Evaluation of Invitro Methods of Antioxidant And Antiulcer Activity of Poly Herbal Plants From North India

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Abstract

Ulcers can be caused by a number of different things in humans, including stress, long-term use of anti-inflammatory medicines, etc. It is generally acknowledged that ulcer is the outcome of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism, even if the origin of ulcer is unknown in most cases. As a result, scientists are still looking for ways to improve upon existing anti-ulcer medications by assessing the gastroprotective qualities of various medicinal plants. Traditional medicine relies on Spondias mombin L and Calpurnia aurea to cure inflammation and gastrointestinal disorders. The acid-neutralizing capacity (ANC) of the extract was found to be significantly lower at a concentration of 1500 mg compared to 15.7 with standard Aluminium hydroxide + Magnesium hydroxide (500mg) when assessing for anti-ulcer activities using aqueous extract and in-vitro method as the acidneutralizing capacity and H+/K+ - ATPase inhibition activity method. Maximum percentage inhibition of H+/K+-ATPase activity was seen with 100 g of extract compared to 69.56% with regular Omeprazole.

Keywords: Ulcer Activity, Herbal Extract, Omeprazole, Invitro

Introduction

Ayurveda is just one of the many ancient medical practises that have been around for a long time but are still used today. This ancient Vedic knowledge, which is more commonly known as Ayurvedic Medicine, has been around for a significant amount of time and is frequently acknowledged as being one of the first medical sciences.[1] Ayurveda, also known as the "Mother of All Healing," is an ancient Indian medical practise that has been practised for generations.[2] The term "the science of life" comes from the Sanskrit words "ayur," which means "life," and "veda," which means "science or knowledge." This field of study focuses on achieving a state of internal and external equilibrium through a number of different methods.[3] Herbal remedies have a long and illustrious history that has been well documented and can be traced back to at least the Stone Age in many parts of the world. Herbal medicine was practised in ancient China, Greece, Egypt, and India, as well as in traditional healing rituals practised by Native Americans and Africans.[4] Herbal medicine was used for a wide variety of therapeutic purposes. A number of ancient Indian texts, such as the Vedas and the Samhitas, attest to the fact that herbs are among the Ayurvedic system's most powerful active pharmaceutical components. At the beginning of the 19th century, scientists began to isolate and modify active compounds found in herbal remedies using newly developed chemical analysis tools.[5-6] These procedures were performed on herbal remedies. This marked the beginning of a transition away from using natural medicinal herbs and towards using synthetic pharmaceuticals. During this time, there was a gradual shift away from the use of herbal remedies.According to the findings of a study [7], despite having a potent pharmacological effect, synthetic drugs are more expensive than their natural counterparts and have a greater number of unintended side effects. Peptic ulcer disease has become the focus of both laboratory and clinical investigations in recent years [8] as a direct result of the disease's high incidence rate among people all over the world. Peptic ulcer is a problem in the public health system that has a high prevalence of morbidity and a significant mortality rate. Peptic ulcers are commonly caused when there is an imbalance in the stomach's destructive and protective components [9]. Exogenous factors include excessive alcohol consumption, indiscriminate use of NSAIDs, stress, smoking, and an infection with Helicobacter pylori. Endogenous factors include HCl, pepsin, biliary reflux, lipid peroxidation, and the creation of active oxygen species (ROS) [10]. The mucus-bicarbonate barrier, mucin synthesis, surface phospholipids, prostaglandins (PGs), nitric oxide (NO), mucosal blood flow, cell renewal, growth factors, and antioxidant enzymes are all components of the defence system [11]. Oxidative stress brought on by gastric ulceration causes an increase in the formation of reactive oxygen species (ROS), which has the potential to compromise the epithelial cells' ability to maintain their integrity. An abnormally high production of ROS metabolites has the potential to compromise the natural antioxidant defences of the body [12]. In addition, reactive oxygen species encourage the accumulation of neutrophils in mucosal tissues when gastric ulceration is present. Recent studies [13,14] have shown that the damage caused by ulcers is significantly exacerbated by proinflammatory cytokines, which are responsible for activating neutrophils. Peptic ulcers can be effectively treated with substitutes that control acidic hypersecretion and its immediate

effects on the mucosa of the stomach.[15-16] There are primarily two different kinds of medications that can be used to treat the symptoms that are brought on by acid.

