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EXTRACTION PHYTOCHEMICAL ANALYSIS AND ANTI ULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ACACIA NILOTICA*

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ABSTRACT

Ulcer is a common gastrointestinal disorder which is seen among many people. It is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. It may be due to the regular usage of drugs, irregular food habits, stress, and so forth. Peptic ulcers are a broad term that includes ulcers of digestive tract in the stomach or the duodenum. Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of the most commonly used medicines and proven to be effective for certain disorders. Some people use NSAIDs on daily basis for preventive purpose. But a variety of severe side effects can be induced by NSAIDs. Studies have shown that edible natural ingredients exhibit preventive benefit of gastric ulcer. Therefore present study was designed to evaluate antiulcer activity of hydroalcoholic extract of *Acacia nilotica* in rats. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. The total phenolics content of *Acacia nilotica* extract was (0.586 mg/100mg), followed by flavonoids (0.874mg/100mg) respectively. Further hydroalcoholic extract of 100 and 200mg/kg/p.o significantly ($p<0.01$) reduced the gastric pH, ulcer index in Indomethacin induced ulcer models in rats. The findings of this study confirmed that *Acacia nilotica* extract has anti-ulcer pharmacologic activity due to one or more of the secondary metabolites present in it. Therefore, this study validates its anti-ulcer use in Indian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

Key words: *Acacia nilotica*, Hydroalcoholic extracts , Antiulcer, Aspirin-induced gastric ulcer.

INTRODUCTION:

Peptic ulcer is a gastro intestinal disorder due to an imbalance between the aggressive factors like acid, pepsin, Helicobacter pylori and defensive factors like bicarbonate secretion, prostaglandins, gastric mucus, innate resistance of the mucosal cell factors (Dashputre and Naikwade, 2011). Normally peptic ulcer develops when aggressive factors overcome the defensive factors (Izzo and

Borrelli, 2000). Several factors are also associated in the occurrence of peptic ulcer including stressful lifestyle, alcohol consumption, use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDS), *Helicobacter pylori* infections, and smoking, lower socio-economic status and family history (Mota et al., 2009). Although ulcer is not a deadly disease, it can lead to more serious complications like gastrointestinal bleeding, perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction (Everheart, 1994). Medications are used to relieve the pain, heal ulcerations and delay recurrence of ulcerations. These include antibiotics (Tarin and Chichioco-Hernandez, 2011) antacids and proton pump inhibitors (Yuan et al., 2006). Several drugs are available in the market for gastric ulcer therapy; however, most of these drugs are associated with unwanted side effects (Shirode et al., 2008). *Acacia nilotica* especially and other *Acacia* species are used in local traditional medicine by people as remedy for various disorders like cancers of (ear, eye or testicles) and indurations of liver and spleen, condylomas and excess flesh. It may also be used for colds, congestion, coughs, diarrhea, dysentery, fever, gallbladder, hemorrhage, hemorrhoids, leucorrhea, ophthalmia, sclerosis, smallpox and tuberculosis (Rao et al., 2019). Hence, the objective of the present investigation is to evaluate the anti-ulcer activity of *Acacia nilotica* against NASAID (Indomethacin) induced gastric ulcer in Wister albino rat model.

Materials and methods

Plant material

Flowers of *Acacia nilotica* were collected from local area of Bhopal (M.P.) in the month of July, 2019.

Chemicals and reagents

All the drugs, solvents and chemicals used in the study were of analytical grade. Ranitidine was obtained as a gift sample from Scan Research Lab, Bhopal, MP, India. All other chemicals e.g. Methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India. Tris buffer, Topfer's reagent, Folin's Reagent and Phenolphthalein were purchased from Hi-Media Pvt. Ltd., Mumbai, India. Indomethacin (Sigma Chemical Co, St Louis, MO, USA) were used in present study.

Material and Methods

Extraction by maceration process

60.2 gram flower of *Acacia nilotica* were exhaustively extracted with hydroalcoholic extract solvent (Ethanol 70%) and using drug – solvent ratios (1:2) using maceration process (10hrs). The extracts were evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts (Sharma et al., 2020).

Determination of percentage yield

The percentage yields of each extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Qualitative evaluation

Phytochemical tests were done as per the methods given in.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

b) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Sharma etal., 2020).

Total Phenolic content estimation:

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25mg/ml was prepared in methanol.

Preparation of Extract: 10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at

765 nm using a spectrophotometer (Parkhe et al., 2018).

Total flavonoid content estimation:

Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25mg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extracted dissolve in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm (Parkhe et al., 2018).

***In Vivo* antiulcer activity**

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Preliminary experiments were carried out on rats (n=6). A Hydroalcoholic flower extract of *Acacia nilotica* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD, 2001). Animals were kept fasting providing only water, amoxicillin microspheres were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-ulcer effect.

