CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTIULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CASSIA AURICULATA*

Rishi Nema*, Mr. Arpit Shrivastava, Shubhankar Soni, Dr. Harshita Jain
Adina Institute of Pharmaceutical Sciences, Sagar (M.P.)

Article Received: August 2022 **Accepted:** September 2022 **Published: October** 2022

Abstract
Ulcer is the most common gastrointestinal disturbance resulting from an inadequate gastric mucosal defense. Several drugs are available in the market to address the disease; however, these drugs are associated with unnecessary side effects. Previous research has confirmed the efficacy of plant extracts for possible treatment of the disease. This research aims to evaluate the anti-ulcer properties of medicinal plants Cassia auriculata. The present study is aimed to evaluate the antiulcer activity of hydroalcoholic extract of Cassia auriculata plant in rats. The antiulcer activity of the Cassia auriculata plant extract was evaluated in pyloric ligation model and ethanol induced gastric ulcer model. In pyloric ligation model, the rats were divided into four groups of six each. Group I rats were treated with vehicle and served as negative control and Group II rats were treated with standard drug Ranitidine 50 mg/kg , Group III and IV treated with hydro alcoholic extract of Cassia auriculata (200 and 400 mg/kg, p.o respectively. The hydroalcoholic extract at the dose of 200 and 400 mg/kg showed 30.57 and 62.76 % protection respectively against pyloric ligation-induced ulcer. In conclusion, the hydroalcoholic extract of I. Cassia auriculata has antiulcer activity.

Key words: *Cassia auriculata, Hydroalcoholic extract, Antiulcer activity*

Corresponding author:
Rishi Nema,
harshi.jain1987@gmail.com

QR code

Please cite this article in press Rishi Nema et al ***Extraction, Phytochemical Screening And Antiulcer Activity Of Hydroalcoholic Extract Of Cassia Auriculata.,*** *Indo Am. J. P. Sci, 2022; 09(10).*

INTRODUCTION:

A history of heartburn, gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion for peptic ulcer. Medicines associated with ulcer include NSAIDs (non-steroid anti-inflammatory drugs) that inhibit cyclooxygenase, and most glucocorticoids (e.g. dexamethasone and prednisolone). A major causative factor (60% of gastric and up to 50-75% of duodenal ulcers) is chronic inflammation due to *Helicobacter pylori* that colonizes the antral mucosa[1]. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis). Gastrin stimulates the production of gastric acid by parietal cells. In H. pylori colonization responses to increased gastrin, the increase in acid can contribute to the erosion of the mucosa and therefore ulcer formation. In Western countries the percentage of people with *Helicobacter pylori* infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80 etc.).

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue. A gastric ulcer would give epigastric pain during the meal, as gastric acid production is increased as food enters the stomach. Symptoms of duodenal ulcers would initially be relieved by a meal, as the pyloric sphincter closes to concentrate the stomach contents; therefore acid is not reaching the duodenum. Peptic ulcer disease (PUD) is an illness that affects a considerable number of people worldwide. It develops when there is an imbalance between the ‘‘aggressive’’ and ‘‘protective’’ factors at the luminal surface of the epithelial cells. Aggressive factors include Helicobacter pylori, HCl, pepsins, nonsteroidal anti-inflammatory drugs (NSAIDs), bile acids, ischemia, hypoxia, smoking and alcohol. While defensive factors include bicarbonate, mucus layer, mucosal blood flows, PGs and growth factors. Burning or gnawing feeling in the stomach area lasting between 30 minutes and 3 hours commonly accompanies ulcers[2].

Gastric ulcers the widest state disease and are a very common global problem today. Peptic ulcer is a lesion of the gastric /duodenal mucosa occurs at a site where the mucosal epithelium is exposed to acid and pepsin. Peptic ulcers occur due to imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. The aggressive and protective factors in the stomach are acid pepsin secretion, mucosal barrier, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. Sometimes the gastric mucosa is continuously exposed to potentially injurious agents such as pepsin, bile acids, food ingredients, bacterial products and drugs. Factors such as stress, smoking, nutritional deficiency and ingestion of NSAID’S all can increase the incidence of gastric ulcers. It is reported that prolonged anxiety, emotional stress, haemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation.

Physical, chemical and psycological factors may lead to gastric ulceration in humans and experimental animals. Reactive oxygen species (ROS) are reported in the pathophysiology of human diseases such as neurodegenerative inflammation, viral infections autoimmune GI. inflammation and gastric ulcer.