Material & Methods:

We obtained two of the polyherbal plants under study, Spondias mombin L and Calpurnia aurea, from the forest research institute's central nursery in Dehradun, India.

Extract Process

After being allowed to air-dry for fourteen days at room temperature and in the shade, the leaves of Spondias mombin L and Calpurnia aurea were ground into a coarse powder. After that, 600 grammes of powder needed to be macerated with 80 percent methanol in a conical flask using a micro orbital shaker for a total of three days. On day three, we used gauze to separate the supernatant from the marc, and then we filtered the mixture through Whatman No. 1 filter paper to remove any remaining impurities. The remaining residue was macerated once more for a total of six days in the same solvent that had been used for the first two macerations. After being combined, the supernatant from each succeeding filter was dried in a hot oven set to 40 degrees Celsius, and then lyophilized to remove any remaining moisture. After calculating and recording the yield percentage, it was placed in a plastic desiccator for safekeeping [18]. The procedure described in Ayal et al. [18] was altered slightly so that the hydromethanolic extract could be further fractionated with the aid of ethyl acetate, chloroform, and distilled water. In a separatory funnel, 80 grammes of extract were diluted with 360 millilitres of distilled water, and then 360 millilitres of chloroform were partitioned through three times. This process was repeated twice. Condensation of the precipitate was then carried out in an oven at a temperature of 40 degrees Celsius and a pressure of 30 millibars in order to obtain the chloroform fraction. The remainder was segmented into three equal parts by using the same volume of ethyl acetate in each step. In a process that was very similar to the one that was used to dry the chloroform fraction, the filtrates were placed in a hot oven and allowed to dry. After leaving the remaining aqueous fraction to lyophilize for the night, the yields of each of the different fractions were calculated using a percent system. After that, the components were placed in an airtight container and kept at a temperature of 4 degrees Celsius until they were required for the research. Tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids were discovered as a result of confirmatory qualitative phytochemical screening of plant extracts using well-established methods.

Test for Tannins

With the addition of 0.1% ferric chloride, 200 mg of the plant extract was heated with 10 mL of distilled water. The liquid was then examined for blue-black colouring, which would indicate the presence of tannins.

Test for Alkaloids

100 mL of water was used to dissolve the plant extract, which was then filtered before being boiled in steam with 2 mL of the filtrate and 3 drops of 1% HCl. The Mayer-Wagner reagent was then added to 1 mL of the heated mixture. Alkaloids could be detected by the formation of a cream- or brown-red coloured precipitate.

Test for Saponins

The extract was mixed with 5 mL of distilled water and 0.5 mL of agitated. The appearance of saponins was then confirmed by the creation of foam.

Test for Flavonoids and Glycosides

After being combined with 10 mL of ethanol, 200 mg of the plant extract was filtrated. Magnesium ribbon, concentrated HCl, and filtrate were combined in two mL. Pink or red colour formation suggests the presence of flavonoids. When 0.5 mL of crude extract was mixed with 1 mL of distilled water and 1 mL of NaOH, the appearance of a yellowish colour showed the existence of glycosides.

Test for Steroids

The presence of steroids is demonstrated by the creation of a bilayer (red top layer and greenish bottom layer) after 1 mL of the crude extract was mixed with 10 mL of chloroform and 10 mL of sulfuric acid.

Test for Terpenoids

The production of a reddish-brown colour in the terpenoids test, which involved combining 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid, indicated the presence of terpenoids.

Test for Phenols

Three drops of FeCl3 and one millilitre of K2Fe (CN6) were added to around 1 mL of the extract. The development of greenish-blue forms proved that phenols were present.

DPPH radical scavenging assay

The ability of the extracts to neutralise free radicals was determined by carrying out an experiment known as the DPPH radical scavenging assay, which was outlined by Blois [23] and Desmarchelier et al. [24]. The decolorization of a methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to evaluate the plant extractives' capacity to donate hydrogen atoms. DPPH is an acronym for 2,2-diphenyl-1-picrylhydrazyl. In a solution of methanol, DPPH produces a colour that can be described as violet or purple. This colour,

