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### IN-VIVO ANTIULCER ACTIVITY OF METHANOL, ACETONE AND ETHYL ACETATE PETALS EXTRACTS OF HIBISCUS PLATINIFOLIUS MILL

A.Sravya\*, B.A.S.S.S.Swaroop, A.Uma Maheswari, CH.Elina Rani, M.Surya Kumari, P.Anusha, P.Sai Hareesh

Department of Pharmacology, JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India.

#### **ABSTRACT**

Hibiscus platinifolius is a traditional medicinal plant throughout world and widely used to treat CNS disorders, cardiovascular diseases, skin diseases, liver diseases, asthma and ulcers. The plant is important in the treatment of ulcers. Therefore methanol, acetone and ethyl acetate extracts of Hibiscus platinifolius petals were assessed for antiulcer activity. In addition, measured the antioxidant activity and determined content of total phenolic and flavonoids in the extract in order to correlate them with the assayed activities. Methanol extracts exhibit antiulcer activity by pylorus ligation method and ethanol induced method at the dose of 500mg/kg when compared to standard which may be due to the presence of high phenolic content and high flavonoid content and other constituents.

Keywords: Hibiscus platinifolius, total phenolic, flavonoids, antiulcer activity

### Author for correspondence A.Sravya,

Department of Pharmacology, JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India. Email id: sravya.allu27@gmail.com

#### INTRODUCTION

Plants have been the basis of different traditional medicinal systems throughout the world and continue to provide mankind with new remedies. World Health Organization (WHO) defines traditional medicine (including herbal drugs) as therapeutic practices that have been in existence, often for hundreds of years,

Before the development and spread of modern medicine and are still in use today. Herbal medicine is the synthesis of therapeutic experience of generation of practicing physicians of indigenous systems of The traditional preparations include medicine. medicinal plants, minerals, organic matter etc. Herbal drugs have been in use in Indian, Chinese, Syrian, Roman, Egyptian, Greek texts for thousands of years as per recorded evidences. The Indian texts include Rigveda. CharakSamhita Atharvaveda. SushrutaSamhita. Folk medicines also play a vital role in healthcare system of ethnic people. Thus herbal medicines have been derived from rich traditions of ancient civilizations and scientific heritage. Herbal medicines serve as major remedy in traditional system of medicine, even in 21st century these are the primary

source of health care system in rural areas and poor countries. According to WHO, about 80% of the world populations still depend on herbal medicines for primary health care Herbal medicine practices continue still today because of their biomedical superiority over modern medicine. Herbal medicines are used for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in plants are a part of the physiological functions of living system and hence they are believed to have better compatibility with the human body. These drugs are made from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity. India is one of the world's 12th biodiversity zones with the presence of over 45 thousands diverse plant species, though only half of the plants are used for their medicinal values. Ayurveda, Siddha, Unani and Folk medicines are the indigenous medicinal systems in India. About 8,000 herbal medicines have been codified in Ayurveda. The Rigveda has documented 67 medicinal plants, Yajurveda 81 species, Atharvaveda 290 species, CharakSamhita and SushrutaSamhita had discussed properties and uses of 1100 and 1270 species respectively are still used in classical formulations. Medicinal plants play a key role in the development of potent therapeutic agents. Plant based drugs provide excellent contribution to modern therapeutics. Plant derived drugs are used to cure mental illness, skin diabetes. diseases, tuberculosis. jaundice, hypertension and cancer. The use of plant material used as indigenous cure in folklore or traditional system of medicine.