Experimental designs

Indomethacin induced gastric ulcer

Group –1: Control

Group –2: Cimetidine (Standard)

Group –3: Hydroalcoholic flower extract of *Acacia nilotica (*100mg/kg, p.o.)

Group –4: Hydroalcoholic flower extract of *Acacia nilotica* (200mg/kg, p.o.)

The animals were fasted for 24 h prior to the experiment. Under anaesthesia, ulcers were induced by applying indomethacin (5 mg/kg. p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with Cimetidine (100 mg/kg, p.o.), low dose of Hydroalcoholic flower extract of *Acacia nilotica* (100 m/kg p.o.) or high dose of Hydroalcoholic flower extract of *Acacia nilotica* (200 m/kg p.o.) [once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on the 5^{th} day, the stomachs removed and cut open along the greater curvature (Khare *et al.,* 2008). The ulcer index was determined using the formula (Kujur et al., 2019):

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer, 1 = superficial mucosal erosion,

2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

Results and Discussion

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *Acacia nilotica* extracts was 6.24% w/w. The results of preliminary phytochemical screening of hydroalcoholic extract of *Acacia nilotica* flowers are shown in Table 1. The extract showed the presence of polyphenolic compounds, saponins, flavonoids and alkaloids. The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = Y = 0.0002X-0.025, R^2= 0.980,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **=0.004 X-0.001, R^2=0.996**, where X is the absorbance and Y is the quercetin equivalent (QE). Results were shown in Table 2.

Table 1 Result of phytochemical screening of extracts of *Acacia nilotica*

S. No.	Test	Results
1	Alkaloids	-ve
2	Carbohydrates	+ve
3	Glycosides	-ve
4	Saponins	+ve
5	Phenols	+ve
6	Flavonoids	+ve
7	Proteins	-ve
8	Diterpenes	-ve

Table 2 Total phenolic and total flavonoid content of flowers extract of *Acacia nilotica*

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extract
1	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	0.586
2	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.874

The present study investigated the effect of Hydroalcoholic flower extract of *Acacia nilotica* on the ulcers. Hydroalcoholic flower extract of *Acacia nilotica* showed effect on the healing of gastric ulcers induced by indomethacin. It acts through the inhibition of cell wall biosynthesis that leads to the death of the bacteria. Hydroalcoholic flower extract of *Acacia nilotica* was effective in reducing the ulcer area and the ulcer score. Amoxicillin has

an antiulcer effect. It increased healing of indomethacin induced ulcer, results of Anti-ulcerogenic effect and effect of pH shown in Table 3.

Table 3 Anti-ulcerogenic effect of Hydroalcoholic flower extract of *Acacia nilotica* against ulcerogenic agents in rats (Ulcer index)

Treatment and dose	Indomethacin
Control	3.50 ± 5.0
Cimetidine (100 mg/kg, p.o.)	1.50 ± 5.0***
Hydroalcoholic flower extract of *Acacia nilotica* (100 mg/kg, p.o.)	2.85 ± 5.0**
Hydroalcoholic flower extract of *Acacia nilotica* (200 mg/kg, p.o.)	2.10 ± 5.0***

Values are expressed as mean ±S.E.M. (n = 6).
Percent inhibition calculated as compared to control group.***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$ (One-way ANOVA followed by Tukey's post hoc test).

Table 4: Anti-ulcerogenic effect of Hydroalcoholic flower extract of *Acacia nilotica* against ulcerogenic agents in rats (PH)

Treatment and dose	Indomethacin
Control	1.20 ± 1.0
Cimetidine (100 mg/kg, p.o.)	6.80 ± 1.0***
Hydroalcoholic flower extract of *Acacia nilotica* (100 mg/kg, p.o.)	4.50 ± 1.0*
Hydroalcoholic flower extract of *Acacia nilotica* (200 mg/kg, p.o.)	5.85 ± 1.0***

Values are expressed as mean±S.E.M. (n = 6).
Percent inhibition calculated as compared to control group.***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$ (One-way ANOVA followed by Tukey's post hoc test).

Conclusion

The phytochemical analysis showed that the *Acacia nilotica* plant extract contains a mixture of phytochemicals as Carbohydrates, Saponins, Phenols and Flavonoids. The total phenolics and total flavonoids content for the Hydroalcoholic extract was found to be 0.586 and 0.874 mg/100mg in flowers extract of *Acacia nilotica.* The present study investigated the effect of Hydroalcoholic flower extract of *Acacia nilotica* on the ulcers. Hydroalcoholic flower extract of *Acacia nilotica* showed effect on the healing of gastric ulcers induced by indomethacin. It acts through the inhibition of

cell wall biosynthesis that leads to the death of the bacteria. Hydroalcoholic flower extract of *Acacia nilotica* was effective in reducing the ulcer area and the ulcer score. Amoxicillin has an antiulcer effect. It increased healing of indomethacin induced ulcer.

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