reaction mixtures of control, standard (Aspirin), and different concentrations of methanolic seed extract of *Z. mays* (i.e., 100-1000 µg/mL) were incubated at 37ºC for 15 minutes and heated at 70ºC for 5 minutes. After cooling the turbidity was measured at 660 nm. Percentage inhibition of albumin denaturation was calculated using the following formula (Chandra *et al.*, 2012).

Inhibition (%) = $(1 - A2/A1)$ X 100

Where,

A1 = Absorption of the control sample

A2 = Absorption of the test sample

Results and Discussion

The results of qualitative phytochemical analysis of methanolic seed extract of *Z. mays* was represented in Table 1. Results revealed that the major phytochemicals found in methanolic seed extract of *Z. mays* were found to be alkaloids, flavonoids, glycosides, proteins & amino acids, steroids, phenolic compounds, tannins and terpenoids. While, the phytochemicals saponins were found to be absent in methanolic seed extract of *Z. mays* (Table 1). The results of quantitative estimation of methanolic seed extract of *Z. mays* was represented in Table 2. Results depicted that total phenolic quantity was found to be highest (21.46 GAE) followed by total flavonoids (13.64 GAE), and quantities of tannins (2.18 GAE). The results of *in-vitro* anti-inflammatory activities of standard and methanolic seed extract of *Z. mays* was presented in Table 3 and Figure 3.

Results revealed that the mean inhibition (%) exhibited by standard was found to be 101.70, 133.92, 187.15, and 309.00 at the concentrations of 250 µg/mL, 500 µg/mL, 750 µg/mL, and 1000 µg/mL respectively. Similarly, the mean inhibition (%) exhibited methanolic seed extract of *Z. mays* at concentrations of 250 µg/mL, 500 µg/mL, 750 µg/mL, and 1000 µg/mL was found to be 43.22, 102.86, 223.52, and 565.11 respectively. These findings depicted that there was dose dependent inhibition (%) was observed in standard as well as methanolic seed extract of *Z. mays.* Furthermore, the inhibition (%) *of* methanolic seed extract of *Z. mays* at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) of methanolic seed extract of *Z. mays* was better than that of standard drug i.e., Aspirin.

A number of factors, such as bacterial infection, chemical injury, and environmental pollution, can cause inflammation, which is a complicated process that can cause cell damage or death. The most widely used drugs in the world today are NSAIDs (O'Byrne and Dalgleish, 2001; O'Byrne and Dalgleish, 2000). The most frequent inflammatory-related complaints are pain and fever. The NSAIDs used to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease. Market demand exists for orally active molecules that are more effective than currently available medications at treating the underlying causes of inflammatory disease as opposed to just the symptoms. Different methods such as inhibition of phosphatases, aminotransferases, cotton pellet granulation techniques, inhibition of heat-induced hemolysis, inhibition of albumin denaturation, membrane stabilizing, platelet aggregation, have been used to study the anti-inflammatory potentials of drugs or agents (Oyedapo *et al.*, 2010). With this scenario, in the current study we aimed for qualitative phytochemical analysis and determination of *in-vitro* anti-inflammatory activity of seed extract of *Z. mays* using albumin denaturation method.

Table.1 Qualitative photochemical analysis of methanolic seed extract of *Z. mays*

Phytochemical Components	Methanolic Seed Extract of *Z. mays*
Alkaloids	+
Flavonoids	+
Glycosides	+
Proteins and Amino acids	+
Saponins	-
Steroids	+
Phenolic compounds	+
Tannins	+
Terpenoids	+

'+': Present; '-': Absent

Table.2 Quantitative estimation of phytochemicals of methanolic seed extract of *Z. mays*

Phytochemical Components	Methanolic Seed Extract of *Z. mays*
Total phenolics	21.46 GAE
Total flavonoids	13.64 GAE
Tannins	2.18 GAE

Table.3 Effect of methanolic seed extract of *Z. mays* on *in-vitro* anti-inflammatory activity

