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				Posearch Article

## BIOPROSPECTING OF MEDICINAL PLANT COUROUPITA GUIANENSIS FOR ITS POTENTIAL ANTI -ULCER ACTIVITY

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**ABSTRACT:** In the present investigation anti ulcer activity of methanol extract (flower) of the medicinal plant *Couroupita guianensis* was evaluated in Wistar rats .The experimental rats were divided into four groups (n= 6). The rats were administered with distilled water (Control), Omeprazole (10 mg/kg) (**Positive control**), MECG (200mg/kg b.w) MECG (400mg/kg b.w) and was followed by the ligation of pylorus region of the stomach for ulcer induction. The stomach of the sacrificed rats were microscopically analysed ,gastric juice parameters such as gastric juice volume, pH, free acidity and total acidity and the biochemical parameters such as carbohydrate ,protein and pepsin level were quantified. The results of the present investigation revealed that the ulcer protection was 86.82% in rats treated with MECG 400 mg/ml and the ulcer score level was found  $4.93\pm0.22$  %. .The MECG reduced gastric juice, free acidity, total acidity and P<sup>H</sup> in the experimental Wistar rats. The total acidity range and free acidity level was recorded 88.50± 5.09 and  $61.83\pm4.71$  respectively at the concentration of 400 mg / ml.The biochemical marker enzyme pepsin level was reduced to  $15.36 \mu$ g/ml when compared with the ulcer induced control group (24.62  $\mu$ g/ml).

Key words: Couroupita guianensis; anti-secretory, gastric juice markers, Biochemical markers

## INTRODUCTION

*Couroupita guianensis* is one of the medicinal plants which is native to South India and Malaysia, is commonly known as Nagalinga pushpam in Tamil. It grows to a height of 20 meters. Leaves are alternate, oblong up to 20 cm long, entire to slightly serrate and hairy on the veins beneath. Inflorescence is racemose, arising from the trunk and other large branches. Flowers are reddish with a yellow tinge on the outside, fragrant, with stamens borne on an overarching androphore. Fruit is a large, reddish-brown globose, 15 to 24 cm, with a woody capsule, and each containing 200 to 300 seeds (Elumalai et al, 2012). The leaf of this plant has been found with potential anti oxidant activity, anthelmintic activity, immuno modulator and anti-nociceptive activity (Rajamanickam et al., 2009; Pradhan et al., 2009; Mariana et al., 2010). The bioactive compound isatin isolated from the medicinal plant *Couroupita guianensis* showed antioxidant activity and was cytotoxic to the HL60 cancer cells (Premanathan et al., 2012). In traditional medicine, the leaves of this plant have been used in the treatment of skin diseases, stomachache, and intestinal gas formation, antithrombotic and vasodilatory actions (Golatkar et al., 2003). The flower extracts of this plant had been screened for larvicidal activity against vector (*Desal* et al., 2003). Native Amazoniam people used the infusion or teas obtained from leaves, flower and bark of C.guianensis to treat hypertension, tumours, pain and inflammatory processes (Sanz et al., 2009).

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Ramalakshmi et al, (2013) reported that, the flower extracts of the plant *Couroupita guanensis* showed potential antimicrobial activity against the fish- borne pathogens *viz.*, *Vibrio alginolyticus* and *Plesiomonas shigelloides*. Still there are only few reports regarding pharmacological potential of the medicinal plant *Couroupita guianensis*. Hence, the present investigation is aimed to find out the anti - ulcer potential of flower extracts of the traditional medicinal plant *Couroupita guianensis* in Wistar rats.

## MATERIALS AND METHODS

#### Plant collection

The flowers of the medicinal plant *Couroupita guianensis* (Fig.1) (Elevation - 102 meters, latitude 8.7792<sup>0</sup> N, Longitude-77.4031<sup>0</sup> E) collected from the temple premises of Kumararkoil located nearby to Tenkasi area, Tirunelveli District, Tamilnadu during the month of March 2013 in the morning time and the flowers were brought about to the laboratory without the exposure of the plant material to scorching sun. Then the flowers were air dried under shade condition for about 10 days.

