Phytochemical and Pharmacological Evaluation of Cucurbita pepo and Benincasa hispida their Antioxidant activity and Gastroprotective activity

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Abstract

The ethanol extract of Benincasa hispida at varied concentrations showed remarkable inhibitory effect of superoxide radical activity scavenging compared to petroleum ether and ethyl acetate extract. From this work we conclude that all the extracts were exhibiting significant scavenging activity towards 1, 1-di phenyl picryl hydrazyl, Nitric oxide, Hydroxyl, Super oxide radicals. The activity was found to be concentration dependent. In DPPH model the free radical scavenging capacity was found to be highly significant when compare other three models. In all the three selected plants Ethanol extract was found to have high scavenging activity than Ethyl acetate and petroleum ether extracts. Scavening activity of ethanol extracts may be due to presence of the flavonoids and phenolic. Acute toxicity studies were performed for extracts of selected three plants according to the toxic classic method as per guidelines 423 prescribed by OECD. Acute toxicity studies of extracts of Cucurbita pepo and Benincasa hispida fruit were performed in animals at dose levels of 50, 300 and 2000 mg/kg as per OECD guide lines. No mortality was observed in animals dosed with the extracts of Cucurbita pepo and Benincasa hispida fruits at dose levels of 50, 300 and 2000 mg/kg (p.o). The treated animals did not demonstrate any significant changes in behavioral pattern and exhibited normal activity. Also, there were no clinical signs of tremors, convulsions, exophthalmos, salivation, diarrhea and lethargy. There was no significant difference in the mean body weights between treated groups and control group and the rats exhibited normal body weight gain during the study. No lethal effects or mortality was observed in animals throughout the test period following single oral administration at all selected dose levels of all extracts. The animals were examined for long term toxicity (14 days). None of these extracts showed any mortality even at the dose of 2000mg/kg. From the results of acute toxicity studies 1/10th , 1/20th doses were selected for the experimental study. Gastro Protective Activity of extract of Cucurbita pepo fruit and Benincasa hispida fruits were performed on various ulcer induce model including aspirin + Pylorus ligation induced, acetic acid induced chronic ulcer, HCl- ethanol induced ulcer. Ethanol extracts of Cucurbita pepo fruit and Benincasa hispida fruits were found more potent in antioxidant activity so ethanol extract were selected for further gastroprotective activity. Ethanol extracts of both plant at dose of 100 200 and 300 mg/kg were tested for gastroprotective activity using above ulcer model.

INTRODUCTION

Peptic ulcers are sores or lesions in the gastrointestinal mucosa extending throughout the muscularis mucosae, typically characterized by different stages of necrosis, neutrophil infiltration, blood flow reduction, increased oxidative stress and inflammation. PU manifest as a non-fatal disease, majorly represented by periodic symptoms of epigastric pain, which are often relieved by food or alkali, besides to trigger much discomfort to patients, disrupting their daily routines and also causing mental agony. The disease is mostly categorized based on its anatomical origins, such as gastric (found along the lesser curvature of the stomach) and duodenal (occurring in the duodenal bulb—the most exposed area to gastric acid) ulcers.

Studies have shown that peptic ulcer disease occurs because of an imbalance between aggressive injurious (e.g., pepsin, HCl) and defensive mucosa-protective factors (e.g., prostaglandins, mucus and bicarbonate barrier and adequate blood flow). All ulcers of the upper gastrointestinal tract were originally thought to be caused by the aggressive action of pepsin and gastric acid on mucosa However, the denomination “peptic ulcer” has lately pointed to Helicobacter pylori infection, where the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (ASA) are some of the disease-causing factors. Thus, based on the latest advances on this field and stress the fact that PUD is an important cause of morbidity and health care costs, the present report aims to provide a general overview on peptic ulcers, namely considering their epidemiology, main symptoms and clinical features, pathogenesis, where a particular emphasis will be given to H. pylori infection, pharmacological agents used in an effective management and also pointing out the latest challenges and opportunities of using plant phytochemicals as upcoming antiulcerogenic agents. Lastly, a special emphasis was given on plant products safety and security,

in order to trigger the interest in deepening skills on this matter and to ensure an effective managing competence for health-related systems [1].

Material and Methods

Procurement and Authentication of Plant material (Cucurbita pepo and Benincasa hispida): Pharmacognostic study helps in confirmation and determination of identity, purity and quality of a crude drug. Morphological characterization of Cucurbita pepo reveals that it is a sprawling vine with yellow fruit-bearing flowers. The of Cucurbita pepo have a mild flavor. Size and weight of fruit may vary.

Benincasa hispida is a large climbing or trailing herb with stout hispid stems. Fruits are 30 to 45 cm long broadly, cylindric, not ribbed hairy, ultimately covered with a waxy bloom. Fruit is covered in a fuzzy coating of fine hairs when young. The immature melon has thick white flesh that tastes sweet. By maturity, the fruit loses its hairs and develops a waxy coating, giving rise to the name wax gourd. The fruit may grow as large as 80 cm in length. It has yellow flowers and broad leaves[2].

