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In vitro evaluation of anti-inflammatory activity of cornsilk and clove in combination

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Abstract
Inflammation is a healthy process resulting from some disturbances or disease. The signs of inflammation are redness, heat, swelling, pain, loss of function. Inflammation process plays a protective role in our body and in some conditions produce some negative effect such conditions include the inflammatory disorders. For overcoming such problems, many natural plant compounds are used to inhibit inflammatory pathways with less side effects. The secondary metabolites like phenolic compounds and flavonoids are responsible for having anti-inflammatory properties. The aim of this study was to evaluate the anti-inflammatory activity of Corn silk (*Stigma maydis*) and clove (*Syzygium aromaticum*) in combination by using *In vitro* evaluation method "Egg albumin denaturation assay". Firstly corn silk and clove are collected and the extraction is performed by maceration and hydro distillation process respectively, followed by phyto chemical screening. Different concentrations (10, 20, 30 µg/ml) of plant extracts are prepared and diclofenac was used as standard drug. The percentage inhibition of protein denaturation determine the anti-inflammatory activity. The combined extract of corn silk and clove showed a significant inhibition of protein denaturation than individual extract.

Keywords: Corn silk, inflammation, clove, extract, *in vitro*, clove oil

Introduction
Inflammation is a healthy process resulting from some disturbances or disease. Inflammation is a vital part of the immune systems response to injury and infection caused by physical trauma, noxious chemicals or microbiological agents. The response consists of changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (Leukocytes) from the circulation to the site of tissue damage. It is the body's way of signaling the immune system to heal and repair damaged tissue, as well as defend itself against foreign invaders, such as viruses and bacteria. The signs of inflammation are redness, heat, swelling, pain, loss of function. Inflammation process plays a protective role in our body and in some conditions produce some negative effect such conditions include the inflammatory disorders. For overcoming such problems, many natural plant compounds are used to inhibit inflammatory pathways with less side effects.

Aim
The aim of this study was to evaluate the anti-inflammatory activity of Corn silk (*Stigma maydis*) and clove (*Syzygium aromaticum*) in combination by using *In vitro* evaluation method "Egg albumin denaturation assay". The plant materials (Corn silk and clove) were choosen based on the individual anti-inflammatory properties.
The secondary metabolites like phenolic compounds and flavonoids are responsible for having anti-inflammatory properties.
Diclofenac sodium was taken as standard drug.
The presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, carbohydrates and proteins were seen both in corn silk and clove.
Corn silk extract and clove extract have shown greater anti-inflammatory activity in combination.

Materials and Methods

Procurement of materials: The selected plant materials corn silk and clove were collected. The Collected corn silk was shade dried for about 15days to remove the moisture content. Then they were grinded into coarse powder using mechanical mixer.

Preparation of extracts

Preparation of corn silk extract: About 50gm of coarsely powdered corn silk was weighed and taken into stoppered conical flask along with 250ml of methanol and covered it with cotton and aluminium foil, then allowed to stand at room temperature for a period of 7 days with frequent agitation until the matter has dissolved, then the mixture was filtered, and the filtrate is collected in beaker and transferred to china dish and then, it was evaporated for about 2 days and the residue was collected.

Fig 1: Preparation of corn silk extract

Preparation of clove extract

About 50gm of clove powder was weighed and taken into round bottom flask of 250ml and100ml of distilled water were added into it along with the porcelain chips, then the round bottom flask was connected to the clevenger apparatus along with the inlet and outlet pipes connected to the apparatus and kept it on mantle. The heat was applied to the round bottom flask, the material was boiled in the distilled water until oil distillation ceased after 30min-1hr.Then the oil was separated using separating funnel and collected into beaker.