Peptic ulcers are the localized erosions of mucous membranes of the stomach or duodenum. The pain associated with ulcers is caused by irritation of exposed surfaces by the stomach acids. Before the appearance of effective antiulcer drugs in the 1960s, ulcer sufferers often suffered intense pain for many years, and if left untreated, the ulcer could result in severe bleeding and even death. Antiulcer therapy has been a huge money spinner for the pharmaceutical industry, with drugs such as cimetidine, ranitidine, and the proton pump inhibitors (PPIs). None of these drugs available until the 1960s, however, and it is perhaps hard for us now to appreciate how dangerous ulcers could be before that. In the early 1960s, the

conventional treatment was to try to neutralize gastric acid in the stomach by administering antacids. These were bases such as sodium bicarbonate or calcium carbonate. The dose levels required for neutralization were large and caused unpleasant side effects. Relief was only temporary, and patients were often advised to stick to rigid diets such as strained porridge and steamed fish. Ultimately, the only answer to severe ulcers was a surgical operation to remove part of the stomach. The first effective antiulcer agents were the H<sub>2</sub> histamine antagonists which appeared in the 1960s. These were followed in the 1980s by the PPIs. The discovery of H. pylori then led to the use of antibacterial agents in antiulcer therapy. The current approach for treating ulcers caused by H. pylori is to use a combination of drugs which includes a PPI such as omeprazole, and two antibiotics such as amoxicillin and metronidazole (1-5).

Research on free radicals and to find new antioxidant phytochemicals have arouse significant interest among scientist in past decade, their broad range of effect in biological system have drawn the attention of many researchers. There are extensive evidences to implicate free radicals in the development of chronic and acute diseases such as cancer, cardiovascular diseases, rheumatoid arthritis, inflammation, cataracts, diabetes, Alzheimer's disease and aging. Damage caused by reactive oxygen or nitrogen species leads to the pathological changes which are associated with many diseases. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and used in industrial processing commonly responsible for potential health risk such carcinogenicity, inflammations and atherosclerosis.

#### MATERIALS AND METHODS

#### **Collection of Plant Material**

Hibiscus platinifolius leaves were procured in spring season, from Joharapuram locality in Kurnool. Flowers were identified and authenticated by botanist Dr. P. VENU at Botanical Survey of India (BSI), Hyderabad.

#### **Preparation of plant extracts**

Leaves of *Hibiscus platinifolius* were shade dried under room temperature for one week and petals were powdered mechanically. The finely powdered petals were kept separately in an air tight container until the

time of use. About 60 gms of finely powdered petals were extracted with organic solvents in polar dependent manner by using methanol solvent in Soxhlet apparatus for 28 hours individually and finally solvents were evaporated and concentrated by using distillation apparatus. These extract were used for phytochemical screening and antiulcer activities.

#### **Experimental Animals**

Experiments were performed in both sexes of Wistar rats (150-250 gms). The animals were housed for one week before starting the experiment. All animals had free access to pallet chow and water until the day before experiment, when only water was made available to them. The animals were housed in large spacious hygienic cages during the course of the experimental period. All animal procedures have been approved by Institutional Animal Ethical Committee (Registration No: 1305/ac/09/CPCSEA) in accordance with animal experimentation and care.

# Qualitative phytochemical screening of methanol, acetone and ethyl acetate extracts

Methanol, acetone and ethyl acetate extracts of *Rosa damascena mill*. Were screened for their chemical constituents. Phytochemical screening was done as explained in literature. A small amount of dried extract was used to determine the alkaloids, carbohydrates, phenols, flavonoids, steroids and triterpenoids.

## In vivo anti-ulcer activity (6, 7) Pylorus ligation method

In this method Wistar rats of both sexes weighing between 150-250 gm were selected and divided into 5 groups and each group contains 6 animals. Animals fasted for 24 hrs. Group 1 serves as normal, group 2 received vehicle (saline), group 3 administered with Ranitidine (20 mg/kg) which serves as positive control, group 4 & 5 received methanol extract (250mg/kg and 500mg/kg), administered orally. After 30 minutes rats were lightly anesthetized by using chloroform and the abdomen was opened without damaging any blood supply. Then its pylorus was ligated. The abdomen was closed by suturing and the rats were allowed to recover for 4hr. After 4 hrs, the animals were anesthetized using chloroform and then euthanized by cervical dislocation. The abdomen was opened and a ligature was placed around the esophagus junction. The stomachs were removed and calculate the ulcer index and percentage inhibition.