Figure 1: Cucurbita pepo bearing branch

Figure 2: Benincasa hispida fruit

Table 1: Organoleptic identification of Cucurbita pepo fruit

S. No.	**Parameters**	**Cucurbita pepo**
1	Shape and size	Fruits are ovoid ribbed size 13-45cm long covered withhard peel
2	Colour	outer (greenish yellow) inner (yellow)
3	Odour	Characteristics
4	Taste	Sweet

Table 2: Organoleptic identification of Benincasa hispida fruit

S. No.	Parameters	*Benincasa hispida*
1.	Shape and size	Fruits are 30 to 50 cm long broadly, cylindric, not ribbedhairy, ultimately covered with a waxy bloom
2.	Colour	outer (greenish waxy) inner (white)
3.	Odour	Characteristics
4.	Taste	Sweet

Physicochemical parameter studies on selected plants: Total ash of Cucurbita pepo fruit was found 6.4%. Water soluble ash was found 2.91 % whereas 0.91% was acid insoluble ash. The ethanol soluble extractive values were found to be 9.6% and water-soluble extractive values were found to be 13.8 %. The moisture content of the powder estimated as percentage loss on drying (LOD) was found to be 29.2 % w/w (Table 5.3).

Total ash of Benincasa hispida fruit was found 5.7%. Water soluble ash was found 2.68 % whereas 0.98% was acid insoluble ash. The ethanol soluble extractive values were found to be 8.3 % and water-soluble extractive values were found to be 10.9% and loss on drying (LOD) was found to be 27.86% w/w (Table 4).

Table 3: Physicochemical parameters of Cucurbita pepo fruit

S. No.	Physicochemical parameter values	values (% w/w)
1	Total ash	6.4
2	Water soluble ash	2.91
3	Acid insoluble ash	0.91
4	Foreign organic matter determination	1.05
5	Ethanol soluble extractive	9.6
6	Water-soluble extractive	13.8
7	Loss on drying (%)	29.2

Table 4: Physicochemical parameters of Benincasa hispida

S. No.	Physicochemical parameter	values (% w/w)
1	Total ash	5.7
2	Water soluble ash	2.68
3	Acid insoluble ash	0.98
4	Foreign organic matter determination	0.98
5	Ethanol soluble extractive	8.3
6	Water-soluble extractive	10.9
7	Loss on drying (%)	27.86

Extraction of the Drugs: The coarse powder of the fruit of Cucurbita pepo and Benincasa hispida were subjected to successive solvent extraction using solvents of ascending polarity. After extraction the percentage yield of each extract was calculated with reference to the air dried drug used for the study. The percentage yield and other characteristic features of the extracts are tabulated below[3].

Table 5: Extractive value of Cucurbita pepo fruit

S. No.	Extract	Yield (%W/W)
1	Petroleum ether	6.5
2	Ethyl acetate	9.7
3	Ethanol	18.3

Table 6: Extractive value of Benincasa hispida fruit

S. No.	Extract	Yield (%W/W)
1	Petroleum ether	5.2
2	Ethyl acetate	8.5
3	Ethanol	15.8

Preliminary Phytochemical Screening: The qualitative phytochemical screening of the tubers for the presence of alkaloids, carbohydrate, reducing sugars, glycosides like anthraquinones, flavanoids, saponins, tannins, phenolic compounds, fixed oils, fats, proteins, amino acids and sterols in petroleum ether, ethyl acetate and ethanol extracts of the fruit of Cucurbita pepo and Benincasa hispida were carried out[4].

Table 7: Phytochemical analysis of Cucurbita pepo fruit extracts

Tests of Phytoconstituents	Petroleum ether extract	Ethyl acetate extract	Ethanol extract
1. Alkaloids			
a) Mayer's reagent	-	-	+
b) Dragendorff's reagent	-	-	+
2. Flavonoids			
a) Shinoda test	-	+	+
3. Saponins			
a) Froth test	-	-	+
4. Carbohydrate			
a) Molisch's test	-	+	+
c) Test for gums	-	+	+
d) Test for mucilage	-	+	+
5. Phytosterols			
a) Libermann-Burchard test	+	-	-
c) Salkowski reaction:	+	-	-
6. Tannins and Phenolic			
a) With Lead acetate	-	+	+
7. Cardiac glycoside			
a) (a) Borntrager's test	-	+	+
b) Legal's test	-	+	+
8. Coumarins			
a) With ammonia	-		-
b) Hydroxylamine HCl	-		-
9. Proteins			
a) Biuret test	-	+	+
10. Triterpens			
a) Vanillin sulphuric acid	+		-

Table 8: Phytochemical analysis of Benincasa hispida fruit extract

Tests of Phytoconstituents	Petroleum ether extract	Ethyl acetate extract	Ethanol extract
1. Alkaloids			
a) Mayer's reagent	-	-	+
b) Dragendorff's reagent	-	-	+
2. Flavonoids			
a) Shinoda test	-	+	+
3. Saponins			

a) Froth test	-	-	+
4. Carbohydrate			
a) Molisch's test	-	+	+
c) Test for gums	-	+	+
d) Test for mucilage	-	+	+
5. Phytosterols			
a)Libermann-Burchardtest	+	-	-
c) Salkowski reaction:	+	-	-
6. Tannins and Phenolic			
a) With Lead acetate	-	+	+
7. Cardiac glycoside			
a) (a)Borntrager's test	-	+	+
b) Legal's test	-	+	+
8. Coumarins			
a) With ammonia	-		-
b) Hydroxylamine HCl	-		-
9. Proteins			
a) Biuret test	-	+	-
10. Triterpens			
a) Vanillin sulphuric acid	+		-

Anti-oxidant Activity:

Analysis of the free radical scavenging activities of the selected Cucurbita pepo fruit and Benincasa hispida fruits extracts revealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, NO, Hydroxyl radical and superoxide radical radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher[5].