Fig 2: Preparation of clove extract

Table 1: Phytochemical screening

S.no	Chemical test	Appearance	Results CS	Results clove	Results comb
1.	**Test for alkaloids:**				
	Dragendroff's test	a) orange-brown ppt	-ve	+ve	+ve
	Mayer's test	b)white ppt	-ve	+ve	+ve
	Wagner's test	c)reddish brown ppt	+ve	+ve	+ve
2.	**Test for flavonoids:**				
	Sulphuric acid test	a)yellow/red/blue	+ve	+ve	+ve
	Lead acetate test	b)yellow ppt	+ve	+ve	+ve
	Alkaline reagenttest	c)yellow turns colourless adding dil. HCL	+ve	+ve	+ve
3.	**Test for phenols:**				
	Ferric chloride test	bluish black colour	-ve	-ve	-ve
	Gelatin test	white ppt	+ve	+ve	+ve
4.	Test for steroids a)Libermann test	a)blue colour	-ve	-ve	-ve
5.	Test for tannins a)Gelatin test	a)white ppt	+ve	+ve	+ve
6.	Test for saponins: a)Foam test	a)foam appears	+ve	+ve	+ve
7.	Test for glycosides: a)Keller-killiani test	a) lower- reddishbrown b) upper-acetic layer	+ve	+ve	+ve
8.	**Test for carbohydrates:**				
	Molish test	violet ring formation	-ve	-ve	-ve
	Benedicts test	reddish brown	-ve	-ve	-ve
	Barfoeds test	red ppt	+ve	-ve	+ve
	Fehlings test	red ppt	-ve	+ve	-ve
9.	Test for proteins: a)Millons test	a)brick red	+ve	+ve	+ve

Method of *In vitro* evaluation of anti-inflammatory activity

Using: EGG albumin denaturation assay

The anti-inflammatory activity of crude extracts can be determined by inhibition of the denaturation of egg albumin (protein).

1. The control will be made by mixing 4 ml of distilled water, 0.4ml 1% egg albumin solution, and 5.6 ml of phosphate buffered saline to make a total volume of 10ml.
2. 0.4 ml of 0.4% egg albumin solution, 4 ml of sample extract(corn silk, clove ancombination of both) of

different concentrations(10µg/ml,20µg/ml,30µg/ml), and 5.6ml of phosphate- buffered saline (pH 7.4) will be mixed to form a reaction mixture of a total volume of 10 ml**.**

3. 0.4 ml of 0.4% egg albumin solution, 4 ml of standard drug (Diclofenac) of different concentrations (10µg/ml, 20µg/ml, 30µg/ml), and 5.6ml of phosphate- buffered saline (pH 7.4) will be mixed to form a reaction mixture of a total volume of 10 ml. The reaction mixtures will be then incubated at 37 °C for 30 min and will be heated in a water bath at 70 °C for 15 min.
4. After cooling, the absorbance will be measured at 660nm by a suitable UV/Vis spectrophotometer using distilled water as the blank.

The percentage inhibition will be calculated using the relationship.

$$\%\text{inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

Anti-inflammatory activity
Egg albumin denaturation assay

$$\%\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Where D, is the absorbance of test sample and c is the absorbance of negative control C = 0.872

Table 2: Effect of corn silk extract on albumin denaturation

Sample	Concentration (µg/ml)	Absorbance	Percentage inhibition
Corn silk	10µg/ml	0.623	28%
	20µg/ml	0.556	36%
	30µg/ml	0.395	54%

Table 3 shows that corn silk extract of different concentrations (10, 20, 30µg/ml) and their percentage of inhibition of protein denaturation i.e, 28, 36, 54% respectively.