#### Crude extract preparation

The flowers of the medicinal plant were extracted as per the method of Kamaraj et al., (2011). The powdered flowers of about 200g was teased onto the material backing unit of the soxhlet apparatus and the extraction process was carried out using methanol (boiling point 60-  $80^{\circ}$  C) as solvent for 10 h. The extract obtained was condensed using rotary vaccum evaporator under reduced pressure at  $60^{\circ}$  C and the residue obtained (20g) was stored at  $4^{\circ}$ C in a refrigerator.

## ANTI ULCER POTENTIAL OF MECG

#### **Experimental animals**

Wistar rats (150-200g) were procured in a market from Tirunelveli, Tamilnadu, India. The animals were housed in polypropylene cages (38 x 23 x 10cm) and maintained under standard laboratory conditions (14h dark/10h light cycles; temp  $25\pm2^{\circ}C$ ; 35-60% humidity, air ventilation) and were fed up with standard pellet diet (Sai Enterprisei, Chennai) and fresh water ad libitum. The animals were acclimatized to the laboratory condition for about two weeks prior to the starting of the experiment. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and cares of animals were taken as per guidelines of CPCSEA. Department of Animal Welfare and Government of India.

#### Pyloric ligation (PL)-induced ulcers

Male Wistar rats (n = 6) were divided into four groups. Animals were fasted overnight prior to the commencement of the experiment, and water *ad libitum*. Group 1 control rats received only distilled water (10 ml/kg/day, p.o.) . Group II rats treated with reference drug omeprazole (10 mg/kg/day). Group III and Group IV rats were treated with 200mg/kg and 400 mg/kg/(p.o.) respectively with methanol extracts of *Couroupita guianensis* orally for 3 days before subjecting them to ulcerogen. Pyloric ligation was induced by ligating the pyloric end of the stomach of rats on 3rd day under mild ether anesthesia (35 mg/kg) .Then the animals were caged individually for recovery from induced ulcer and the animals were deprived of water. After 4 h of surgery, the animals were sacrificed and ulcer scoring was performed .The gastric juice was collected for studying the profile of gastric juice markers. The stomach was examined for scoring the ulcer lesions (Jyoti Gupta et al., 2012).

#### **Collection of gastric juice**

The gastric juices from all the experimental groups of rats were collected 4 h after the induction of pyloric ligation. The gastric juice was centrifuged for 10 min at 3000rpm. Then the gastric juice was subjected for the estimation of various gastric juice markers *viz.*,  $P^{H}$ , free acidity and Total acidity.

## Ulcer index

The numbers of ulcer lesions in the stomach region of the experimental rats were counted using a magnifying glass and the diameter of the ulcer was measured using a vernier caliper. Then ulcer index was calculated as by following the scoring method of (Suzuki et al., 1998). The sum length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the protection percentage was calculated from the following formula:

[(UI control - UI treated)/UI control] X100

#### Determination of free acidity and total acidity

Gastric juice of about 1 ml was collected in a 100 ml conical flask and was mixed with few drops of Topfer's reagent and titrated against NaOH (standardized with 0.01 N oxalic acid) until the disappearance of red colour into yellowish orange. The volume of added alkali was noted.

It directly corresponds to the free acidity of the gastric juice. Then few drops of phenolphthalein reagent was added and titrated until the formation of red colour tinge. Again the total volume of alkali added was noted (Hawk et al., 1947). This volume corresponds to total acidity. Acidity was expressed as meq/l/100g.

#### Estimation of total carbohydrates

Gastric juice 0.15ml was taken in a test and was added with 1.0ml of phenol reagent followed by the addition of 5.0ml of sulphuric acid. Then the tubes were kept for incubation at 20°C for 20 min. The absorbance was read at 482nm (Kalra et al., 2011).