DPPH radical scavenging activity of Cucurbita pepo and Benincasa hispida fruits extracts: DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of DPPH was determined by the decrease in its absorbance at 517nm, which is induced by antioxidant. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of l- Ascorbic acid, and plant extracts increased gradually with increase in concentration[6].

Percentage Inhibition of in vitro anti oxidant result of various cucurbita pepo fruit extracts by DPPH Method and ascorbic acid as standard at various concentrations are given in Table 5.9, Table 5.10 and Figure 5.3, Figure 5.4. In case of cucurbita pepo extracts the order of reduction potential was: Ascorbic acid> EOCP > EACP> PECP[7].

Table 9: Results of Cucurbita pepo fruit extracts on DPPH radical scavenging model

Concentration	100µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Ascorbic acid	74.12	77.14	82.14	85.41	96.14
PECP	12.45	18.47	29.45	36.74	48.54
EACP	30.14	34.87	40.28	52.47	62.87
EOCP	64.87	74.87	78.25	80.98	84.65

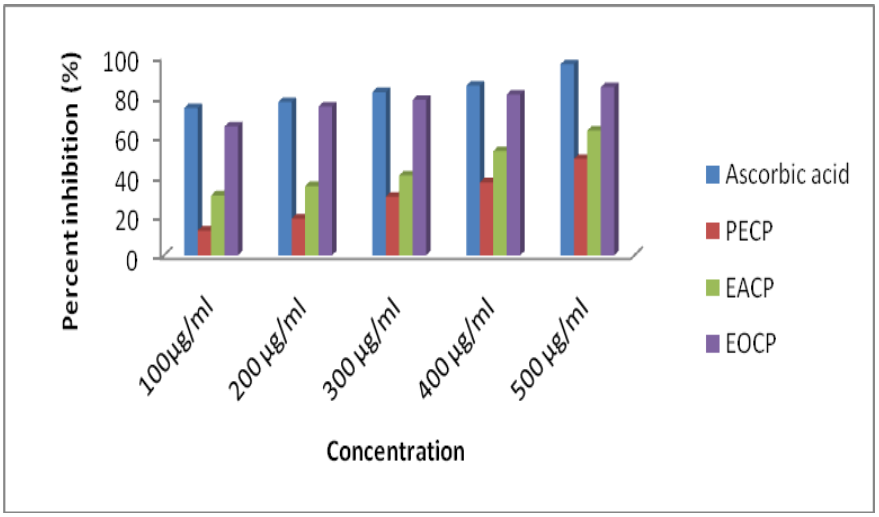

Figure 3: Graphical representation of Cucurbita pepo fruit extracts on DPPH model

Table 10: In vitro 50% inhibition concentration (IC50) of Cucurbita pepo fruit extracts on DPPH radical scavenging model

Extract /compound	50% inhibition concentration (IC_{50}) of DPPH model (µg/ml)
Ascorbic acid	66
PECP	500
EACP	398
EOCP	80.32

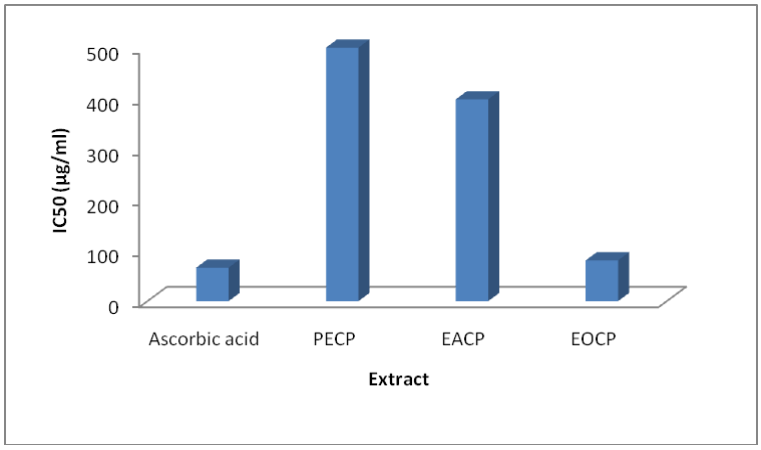

Figure 4: Graphical representation of 50% Inhibitory Concentration (IC50) Cucurbita pepo fruit and Ascorbic acid on DPPH radical scavenging model

Percentage Inhibition of in vitro anti oxidant result of various Benincasa hispida fruit extracts by DPPH Method and ascorbic acid as standard at various concentrations are given in Table 5.11, Table 5.12 and Figure 5.5 , Figure 5.6 . In case of Benincasa hispida extracts the order of reduction potential was: Ascorbic acid> PEBH> EABH> EOBH[8].