Graphical representation 1

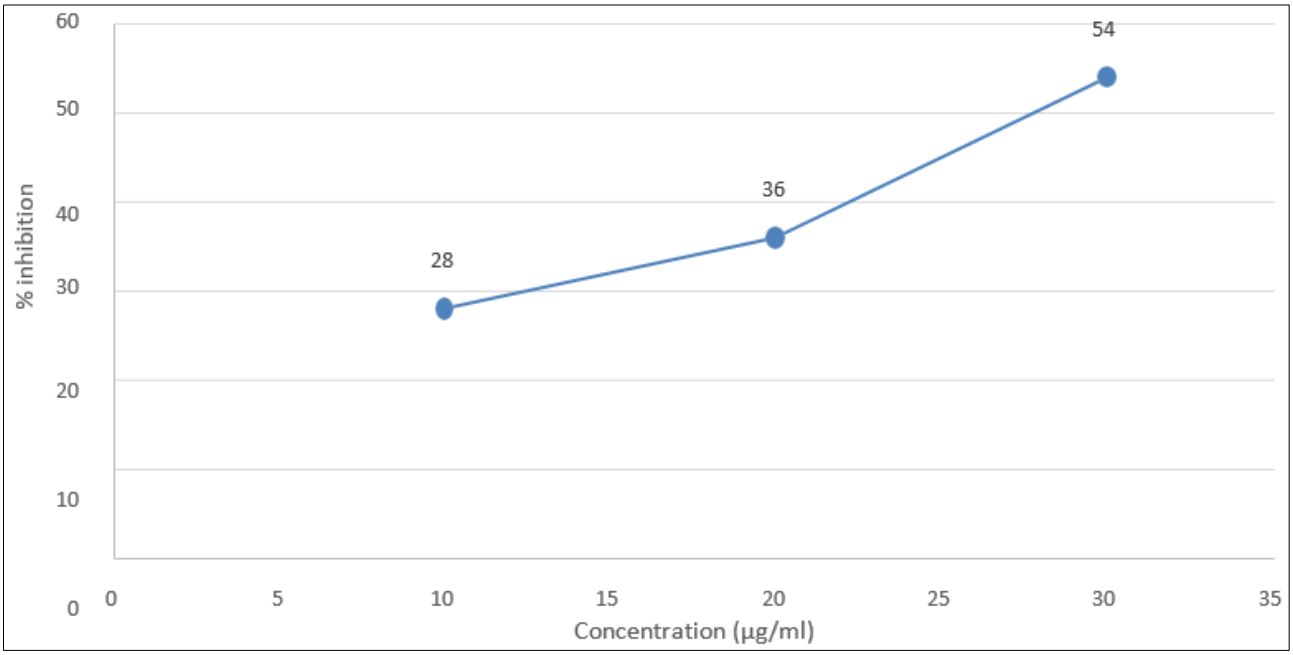

Fig 3: Graph shows that percentage inhibition of protein denaturation of corn silk

Anti-inflammatory activity of clove

Table 4: Effect of clove extract on albumin denaturation.

Sample	Concentration (µg/ml)	Absorbance	Percentage inhibition
Clove	10µg/ml	0.631	27%
	20µg/ml	0.592	32%
	30µg/ml	0.438	49%

Table 4. Shows that clove extract of different concentrations (10, 20, 30 µg/ml) and their percentage of inhibition of protein denaturation i.e, 27,32, 49%respectively.