#### **Estimation of total proteins**

The gastric juice of about 0.1 was diluted with 0.9 ml of distilled water and was added with 4.5 ml of alkaline copper reagent and kept at room temperature for 10 min. Then 0.5 ml of Folin reagent was added. After 20 min and the blue colour developed was read at 640 nm. The concentration of protein was calculated from a standard graph developed from bovine serum albumin (Lowry et al, 1951) and was expressed in  $\mu$ g/ml.

#### Estimation of pepsin

5 ml of 1% BSA substrate prepared in HCl (pH 2.1) was mixed with 1 ml of gastric juice sample (equal volume of gastric juice with HCl at pH 2.1) and incubated at 37 <sup>o</sup>C for 15 min. After the appropriate incubation time was over, the reaction was stopped by addition of 10 ml Trichloro acetic acid. The blank contained a mixer of 10 ml TCA and 1 ml of gastric juice sample, which was incubated for 15 min before the addition of 5 ml of the substrate. The reaction mixture was filtered separately after 30 min. Then the filtrate was added with 10 ml of 0.5 M NaOH and 1 ml of Folin-phenol reagent and was read at 680 nm. Tyrosine was maintained as standard solution. The activity of pepsin was expressed as micrograms of tyrosine equivalents released per ml of gastric juice per minute (Debnath et al., 1974)

## RESULTS

#### Anti ulcer potential of MECG

The anti ulcer activity of MECG was assessed in Wistar rats by pylorus ligation induced method. Ligation of pyloric end of the stomach induces excessive secretion of hydrochloric acid and this leads to auto digestion of gastric mucosa and break down of the gastric mucosal barrier and in turn develops Ulcer lesions (Kumar et al., 2011). The reports of the anti ulcer activity revealed the potential of methanol extract of *Couroupita guianensis* in curing pyloric ligation induced ulcer in Wistar rats. In the group III rats administered with 200 mg / kg of extract of *Couroupita guianensis* 59.33 % of ulcer protection was recorded and in the mean time group IV rats treated with 400 mg/kg of the extract of this plant, the level of ulcer protection was increased to 86.82% which is almost equal to the standard reference drug omeparazole treated rats. For this II group the ulcer protection range was 90.64 % . The number of ulcer lesions calculated in the extract of the medicinal plant *Couroupita guianensis* was 15.21  $\pm$  1.11 and 4.93 $\pm$ 0.22 respectively for the group III (200mg/kg) and Group IV rats (400 mg/kg). The number of ulcer lesions has been reduced in elevated level comparatively than that of the group I control rats (37.40 $\pm$ 2.66). In the group II treated with standard drug omeparazole (10mg/kg) the ulcer score was 3.50  $\pm$  0.45. The extract of this plant displayed almost equal level of activity when compared with the standard reference drug in both the ulcer score count and percentage of ulcer protection (Table 9).

Table 9: Effect of flower extracts of the medicinal plant Couroupita guianensis on pyloric ligation induced ulcer
in experimental animals

Group	Ulcer score	Percentage protection (%)
Control	$37.40 \pm 2.66$	
Omeprazole (10 mg/kg)	$3.50\pm0.45$	90.64
Low dose (200mg/kg b.w)	$15.21 \pm 1.11$	59.33
High dose (400mg/kg b.w)	$4.93\pm0.22$	86.82

Values are expressed as mean  $\pm$  SD (n=6).



Group III Couroupita guianensis (200mg/kg) Group IV Couroupita guianensi s (400mg/kg)

## Fig.3: Effect of flower extracts of the medicinal plant *Couroupita guianensis* in curing ulcer lesions of the stomach of experimental rats