Table 11: Results of Benincasa hispida fruits extracts on DPPH radical scavenging model

Concentration	100µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Ascorbic acid	74.12	77.14	82.14	85.41	96.14
PEBH	11.27	17.87	26.57	34.16	46.87
EABH	29.78	33.74	39.47	50.87	61.87
EOBH	62.47	71.14	75.87	78.14	82.59

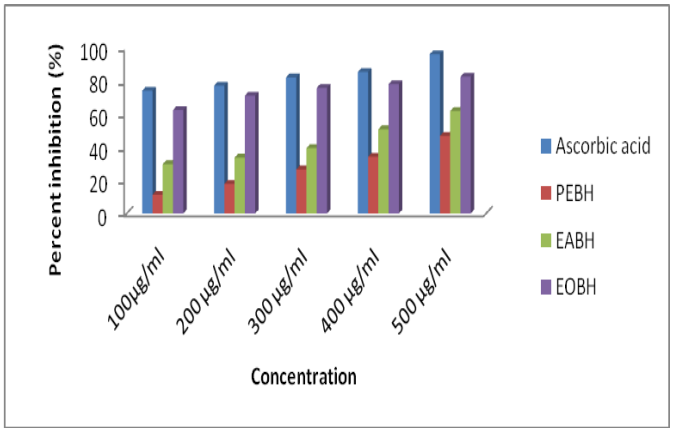

Figure 5: Graphical representation of Benincasa hispida fruits extracts on DPPH model

Table 12: In vitro 50% inhibition concentration (IC50) of Benincasa hispida fruits extracts on DPPH radical scavenging model

Extract /compound	50% inhibition concentration (IC_{50}) of DPPH model (µg/ml)
Ascorbic acid	66
PEBH	508
EABH	406
EOBH	85.64

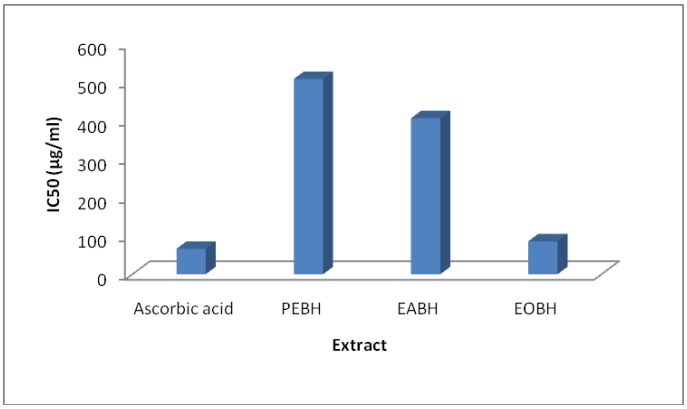

Figure 6: Graphical representation of 50%Inhibitory Concentration (IC50) Benincasa hispida fruits extracts and Ascorbic acid on DPPH radical scavenging model.

Nitric Oxide (NO) radical scavenging activity of Cucurbita pepo and Benincasa hispida fruits extracts:

Nitric oxide plays an important role in various types of inflammatory processes in the body. In the present study the fruit extracts of selected cucurbita pepo and Benincasa hispida checked for its inhibitory effect on Nitric oxide production. Nitric oxide radical generated for sodium nitroprusside at physiological pH was found to be inhibited by the extracts.

The ethanol extract of cucurbita pepo at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. Results (Table 5.13, Table 5.14, Figure 5.7, Figure 5.8) revealed that all the tested extracts showed the percentage of inhibition in a dose dependent manner[9].

Table 13: Results of Cucurbita pepo fruit extracts on Nitric oxide radical scavenging model

Concentration	**100µg/ml**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
Ascorbic acid	76.24	80.14	84.98	90.25	97.54
PECP	12.54	25.64	38.74	41.54	54.87
EACP	52.68	55.74	58.74	65.45	71.05
EOCP	60.87	67.87	71.25	74.65	80.25

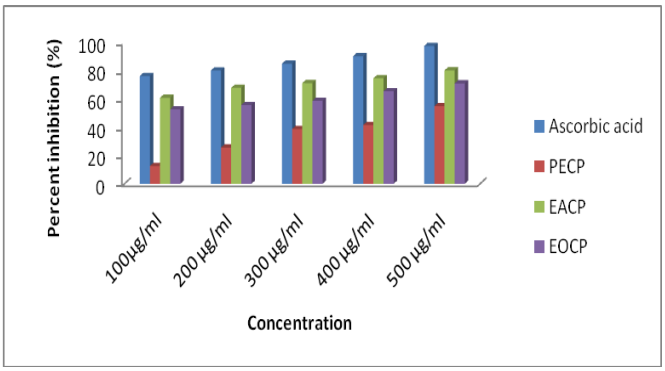

Figure 7: Graphical representation of Cucurbita pepo fruit extracts on Nitric oxide radical scavenging model

Table14: In vitro 50% inhibition concentration (IC50) of Cucurbita pepo fruit extracts on Nitric oxide radical scavenging model

Extract /compound	**50% inhibition concentration (IC_{50}) NO model (µg/ml)**
Ascorbic acid	67
PECP	492
EACP	100.2
EOCP	85

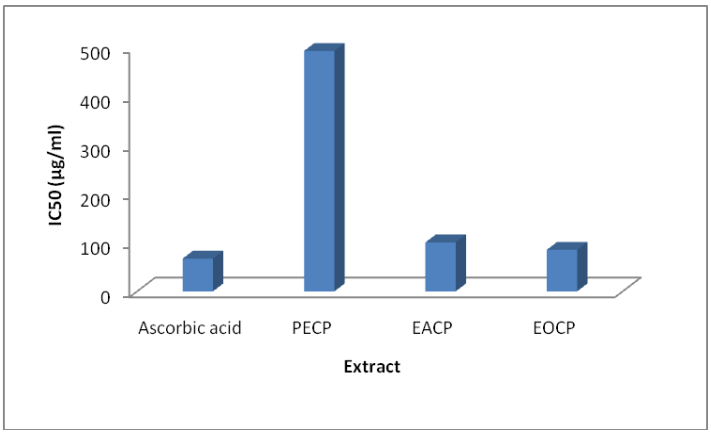

Figure 8: Graphical representation of 50% Inhibitory Concentration (IC50) Cucurbita pepo and Ascorbic acid on Nitric oxide radical scavenging model.