Graphical representation 2

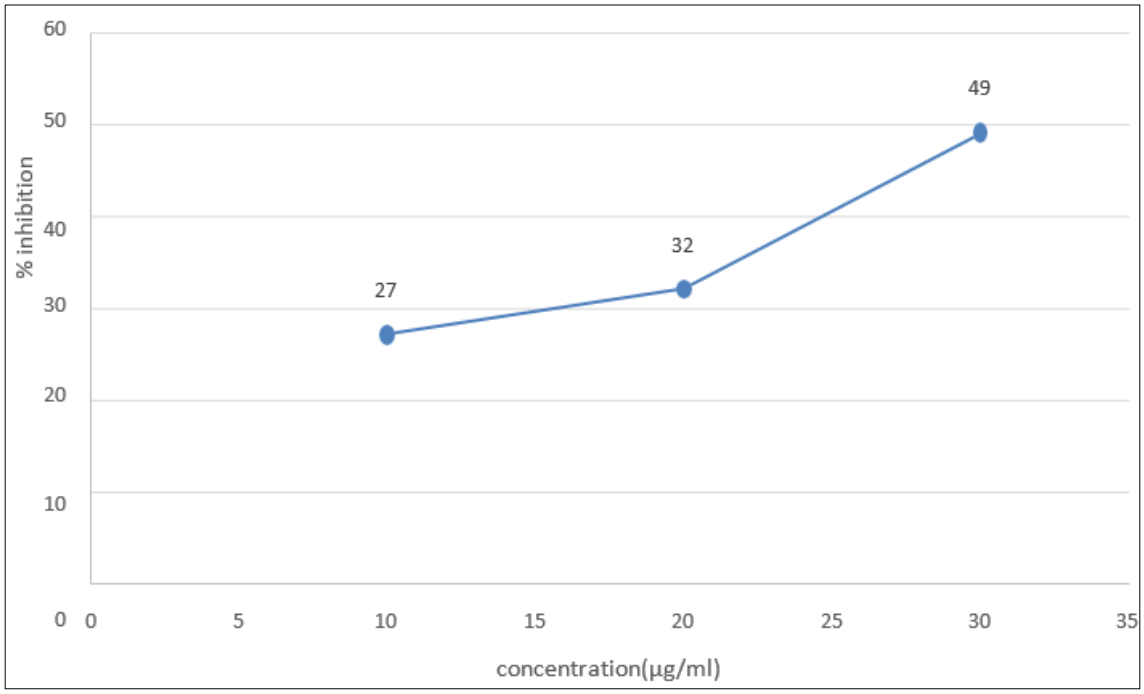

Fig 4: The graph shows that percentage inhibition of clove

Anti-inflammatory activity of corn silk and clove in combination

Table 5: Effect of corn silk and clove extract on albumin denaturation

Sample	Concentration (µg/ml)	Absorbance	Percentage inhibition
Combined extract	10µg/ml	0.582	33%
	20µg/ml	0.511	41%
	30µg/ml	0.328	62%

Table 5 shows that corn silk and clove combined extract of different concentrations (10, 20, 30 µg/ml) and their percentage of inhibition of protein denaturation i.e, 33, 41, 62% respectively.