however, changes to varying shades of yellow when antioxidants are present. Following the formation of a solution by dissolving 0.1 mM DPPH in methanol to form a solution, 2.4 mL of this solution was then added to 1.6 mL of extract in methanol at various concentrations ranging from 12.5 to 150 g/mL. After complete agitation with the vortex, the reaction mixture was left to sit at room temperature and out of the light for half an hour. Using spectrophotometry, the absorbance of the mixture was measured at a wavelength of 517 nm. The benchmark that was used was BHT. For the purpose of computing the percentage of DPPH radical scavenging activity, the following equation was utilised:

$$\%\ \text{DPPH radical scavenging activity} = \{(A_0 - A_1)/A_0\} \times 100$$

where the absorbance of the containing or standard is denoted by A1, and the absorbance of the control is denoted by A0. After that, a graph was created in which the concentration was compared to the percentage of inhibition, and the IC50 was calculated using the graph. The experiment was carried out three times, each time with a different concentration.

In-vitro Ant-ulcer Activity

Preparation of H+/K+ - ATPase Enzyme: **Percentage of inhibition = [Activity (Control)- Activity test / Activity(Control)] x 100**

Result & Discussion

In order to prepare the fresh goat stomach that had been purchased from the local butcher, the gastric mucosa of the fundus was cut off and opened, and the inner layer of the stomach was scraped out for the parietal cell. This was done in order to prepare the stomach for the parietal cell. After homogenising the stomach parietal cell in a Tris buffer containing 16 mM and having a pH of 7.4, as well as 10% Triton X-100, the mixture was centrifuged at 6000 rpm for ten minutes. After that, the solution in the supernatant was put to use to inhibit H+/K+ ATPase. The amount of protein can be calculated using Bradford's method, and the BSA value serves as a point of comparison. An analysis of the inhibition of the H+/K+ ATPase The reaction mixture of the sample that contained 0.1 ml of enzyme extract (300 g) and plant extract at various concentrations was per-incubated for 60 minutes at 37 degrees Celsius. The concentrations of the plant extract were as follows: 20 g, 40 g, 60 g, 80 g, and 100 g. As the substrate, 2 mM ATP was added to the mixture, as well as 200 mL each of 2 mM MgCl2 and 10 mL each of KCl, all of which were added in order to initiate the reaction. The reaction was terminated with 4.5% ammonium molybdate after being carried out for 30 minutes at 37 degrees Celsius. The mixture was then centrifuged at 2000 rpm for ten minutes to separate the inorganic phosphate, which was then measured at 660 nm using the Fiske-Subbarow technique. Next, 60% perchloric acid was added to the mixture. In a nutshell, after ten minutes at room temperature, 0.4 millilitres of ANSA, 1 millilitre of 2.5% ammonium molybdate, 4 millilitres of Millipore water, and one millilitre of supernatant were added. The absorbance of inorganic phosphate at 660 nm has been measured at various extract doses, and the enzyme activity has been calculated as the amount of Pi that is released in micromoles per hour. The results were analysed and compared to omeprazole, a well-known anti-ulcer PPA

inhibitor. The results were expressed as Mean SEM. After applying the formula, it was determined that there was a 16% inhibition of the enzyme.

Table:1 Phytochemical Analysis

	Methanol Solvent	
Test	***Spondias mombin* L**	***Calpurnia aurea***
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+
Glycosides	+	-
Carbohydrates	+	+
Steroids	+	-
Tannins	-	-
Triterpenoids	-	+

DPPH Radical Scavenging Assay

The data from Table 2 and Figure 1 show that the combination has the highest levels of DPPH. The data was gathered from the percentage of inhibition. The results were evaluated in comparison to the standard practise of using ascorbic acid. A higher percentage of inhibition is indicative of greater scavenging activity or antioxidant potential. The findings were statistically significant, as determined by a p-value of 0.05.