The inhibitory effect of Benincasa hispida fruits extracts on Nitric oxide radical scavenging model were shown in Table 12, Table 20 Figure 6. The ethanol extract of Benincasa hispida at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. The ethanol extract of cucurbita pepo at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. The ethanol extract of cucurbita pepo showed more activity than ethanol extract of Benincasa hispida[9-10].

Table 15: Results of Benincasa hispida fruits extracts on Nitric oxide radical scavenging model.

Concentration	100µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Ascorbic acid	76.24	80.14	84.98	90.25	97.54
PEBH	9.87	21.54	35.41	37.84	51.42
EABH	50.75	53.12	56.18	62.18	68.36
EOBH	58.47	65.14	69.18	72.58	78.94

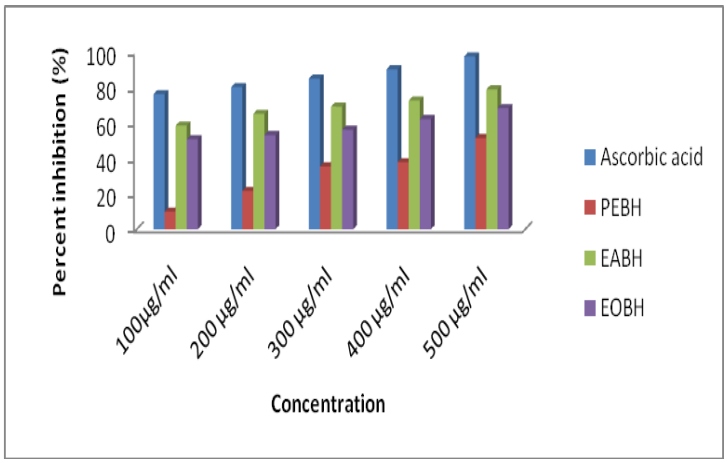

Figure 9: Graphical representation of Benincasa hispida fruits extracts on Nitric oxide radical scavenging model.

Table 16: In vitro 50% inhibition concentration (IC50) of Benincasa hispida fruits extracts on Nitric oxide radical scavenging model.

Extract /compound	**50% inhibition concentration (IC_{50}) NO model (µg/ml)**
Ascorbic acid	67
PEBH	495
EABH	102.54
EOBH	88

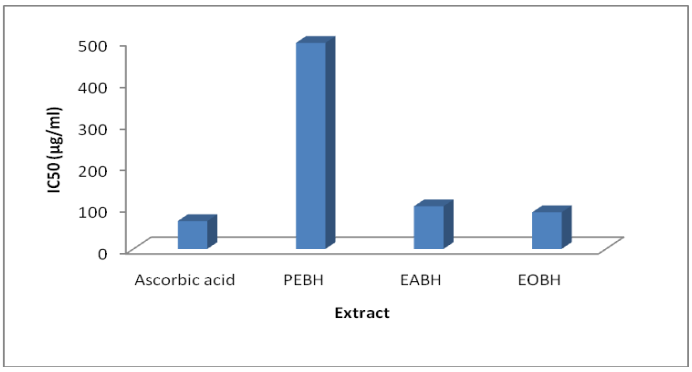

Figure 10: Graphical representation of 50% Inhibitory Concentration (IC50) Benincasa hispida fruits and Ascorbic acid on Nitric oxide radical scavenging model.

Hydroxyl radical scavenging activity of Cucurbita pepo and Benincasa hispida fruits extracts: The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The effect of the selected plant extracts were assessed by means of the iron (II)- dependent DNA damage assay. The fentone reaction generated hydroxyl radicals (OH) which degrade DNA deoxy ribose, using Fe 2+ salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. All the results showed hydroxyl radical scavenging activity in a dose dependent manner[11].

The ethanol extract of cucurbita pepo at varied concentrations showed remarkable inhibitory effect of Hydroxyl radical scavenging activity compared to petroleum ether and ethyl acetate extract. The ethanol extract of cucurbita pepo showed more inhibitory effect of Hydroxyl radical scavenging activity compared ethanol extract of Benincasa hispida.

Table17: Results of Cucurbita pepo fruit extracts on Hydroxyl radical scavenging model

Concentration	**100µg/ml**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
Ascorbic acid	60.25	65.47	70.28	75.15	91.36
PECP	14.78	20.54	24.63	40.28	54.24
EACP	26.48	38.34	50.41	57.26	62.37
EOCP	54.38	60.26	65.38	69.24	75.26

All the results showed hydroxyl radical scavenging activity in a dose dependent manner.