Graphical representation 3

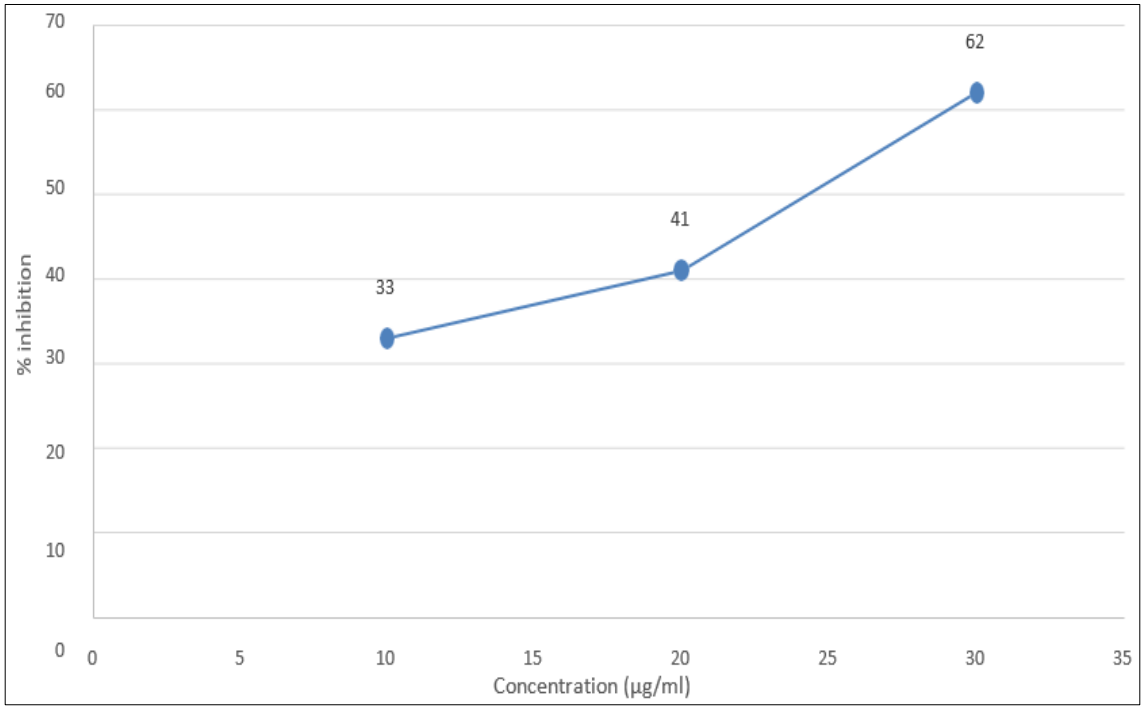

Fig 5: The graph shows percentage inhibition of corn silk and clove in combination

Anti-inflammatory activity of standard (diclofenac)

Table 6: Effect of standard drug (Diclofenac) on albumin denaturation

Sample	Concentration (µg/ml)	Absorbance	Percentage Inhibition
Diclofecan	10µg/ml	0.621	28%
	20µg/ml	0.498	42%
	30µg/ml	0.260	70%

Table 6 shows that standard drug (diclofenac) of different concentrations (10, 20, 30µg/ml) and their percentage of inhibition of protein denaturation i.e, 28,42,70% respectively.

Graphical representation 4

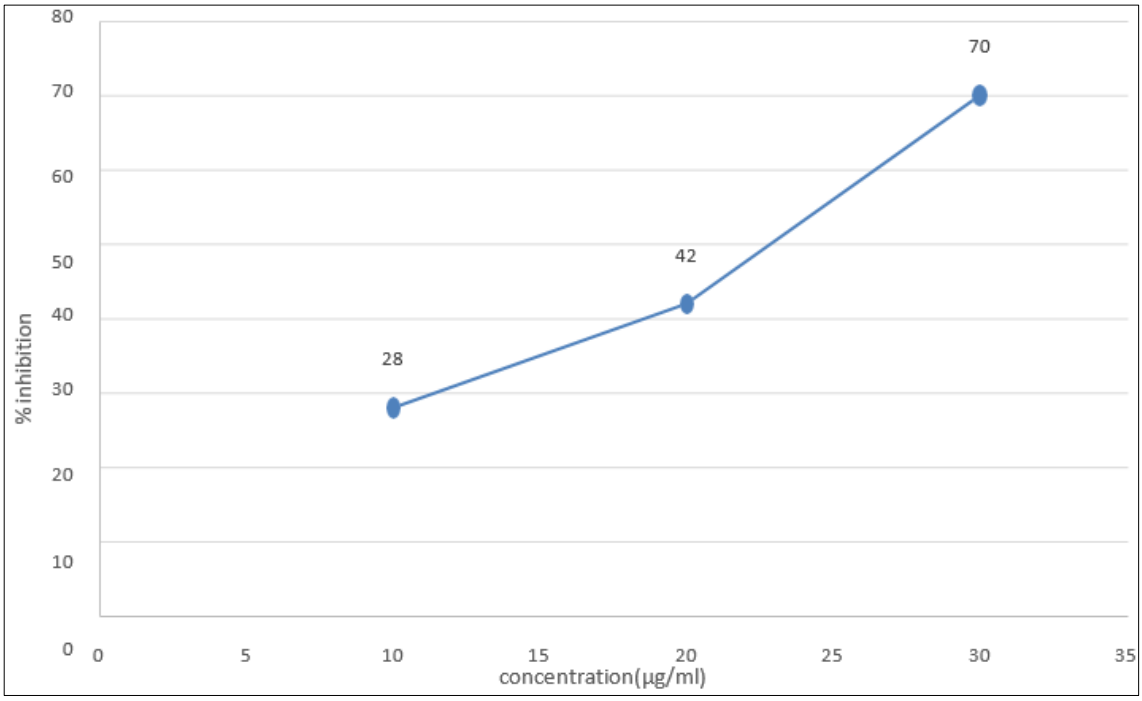

Fig 6: The graph shows the percentage inhibition of standard drug (Diclofenac)