#### Ethanol induced method

In this method Wistar rats of both sexes weighing between 150-250 gm were selected and divided into 5 groups and each group contains 6 animals. Animals fasted for 24 hrs. Group 1 serves as normal, group 2 received vehicle (saline), group 3 administered with Ranitidine (20 mg/kg) which serves as positive control, group 4 & 5 received methanol extract (250mg/kg and 500mg/kg administered orally. One hour later, 1 ml of 80% ethanol was administered orally to each animal. Animals were sacrificed by cervical dislocation, one hour after ethanol administration, stomachs were isolated and cut open along the greater curvature and pinned on a soft board. Finally calculated ulcer index and percentage of inhibition.

#### **Statistical analysis**

The analysis was carried out using Graph Pad PrismV software and values are expressed as mean  $\pm$  SEM (n = 3 for *in-vitro* and n = 6 for *in-vivo* tests). For *in vitro* antioxidant activity IC<sub>50</sub> value was the concentration which causes 50% scavenging effect and determined graphically.

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#### **RESULTS AND DISCUSSION**

#### Physical characteristics of extracts

The methanol extract of *Hibiscus platinifolius*.was thick rosein color, sticky in nature and the percentage yield of the extract was found to be 35.66% w/w.

#### Preliminary phytochemical screening of extracts

Qualitative phytochemical screening was carried out using several tests and results revealed that methanol extract of *Hibiscus platinifolius* contains phenols, flavonoids and absence of alkaloids, carbohydrates and steroids&triterpenoids.

### In-vivo anti-ulcer activity

#### Pylorus ligation method

In this method all extracts showed the antiulcer activity at the doses of 500mg/kg when compared to standard (Ranitidine). Methanol extract showed a percentage inhibition of 72.48% compared to standard (84.10%) at the dose level of 500mg/kg (Table-1).

Table-1 *In-vivo* antiulcer activity of methanol, acetone and ethyl acetate leafs extracts of *Hibiscus platinifolius* mill by pylorus ligation method

S. No	Groups	Treatme	ent	Ulcer index	% inhibition
1	Group-I	Norma	ıl	1.8±0.03	-
2	Group-II	Control		25.8±1.53	-
3	Group-III	Standard		4.1±0.28***	84.10
4	Group-IV	Methanol extract	250mg/kg	15.6±0.98	39.53
5	Group-V	$(T_1)$	500mg/kg	7.1±1.83***	72.48

Significance controls \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compare to the ANOVA T test, (n=6).

#### Ethanol induced method

In this method all extracts showed the antiulcer activity at the doses of 500mg/kg when compared to standard (Ranitidine). Methanol extract showed a percentage inhibition of 61.84% compared to standard (72.76%) at the dose level of 500mg/kg.

Table-2 *In-vivo* antiulcer activity of methanol, acetone and ethyl acetate petals extracts of *Hibiscus* platinifolius mill by ethanol induced method

S. No	Groups	Treatme	ent	Ulcer index	% inhibition
1	Group-I	Normal		1.8±0.03	-
2	Group-II	Control		19.13±2.12	-
3	Group-III	Standard		5.21±1.31***	72.76
4	Group-IV	Methanol extract	250mg/kg	12.63±1.38	33.97
5	Group-V	$(T_1)$	500mg/kg	7.3±0.98***	61.84

Significance controls \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compare to the ANOVA T test, (n=6)

#### **CONCLUSION**

The present work revealed that the extracts of petals of Hibiscus platinifolius by phytochemical screening of methanol extract contain flavonoids and phenols. Methanol extracts exhibit antiulcer activity by pylorus ligation method and ethanol induced method at the dose of 500mg/kg when compared to standard which may be due to the presence of high phenolic content and high flavonoid content and other constituents. The results justified the use of leaves extracts in several cardio tonic, **CNS** disorders, inflammatory, skin diseases, antioxidant and antiulcer diseases traditionally. We suggest that the petals of the plant can be viewed as the potential source of natural antioxidant and antiulcer can afford precious functional components.

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