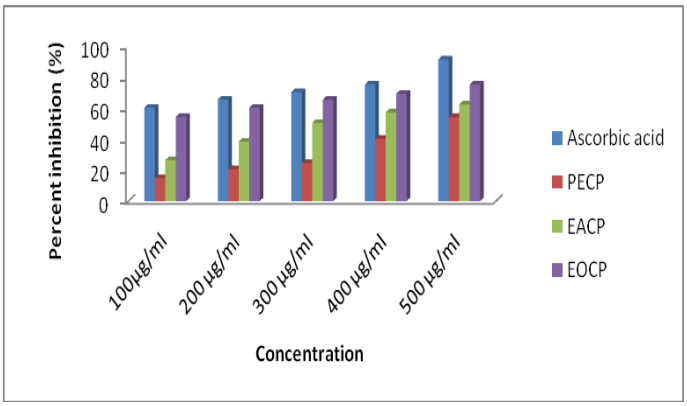

Figure 11: Graphical representation of Cucurbita pepo fruit extracts on Hydroxyl radical scavenging model

Table 5.: In vitro 50% inhibition concentration (IC50) of Cucurbita pepo fruit extracts on hydroxyl radical scavening model

Extract /compound	**50% inhibition concentration (IC_{50}) Hydroxyl radical model (µg/ml)**
Ascorbic acid	82
PECP	503
EACP	398
EOCP	102

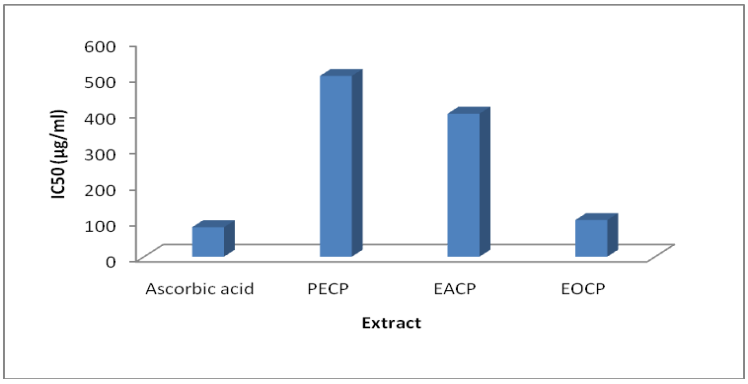

Figure 12: Graphical representation of 50% Inhibitory Concentration (IC50) Cucurbita pepo fruit extracts and Ascorbic acid on Hydroxyl radical scavenging model

The ethanol extract of Benincasa hispida at varied concentrations showed remarkable inhibitory effect of Hydroxyl radical scavenging activity compared to petroleum ether and ethyl acetate extract[12].

Table 19: Results of Benincasa hispida fruits extracts on Hydroxyl radical scavenging model

Concentration	**100µg/ml**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
Ascorbic acid	60.25	65.47	70.28	75.15	91.36
PEBH	12.54	18.62	23.74	37.41	51.64
EABH	25.15	36.15	48.31	55.36	60.87
EOBH	50.27	57.15	61.84	65.63	71.24

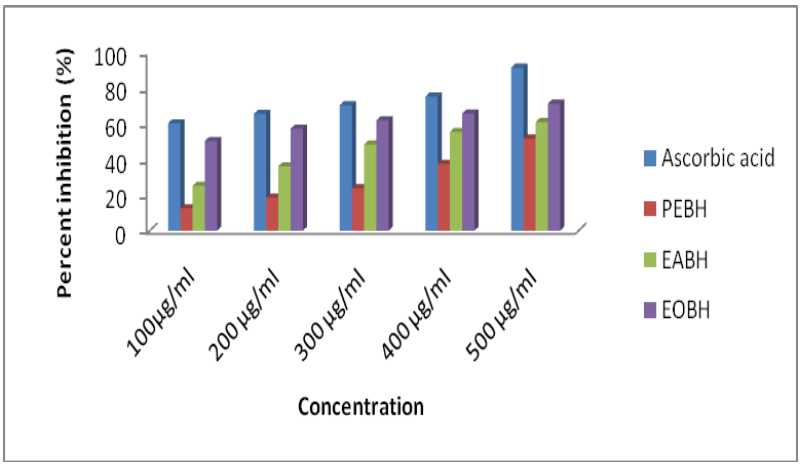

Figure 13: Graphical representation of Benincasa hispida fruits extracts on Hydroxyl radical scavenging model.

Table 20: In vitro 50% inhibition concentration (IC50) of Benincasa hispida fruit extracts on hydroxyl radical scavening model.

Extract /compound	**50% inhibition concentration (IC_{50}) Hydroxyl radical model (µg/ml)**
Ascorbic acid	82
PEBH	508
EABH	403
EOBH	107

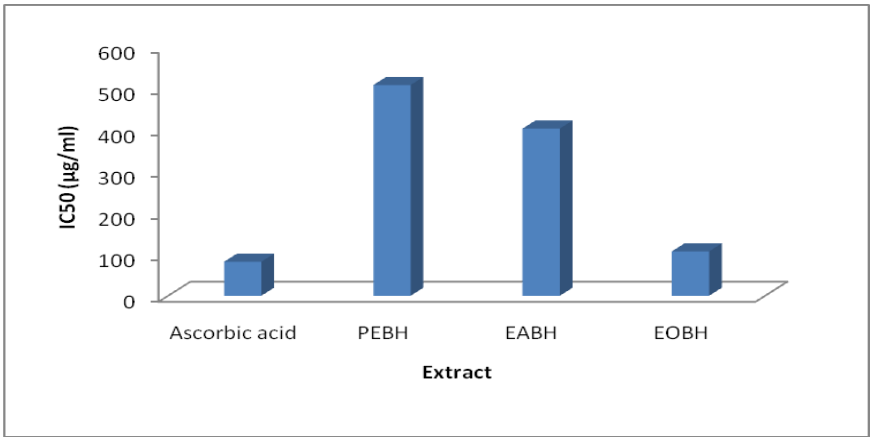

Figure 14: Graphical representation of 50%Inhibitory Concentration (IC50) Benincasa hispida fruits extracts and Ascorbic acid on Hydroxyl radical scavenging model.