Peptic ulcer occurs in that part of the gastrointestinal tract (GIT) which is exposed to gastric acid and pepsin i.e. the stomach and duodenum. The etiology of peptic ulcer is not clearly known. It results probably due to an imbalance between the aggressive (acid, pepsin, bile and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, nitric oxide, innate resistance of the mucosal cells) factors[3]. A variety of psychosomatic, humoral and vascular derangements have been implicated and importance of *Helicobacter pylori* infection as a contributor to ulcer formation and recurrence has been recognized[4].

Cassia auriculata commonly known as Tanner’s Cassia is an important medicinal shrub used in traditional systems of medicine. It holds a very prestigious position in Ayurveda and Siddha systems of medicine. It also growing wild in Central Provinces and Western peninsula and cultivated in other parts of India. It is valuable as a tanning material and as a green manure crop. The plant has been reported to possess antipyretic, antidiabetic, antiperoxidative and antihyperglycemic and microbicidal activity.

Ulcer is the most common gastrointestinal disturbance resulting from an inadequate gastric mucosal defense. Several drugs are available in the market to address the disease; however, these drugs are associated with unnecessary side effects. Previous research has confirmed the efficacy of plant extracts for possible treatment of the disease. This research aims to evaluate the anti-ulcer properties of medicinal plants *Cassia auriculata*.

MATERIAL AND METHODS:

Extraction procedure

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. For the standardization of herbal products, selection of a suitable extraction technique is also important. Extraction is used in the removal of desirable soluble constituents, exclusion those not required with the help of the selected solvents. The collected plant materials were thoroughly washed in tap water and rinsed in distilled water. The cleaned, healthy collected plant samples were cut into small pieces and dried under shade for 3 to 4 weeks. Following procedure will be adopted for the preparation of extract from the shade dried material [5-6].

Defatting of plant material

All materials were shade dried at room temperature. 65.80 gram of dried leaves of *Cassia auriculata* Linn was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried leaves of *Cassia auriculata* Linn were extracted with hydroalcoholic (ethanol: water: 80:20) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods [7].

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

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-coloured precipitate indicates the presence of alkaloids.

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

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) 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.

c) 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.

b) 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.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it 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.

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each 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 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [8].

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [9].

In vivo* antiulcer activity of hydroalcoholic extract of *Cassia auriculata

Animals used

The animals were maintained in colony cages at 25 ± 2ºC, relative humidity 50-55% maintained under 12 h light and dark cycle (06 to 18 h light; 18 to 06 h dark). The animals were fed with standard animal feed (Hindustan Lever Ltd.) and water ad libitum. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional animal ethical committee. All the animals were acclimatized to the laboratory conditions prior to experimentation.

Acute toxicity

Acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420[100]. The study was initiated with a sighting study aimed to determine the dose for the acute toxicity study. The sighting study comprised of female Wistar albino rats dosed in a step wise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. Starting with 5 mg/kg BW, the test article was administered orally to one rat. The rat was then observed for toxic effect for the first 30 min followed by hourly for 8 h for the first 24 h. If they are no signs of toxic effect or mortality observed on the rat within the 24 hours, we then dosed another rat with the next dose (50 mg/kg BW) and a similar procedure was carried out. A stepwise procedure was carried out until the highest dose, 2000 mg/kg BW is reached. If all the rats survived, they were monitored and observed once daily for the next 13 days. The sighting study showed that the rats dosed with 5, 50 300 and 2000 mg/kg BW with the test article survived.

Based on this observation we decided to use the highest dose, 2000 mg/kg BW for the main test, the acute toxicity study. The acute toxicity study comprised of two groups, one control and one treatment group that consisted of 5 female rats in each group. Female rats were chosen because it is the most sensitive gender to see the effect of treatment[10]. The treatment group received mimosa pudica extract that was diluted in water at a dose 2000 mg/kg BW given orally once, in a 2 ml volume. The control group received water delivered in the same volume and same procedure as the treatment group. The experimental animals were observed for 30 min after treatment, followed by observation hourly for 8 h and once daily for the next 13 days.

Anti-ulcer studies (Aspirin plus pylorus ligation induced gastric ulcer in rats)

Wistar albino rats of either sex weighing 150- 200 g were divided into 4 groups, each group consists of 6 animals. All groups of animals received treatments as shown below along with 200 mg/kg of aspirin once daily for three days. Group 1 received 1.0 ml/kg p.o. 1% SCMC as vehicle control; Group 2 received 50 mg/kg, p.o. ranitidine as standard, Group 3 and Group 4 received 200 and 400 mg/kg, p.o. Hydroalcoholic extract of *Cassia auriculata* (HECA) respectively. Ulceration in rats was induced as described by Goel et al., 1985. On the fourth day pylorus part was ligated following 36 h fasting[11]. Four hours after the pyloric ligation the animals were sacrificed by decapitation. Each stomach was opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (20 X). The length and width

(mm) of ulcer on the gastric mucosa were measured by plane glass square (10×10 mm). The Ulcer Area (UA) was calculated. The % of protection (P %) availed to the animals through various treatments were calculated using the formula:

P% = (UA ulcer control-UA treatment)/UA ulcer control × 100

The gastric content was titrated against 0.01 N NaOH using Topfer's reagent as indicator to find out the free acidity and total acidity[12-16].