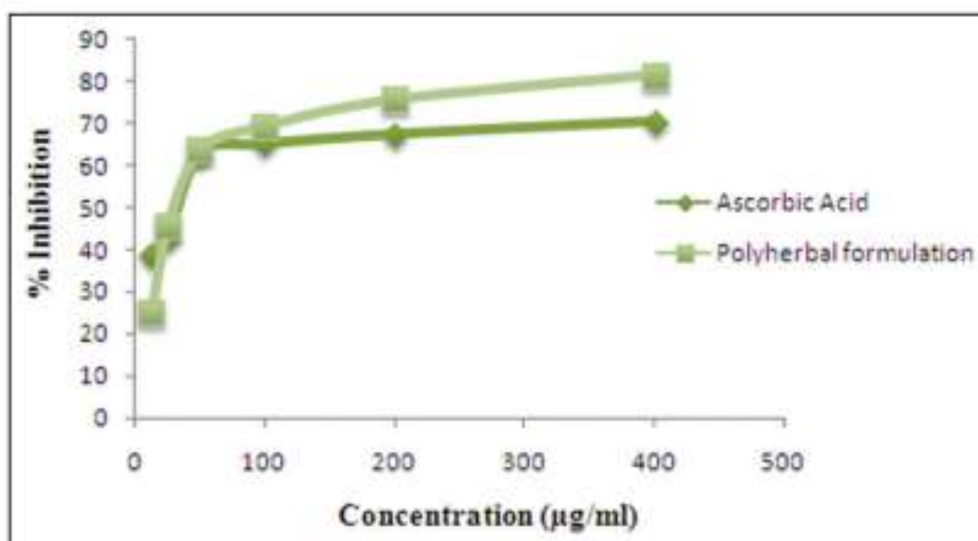

Fig: 1 Antioxidant activity of Poly herbal formulation

Table 2 DPPH Activity of Polyherbal formulation

S.No	Concentration	Ascorbic acid % inhibition	Polyherbal Formulation% inhibition
1	12.5	38.74	26.12
2	25	41.87	44.65
3	50	61.97	68.14
4	100	64.79	68.47
5	200	66.98	76.78
6	400	72.56	81.45
IC_{50}		10.97	36.71

H+/K+ - ATPase Enzyme:

At different concentrations (20g, 40g, 60g, 80g, and 100g), the H+/K+ - ATPase inhibition activity of the aqueous extract was evaluated and contrasted with that of omeprazole, which served as the gold standard. The extract exhibited significant activity that varied significantly with the dose. In comparison to the standard Omeprazole, the extract demonstrated the highest percentage inhibition (62.18 0.54%) when it was present at a concentration of 100 g. At different concentrations (20g, 40g, 60g, 80g, and 100g), the H+/K+ - ATPase inhibition activity of the aqueous extract was evaluated and contrasted with that of omeprazole, which served as the gold standard. The extract exhibited significant activity that varied significantly with the dose. At a concentration of 100g, the extract had a maximum percentage inhibition of 62.180.54%, while the standard omeprazole had a maximum percentage inhibition of 69.561.72%. The findings of the study are presented in Figure 2.

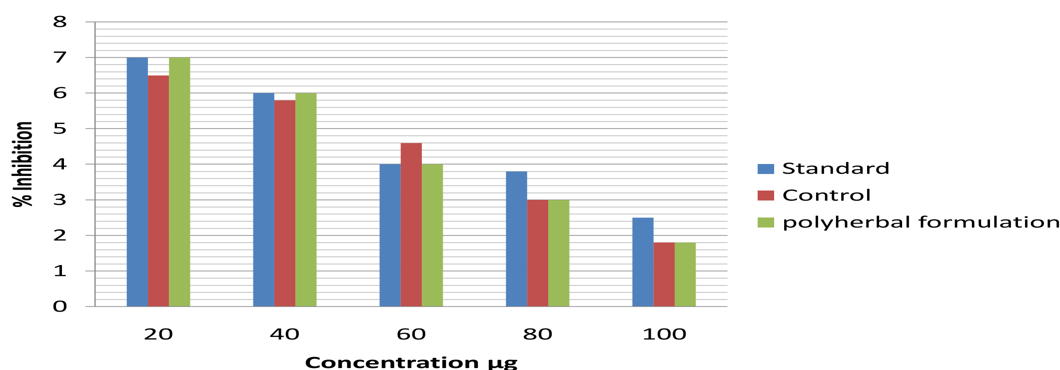

FIG: 2 H+/K+ - ATPASE INHIBITION ACTIVITY OF METHANOLIC EXTRACT IN VITRO

Conclusion

H+-K+ The enzyme ATPase is located on the partial cell's apical secretory membrane. For the treatment of ulcers, there are many medications like PPIs, H2 blockers, etc., but because they have interactions and side effects, it is necessary to produce herbal medications using cutting-edge technology to create effective medications with fewer side effects. In the current study, extracts and omeprazole both inhibited the enzyme in a dose-dependent manner. Methanolic extract significantly demonstrated better inhibition than chloroform extract.

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