Super oxide radical scavenging activity of Cucurbita pepo and Benincasa hispida fruits extracts: Superoxide is a reactive oxygen species, which can cause damage to the cells and DNA leading to various diseases. It was therefore proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical. Several In vitro methods are available for generation of super oxide radicals. In the present study the superoxide radicals were generated by auto-oxidation of hydroxylamine in the presence of NBT (Nitro blue tetrazolium). The reduction of NBT in presence of antioxidants was measured. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The ethanol extract of Benincasa hispida at varied concentrations showed remarkable inhibitory effect of superoxide radical activity scavenging compared to petroleum ether and ethyl acetate extract. The ethanol extract of cucurbita pepo at varied concentrations showed remarkable inhibitory effect of superoxide radical scavenging activity compared to petroleum ether and ethyl acetate extract[13].

Table 21: Results of Cucurbita pepo fruit extracts on Super oxide radical scavenging activity

Concentration	100µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Ascorbic acid	35.25	45.35	54.17	62.87	70.84
PECP	20.68	24.74	39.54	50.47	55.31
EACP	18.98	32.54	35.45	49.75	60.38
EOCP	33.14	36.57	49.54	62.14	65.87

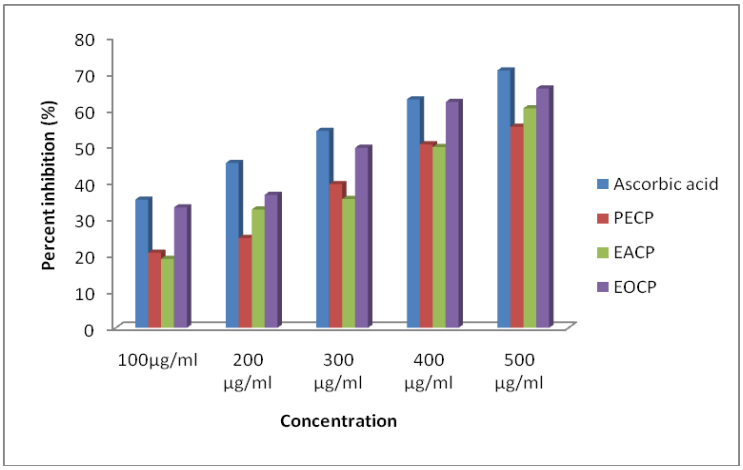

Figure15: Graphical representation of Cucurbita pepo fruit extracts on Super oxide radical scavenging model.

Table 22: In vitro 50% inhibition concentration (IC50) of Cucurbita pepo fruits extracts on Superoxide radical scavenging model.

Extract /compound	50% inhibition concentration (IC_{50}) superoxide model (µg/ml)
Ascorbic acid	160
PECP	499
EACP	509
EOCP	396

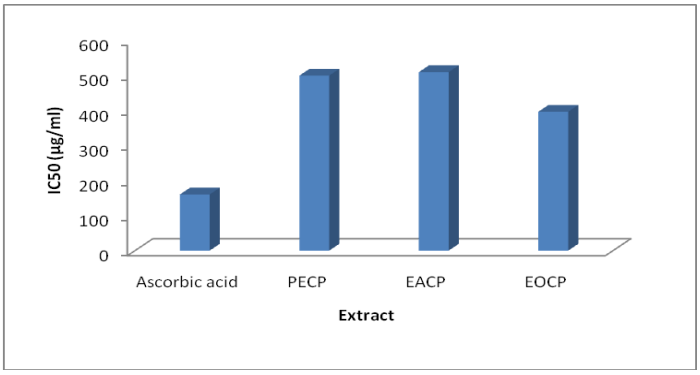

Figure 16: Graphical representation of 50% Inhibitory Concentration (IC50) Cucurbita pepo fruit extracts and Ascorbic acid on Super oxide radical scavenging model

The ethanol extract of Benincasa hispida at varied concentrations showed remarkable inhibitory effect of superoxide radical activity scavenging compared to petroleum ether and ethyl acetate extract[14].

Table 23: Results of Benincasa hispida fruits extracts on Super oxide radical scavenging activity

Concentration	**100µg/ml**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
Ascorbic acid	35.25	45.35	54.17	62.87	70.84
PEBH	18.31	22.23	36.41	47.24	52.63
EABH	16.26	30.31	32.45	47.51	58.24
EOBH	31.87	34.98	47.54	60.45	62.87

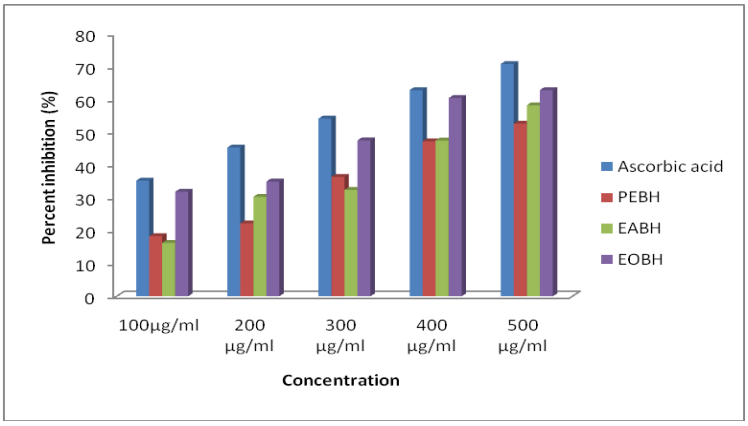

Figure 17: Graphical representation of Benincasa hispida fruits extracts on Super oxide radical scavenging model.