Comparative study of anti-inflammatory activity

Graphical representation 5

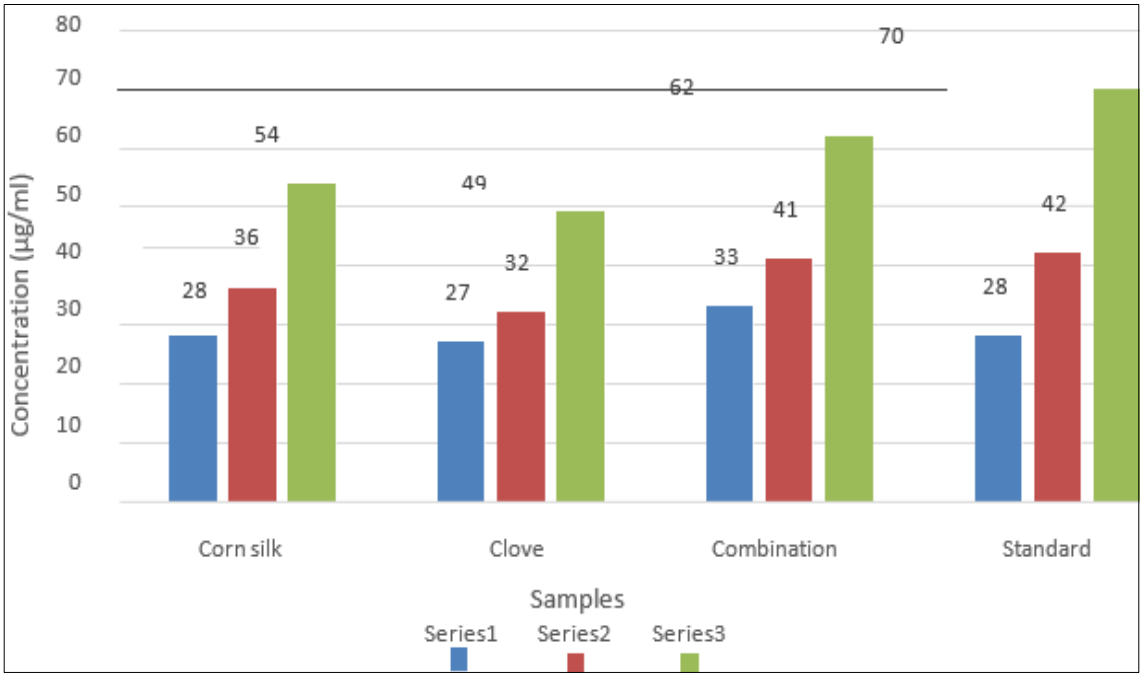

Fig 7: The graph shows comparison of percentage inhibition of cornsilk, clove, combination and standard.

Results

Corn silk extract 10, 20, 30µg/mL has shown activity with percentage inhibition of 28%,36%,54%.

Clove extract 10, 20, 30µg/mL has shown activity with % inhibition of 27% 32%, 49%.

Combined extract 10, 20, 30µg/ml has shown with % inhibition of 33%, 41%, 62%.

Standard drug of 10, 20, 30µg/ml has shown with % inhibition of 28%, 42%, 70%.

Conclusion

In conclusion, by conducting the *In vitro* anti-inflammatory studies, using Egg albumin denaturation assay. The percentage inhibition of protein denaturation gives the anti-inflammatory activity. The phytochemical screening of corn silk, clove and their combination shows the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, carbohydrates, and proteins. The study indicates that the anti-inflammatory activity of corn silk and clove individually is having less activity when compared with the combination of both. Therefore, the combination of corn silk and clove are found to be showing synergistic activity, but when compared with the standard drug the anti-inflammatory activity is slightly less.

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