The extract of *Couroupita guianensis* showed potential activity in reducing the high volume of gastric juice, total acidity, free acidity and P<sup>H</sup> in Wistar rats induced with ulcer by pylorus ligation method. In the group I control rats the volume of gastric juice obtained was 5.78  $\pm$  0.25. In the group II rats treated with the standard reference drug omeparazole it was reduced to  $2.68 \pm 0.20$ . In the III group of rats treated with the extract of the medicinal plant *Couroupita guianensis* the volume of gastric juice was reduced to  $5.03\pm0.25$ . This level is comparatively low than that of the group I control rats. But in the group IV rats treated with 400 mg/kg of extracts of *Couroupita guianensis* the volume of gastric juice level was dramatically reduced to  $3.78 \pm 0.40$ . The P<sup>H</sup> of the gastric juice in the group I control group was  $2.01\pm0.05$  and for the group II rats treated with standard reference drug omiparazole it was  $3.03\pm0.07$ . In the group III (200 mg/kg) and group IV (400mg/kg) rats treated with the extract of *Couroupita guianensis* the P<sup>H</sup> level was decreased to  $2.28\pm 0.36$  and  $2.91\pm 0.04$  respectively. The reduction in P<sup>H</sup> of the gastric juice was comparatively almost equal to the standard drug omeparazole treated group II rats. The free acidity in group I control rats were  $97.33 \pm 4.32$ . In the group II standard ulcer drug treated rats it was  $41.5 \pm 4.32$ . In the group III (200 mg/kg) and group IV (400mg/kg) rats treated with the extract of *Couroupita guianensis* the free acidity level was decreased to  $74.83\pm$ 3.97 and  $61.83\pm4.71$  respectively. The total acidity in group I control was recorded in the range of  $144.17\pm13.95$  and it was reduced to 81.33± 2.56 the group II rats treated with the standard drug omiparazole. In the mean time dramatical decrease in total acidity of the group III and group IV rats were observed in the level of  $88.50 \pm 5.09$  and  $100.67 \pm 2.58$ . The decrease in the free acidity, total acidity and  $P^{H}$  and gastric juice volume by the extract of the medicinal plant *Couroupita guianensis* indicated its potential in curing ulcer in Wistar rats (Table 10).

Group	Volume (ml)	рН	Free acidity (mEq/l/100 g b.w)	Total acidity (mEq/l/100 g b.w)
Control	$5.78 \pm 0.25$	$2.01\pm0.05$	$97.33 \pm 4.32$	$144.17 \pm 13.95$
Omeprazole (10 mg/kg)	$2.68\pm0.20$	$3.03\pm0.07$	$41.5\pm4.32$	$81.83 \pm 2.56$
Low dose (200mg/kg b.w)	$5.03\pm0.25$	$2.28\pm0.36$	$74.83 \pm 3.97$	$100.67\pm2.58$
High dose (400mg/kg b.w)	$3.78\pm0.40$	$2.91\pm0.04$	$61.83 \pm 4.71$	$88.50\pm5.09$

# Table 10: Effect of flower extracts of the medicinal plant Couroupita guianensis on gastric juice parameters of pyloric ligation induced ulcer in Wistar rats

Values are expressed as mean  $\pm$  SD (n=6).

The biochemical markers viz., total carbohydrate, protein and pepsin level also quantified in the animal groups treated with the extract of *Couroupita guianensis*. The carbohydrate level measured in group 1 control rats were 325.56± 3.94 and the protein range was measured 522.20± 1.89. In the standard drug omeparazole treated II group rats the carbohydrate and protein level was increased to 679.89±3.76 and 463.50±0.81 respectively. In the group III and group IV rats treated with the extract of Couroupita guianensis the carbohydrate and protein level was increased comparatively than that of the group 1 control rats. In group III rats treated with 200 mg/kg the carbohydrate and protein level was  $416.78 \pm 2.44$  and  $505.83 \pm 0.70$  respectively. But in the group IV rats treated with high dose of extract of Couroupita guianensis the carbohydrate and protein level was increased as likely that of the group II rats which treated with the standard ulcer curing drug omeparazole. The recorded carbohydrate and protein range was  $513.67 \pm 4.02$  and  $457.20 \pm 1.08$  respectively for the group IV rats treated with (400 mg/kg) of the extract of *Couroupita guianensis* The enzyme pepsin level was found  $24.62 \pm 1.29$  in group I control rats . In the group II rats the level of pepsin has been reduced to  $12.19\pm0.58$  and in the mean time moderate decrease (16.07± 1.29) was recorded in group III rats treated with 200 mg/kg of extract of Couroupita guianensis. However high level of decrease in the pepsin enzyme level was measured in group IV rats treated with 400 mg/kg of the flower extracts of Couroupita guianensis. The pepsin range for this group was  $15.36 \pm 0.78$ . These results indicated the anti secretory activity of the flower extract of Couroupita guianensis in the secretion of digestive enzyme pepsin. In all the studied ulcer curing potential, gastric juice parameters and Biochemical markers remarkable activity was showed by the extract of the medicinal plant Couroupita guianensis (Table 11).