Grouping of Animals (n=6)

Groups	Dose of Vehicle/drug	No. of Animals
Group-I	Received 1.0 ml/kg p.o. 1% SCMC as vehicle control	6
Group-II	Received 50 mg/kg, p.o. ranitidine as standard	6
Group-III	Received 200 mg/kg, p.o. (HECA)	6
Group-IV	Received 400 mg/kg, p.o. (HECA)	6

Statistical Analysis

The statistical analysis of all the result was carried out using one-way ANOVA followed by Dunnet multiple comparisons using graph pad in stat 3 and all the results obtained in the study were compared with the vehicle control group.

RESULTS AND DISCUSSION:

Percentage loss of *Cassia auriculata* Linn was found to be 39.04%. The results showed that all the values obtained were in the limit as given in Ayurvedic Pharmacopoeia of India (API). The total ash value was found to be 4.63%. The values of acid insoluble ash and water soluble ash value were found to be 0.87%, and 3.51% respectively as given in Table 1. Table No. 2 showed the percentage yield of pet. ether, and hydroalcoholic extracts of *Cassia auriculata* Linn 2.53% and 8.45% respectively. From the table no. 2, it could see that, flavonoids, saponins, proteins, carbohydrate and phenol were present in hydroalcoholic extract of *Cassia auriculata* Linn. The phytochemical screening of *Cassia auriculata* Linn revealed negative results for glycosides, alkaloids and diterpenes.

The total flavonoid content and total phenol content were calculated from the absorbance calibration curve generated with different concentrations of quercetin and gallic acid (standards) respectively, which is shown in table 3. The total phenolic content of the hydroalcoholic extract was 0.463 GAE mg/100mg. Similarly, the TFC was assessed 0.874 quercetin equivalents mg/100mg in hydroalcoholic extract. Flavonoids, including flavones, flavanols, and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free hydroxyl groups. Plants rich in secondary metabolites, including phenolic, flavonoids, and carotenoids, have antioxidant activity due to their redox properties and chemical structures.

Peptic ulcers are a deep gastrointestinal erosion disorder. It is due to various factors such as smoke, antiinflammatory drugs, alcohol, stress, fatty foods, Helicobacter pylori infections triggered tissue necrosis, free radical generation, decreased mucus/bicarbonate secretion and cessation of nutrient delivery, etc. Hydrochloric acid together with pepsin is responsible for maintaining the lesion once it is produced.

There are different drugs available for the treatment of peptic ulcer. But several reports on clinical evaluation of these drugs show that there are incidences of relapses, adverse effects (arrhythmias, impotence, gynecomastia, etc.) and drug interactions during ulcer therapy. In the last few years, efforts have been taken to identify new antiulcer drugs from plants with less adverse effects.

So, the present study is aimed to evaluate the antiulcer activity of hydroalcoholic extract of *Cassia auriculata* plant in rats. The antiulcer activity of the *Cassia auriculata* plant extract was evaluated in pyloric ligation model and ethanol induced gastric ulcer model. In pyloric ligation model, the rats were divided into four groups of six each. Group I rats were treated with vehicle and served as negative control and Group II rats were treated with standard drug Ranitidine 50 mg/kg , Group III and IV treated with hydro alcoholic extract of *Cassia auriculata* (200 and 400 mg/kg, p.o respectively.

After 30 min of hydroalcoholic extract of *Cassia auriculata* and Ranitidine treatment pyloric ligation was performed in overnight fasted anesthetized rats. After 4 hours of pyloric ligation animals were sacrificed, abdomen was opened and the esophagus was tied at the end of the stomach. The stomach was isolated and the contents of the stomach were collected in a centrifuge tube. The supernatant was collected and volume of gastric juice and free and total acidity was determined. The stomachs were

removed, opened along the greater curvature and ulcer index were determined.