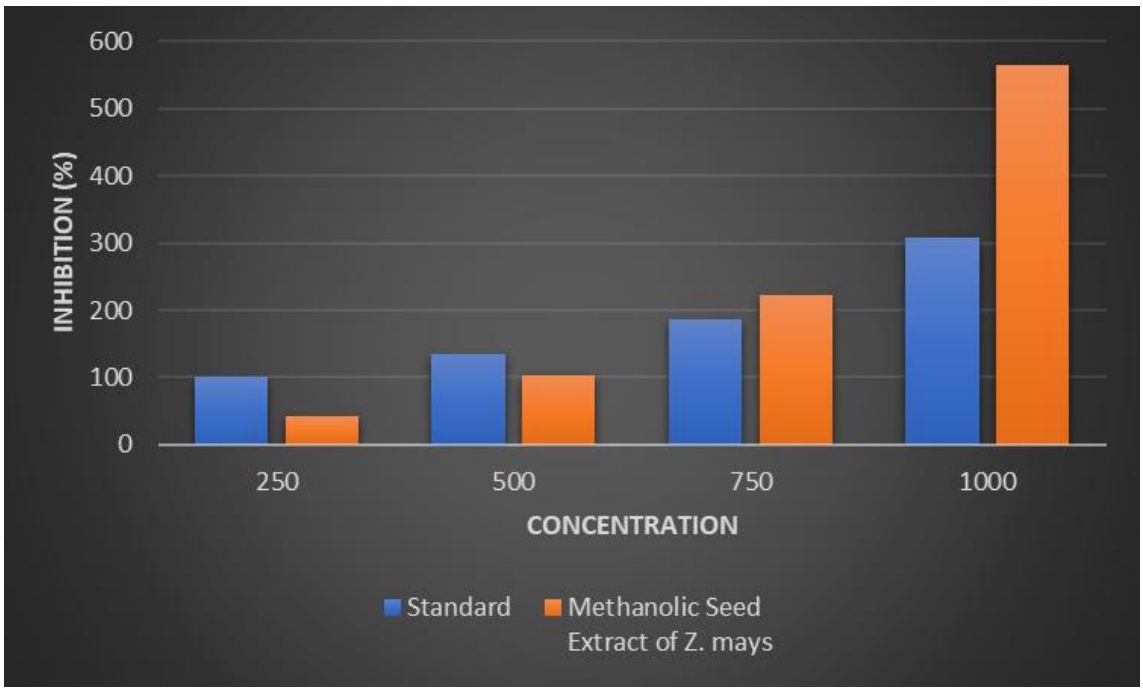
Concentration (µg/mL)	Inhibition (%)	
	Standard	**Methanolic Seed Extract of *Z. mays***
250	101.70	43.22
500	133.92	102.86
750	187.15	223.52
1000	309.00	565.11

Values are expressed as Mean; n=3

Fig.1 Showing plant of *Zea mays*

Fig.2 Showing seeds of *Zea mays*

Fig.3 Effect of methanolic seed extract of *Z. mays* on *in-vitro* anti-inflammatory activity



Denaturation of protein has an unpredictable mechanism which includes modification in electrostatic hydrogen, hydrophobic and disulfide bonding (Sen *et al.*, 2015). Denaturation of protein causes the production of autoantigens in conditions such as rheumatic arthritis, cancer and diabetes which are conditions of inflammation. Hence, by inhibition of protein denaturation, inflammatory activity can be inhibited (Sangeetha and Vidhya, 2016). Concurrently, in our study, there was dose dependent inhibition (%) was observed in standard as well as methanolic seed extract of *Z. mays*. Moreover, the inhibition (%) of methanolic seed extract of *Z. mays* at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) of methanolic seed extract of *Z. mays* was better than that of standard drug i.e., Aspirin.

Furthermore, major phytochemicals found in methanolic seed extract of *Z. mays* were found to be alkaloids, flavonoids, glycosides, proteins and amino acids, steroids, phenolic compounds, tannins and terpenoids. The total phenolic quantity was found to highest followed by total flavonoids, and quantities of tannins. In accordance with our study findings Chanwitheesuk *et al.*, (2005) reported that ethanolic extract of *Z. mays* was found to be rich in biologically active phytoconstituents which include flavonoid, phenolics, Vitamin C and E. The ethanolic extract of *Z. mays* possess and exhibit potent, appreciable and significant anti-inflammatory properties. Therefore, our study results of anti-inflammatory activities of methanolic seed extract of *Z. mays* could be accredited to the phytochemicals present in methanolic seed extract of *Z. mays*.

The results of present preliminary study clearly demonstrated that methanolic seed extract of *Z. mays* possess anti-inflammatory activity. Hence, it could be recommended that methanolic seed extract of *Z. mays* could be employed for the management of inflammatory conditions and could be considered for development of natural anti-inflammatory drugs. However, further studies are recommended to elucidate the exact mechanism of action of particular phytochemical responsible for anti-inflammatory activity of *Z. mays* seeds.

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