Group	Total Carbohydrate (µg/ml)	Total Protein (µg/ml)	Pepsin (µg/ml)
Control	$326.56 \pm 3.94$	$522.20\pm1.89$	$24.62 \pm 1.29$
Omeprazole (10 mg/kg)	$679.89 \pm 3.76$	$463.50\pm0.81$	$12.19\pm0.58$
Low dose (200mg/kg b.w)	$416.78 \pm 2.44$	$505.83\pm0.70$	$16.07 \pm 1.29$
High dose (400mg/kg b.w)	$513.67 \pm 4.02$	$457.20\pm1.08$	$15.36\pm0.78$

 Table 11: Effect of flower extracts of the medicinal plant Couroupita guianensis on biochemical markers of pyloric ligation induced ulcer in Wistar rats

Values are expressed as mean  $\pm$  SD (n=6).

#### DISCUSSION

In the present investigation the traditional medicinal plant *Couroupita guianensis* was bioprospected for its potential anti ulcer activity. The methanol extract of *Couroupita guianensis* flowers showed potential activity in curing the gastric ulcer in experimental Wistar rats. The prime possible reason for this potential anti ulcer activity may be due to the rich presence of phytochemicals such as tannins and flavonoids. Sakat and Juvekar (2009) reported that the phytochemicals such as alkaloids, flavonoids, phenolics, terpenoids and tannins present in the extract of plants stimulates the mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Tannins protect outermost layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation (Asuzu and Onu, 1990).Tannins form a protective pellicle by promoting precipitation of protein on the ulcer in order to prevent ulcer development.

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This pellicle helps in preventing toxic substance absorption and combat the attack of proteolytic enzymes (John and Onabanjo, 1990; Nwafor et al., 1996). Flavonoids able to decrease ulcerogenic lesions by promoting the formation of gastric mucosa inhibit the production of pepsinogen and diminish acid mucosal secretion (La Casa et al., 2000). In our findings the volume of biochemical marker enzyme pepsin was estimated in low level when compared with group 1 control rats. This indicates that the flavonoids present in the MECG inhibited the high level production of pepsinogen and also suppressed the mucosal secretion.

The mechanism of control of gastric juice parameters in the experimental rats are mainly due to the anti secretory activity exhibited by methanol extract of *Couroupita guianensis* flowers. Gastric secretion in oxyntic cells of the gastric glands are mainly due to the presence of receptors such as acetylcholine, histamine and gastrin located at the basolateral membrane of the oxyntic cells (Zakaria et al., 2014). Therefore, its very important to manage the secretion of gastric juice secretion using therapeutic agent for the better control of ulcer diseases (Jain et al., 2007). Our findings in the present investigation clearly demonstrated that MECG inhibited the aggressive factors by reducing gastric juice volume, free acidity, total acidity and pH. The standard anti ulcer drug omeparazole was maintained as positive control which also showed potential gastric protection activiy.

#### CONCLUSION

In the present investigation anti ulcer potential of flower extracts of the medicinal plant *Couroupita guianensis* was tested in Wistar rats. The findings of this research revealed the potential anti ulcer activity of this holy plant .Further research is underway to identify the exact bioactive compound responsible for the anti ulcer activity.

## **CONFLICT OF INTEREST**

We declare that we have no conflict of interest

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