Table 1: Results of percentage yield of *Cassia auriculata* Linn

Extracts	Percentage yield (%)
Pet. ether	2.53%
Hydroalcoholic	8.45%

Table 2: Result of phytochemical screening of hydroalcoholic extract of *Cassia auriculata* Linn

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve +Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins A) Xanthoproteic Test:	 +Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve

Table 3: Estimation of total phenol and flavonoids content of ethanolic extract of *G. glabra*

S. No.	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	0.463	0.874

Table 4: Effect of hydroalcoholic extract of *Cassia auriculata* on gastric secretion, acidity and ulcer score in aspirin plus pylorus ligated rats

Treatment mg/kg	Volume of gastric secretion ml/100g	Free acidity mEq/l/100g	Total acidity mEq/l/100g	Ulcer area (mm2	Protection (%)
Vehicle control (1% SCMC)	2.660 ± 0.346	220.56 ± 14.42	356.66 ± 23.740	0.00±0.0	NA
Ranitidine 50 mg/kg	1.180 ± 0.228***	0.00**	247.41 ±20.392**	193.33±12.50*	78.29 %
HEAC 200 mg/kg	0.843 ± 0.159***	0.00**	290.83 ± 22.436	618.33±11.00*	30.57 %
HECA 400 mg/kg	0.976 ± 0.143***	0.00**	197.41 ±25.818**	331.67±27.86*	62.76 %

Each value is the mean± S.E.M of six determinations. P**<0.01, ***P<0.001 Dunnet test as compared to control.

CONCLUSION:

In conclusion pyloric ligation model, the hydroalcoholic extract of *Cassia auriculata* (400 mg/kg) showed significant ($p<0.001$).rise in gastric pH as compared to control. Both the doses of hydroalcoholic extract showed significant decrease in free acidity, total acidity and ulcer index as compared to control. The hydroalcoholic extract at the dose of 200 and 400 mg/kg showed 30.57 and 62.76 % protection respectively against pyloric ligation-induced ulcer. In conclusion, the hydroalcoholic extract of I. *Cassia auriculata* has antiulcer activity.

REFERENCES:

1. Harold, Kalant, Grant, Denis M., Mitchel, Jane, 2007. In: Principles of Medical Pharmacology, seventh ed. Elsevier Canada Ltd., p. 557, 558, 559.
2. Mahajan N, Sanghai D. 2009. Evaluation of anti-uler potential of leaves of *Jasminum grandiflorum* L. International Journal of Pharmaceutical Sciences. 1(2), 247-249.
3. Anwar Jamal, Aisha Siddiqui, Tajuddin and M A Jafri 2006. A review on gastric ulcer remedies used in Unani System of Medicine. Vol 5(2) 153-159.
4. Richard M Peek, Martin J Blasser 1997Pathophysiology of Helicobacter pylori-induced Gastritis and Peptic Ulcer Disease.The American Journal of Medicine, Vol102, (2),200-207.
5. Mukherjee, P. K., (2007). "Quality Control of Herbal Drugs", 2nd Edition, Business Horizons, 2007, 2-14.
6. Khandelwal, K.R. (2005). Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn: 15, 29, 149, 56, (2005).
7. Kokate, C.K. Ed. Practical Pharmacognosy, 4th Edn., *Vallabh Prakashan*: 112,120, (1994).
8. Mishra AG, Singh R, Meha P, Parkhe G. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superb* seed extract. Asian J Pharm Educ Res. 2017; 6(2):12-7.
9. OECD Test Guideline 425. Guidelines for Testing of Chemicals. Guidelines 425, Acute Oral Toxicity-Up-and-Down Procedure; 2001.
10. Aguwa C.N. and Mittal G.C. Study of antiulcer activity of aqueous extract of leaves of Pyrenacantha standtii (Family I cacinaceae) using various models of experimental gastric ulcer in rats. Eur. J. Pharmacol. 74:215-219 (1981).
11. Salaj Khare, Mohammed Asad, Sunil S. Dhamanigi, V. Satya Prasad. Antiulcer activity of cod liver oil in rats. Indian J Pharmacol. 2008; 40 (5): 209-214.
12. Kwiecien S, Brzozowski T, Konturek PCH, Konturek SJ. The role of reactive oxygen species in action of nitric oxide-donors on stress-induced gastric mucosal lesions. J Physiol Pharmacol. 2002;53:761–73.
13. Silve MIG, de Sousa FCF. Gastric ulcer etiology. In: Chai J, editor. Peptic ulcer disease. Shanghai, China: InTake; 2011. p. 2–28.
14. Bilici D, Suleyman H, Banoglu ZN, Kiziltunc A, Avci B, Ciftcioglu A, Bilici S. Melatonin prevents ethanol-induced gastric mucosal damage possibly due to its antioxidant effect. Dig Dis Sci. 2002;47:856–61
15. Salvayre R., Braquet P., Perochot L. and Douste-Blazy L. Comparison of the scavenger effect of bilberry anthocyanosides with various flavonoids. Flavonoids Bioflavonoids. 11:437-442 (1982).
16. Asuzu I.U. and Onu O.U. Antiulcer activity of the ethanolic extract of Combretum dolichopetalum root. Int J Crude Drug Res. 28:27-32 (1990).