Table 24: In vitro 50% inhibition concentration (IC50) of Benincasa hispida fruits extracts on Superoxide radical scavenging model.

Extract /compound	**50% inhibition concentration (IC_{50}) superoxide model (µg/ml)**
Ascorbic acid	160
PEBH	504
EABH	513
EOBH	399

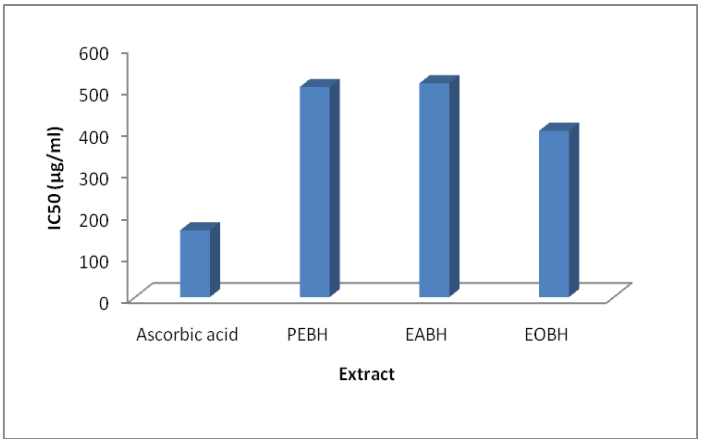

Figure 18: Graphical representation of 50 % Inhibitory Concentration (IC50) Benincasa hispida fruits extracts and Ascorbic acid on Super oxide radical scavenging model.

From this work we conclude that all the extracts were exhibiting significant scavenging activity towards 1, 1-di phenyl picryl hydrazyl, Nitric oxide, Hydroxyl, Super oxide radicals. The activity was found to be concentration dependent. In DPPH model the free radical scavenging capacity was found to be highly significant when compare other three models. In all the three selected plants Ethanol extract was found to have high scavenging activity than Ethyl acetate and petroleum ether extracts. Scavenging activity of ethanol extracts may be due to presence of the flavonoids and phenolic[15].

Acute Toxicity studies of Cucurbita pepo and Benincasa hispida fruit extracts as per as per guidelines 423 prescribed by OECD

Acute toxicity studies were performed for extracts of selected three plants according to the toxic classic method as per guidelines 423 prescribed by OECD. Acute toxicity studies of extracts of Cucurbita pepo and Benincasa hispida fruit were performed in animals at dose levels of 50, 300 and 2000 mg/kg as per OECD guide lines. No mortality was observed in animals dosed with the extracts of Cucurbita pepo and Benincasa hispida fruits at dose levels of 50, 300 and 2000 mg/kg (p.o). The treated animals did not demonstrate any significant changes in behavioral pattern and exhibited normal activity. Also there were no clinical signs of tremors, convulsions, exophthalmos, salivation, diarrhea and lethargy. There was no significant difference in the mean body weights between treated groups and control group and the rats exhibited normal body weight gain during the study. No lethal effects or mortality was observed in animals throughout the test period following single oral administration at all selected dose levels of all extracts. The animals were examined for long term toxicity (14 days).. None of these extracts showed any mortality even at the dose of 2000mg/kg. From the results of acute toxicity studies 1/10th , 1/20th doses were selected for the experimental study[16].

Gastro Protective Activity

Gastro Protective Activity of extract of Cucurbita pepo fruit and Benincasa hispida fruits were performed on various ulcer induce model including aspirin + Pylorus ligation induced, acetic acid induced chronic ulcer, HCl- ethanol induced ulcer. Ethanol extracts of Cucurbita pepo fruit and Benincasa hispida fruits were found more potent in antioxidant activity so ethanol extract were selected for further gastroprotective activity. Ethanol extracts of both plant at dose of 100 200 and 300 mg/kg were tested for gastroprotective activity using above ulcer model[17].

Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) and Benincasa hispida fruit (EOBH) on Aspirin + Pylorus ligation induced ulcer

Aspirin+pylorus ligation-induced gastric ulcer model is a useful model to induce severe ulceration in experimental animals. Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H+ ions. The inhibition of mucosal prostaglandin production occurs rapidly following oral administration of aspirin. This is correlated with the rapid absorption of these drugs through the mucos. In pylorus ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration[18-20].

Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H+ ions. In pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration. Aspirin was administered to PL rats; thus, aspirin further aggravated the acidity and the resistance of the gastric mucosa was decreased thereby causing extensive damage to the glandular regions of the stomach[21].

Ethanol extracts of Cucurbita pepo fruit and Benincasa hispida fruits at a dose of 100 200 and 300 mg/kg b.w., were tested for gastroprotective activity using pyloric ligation rat model. Peptic ulcer is results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid[22-23].

Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on aspirin + Pylorus ligation induced ulcer

Antiulcer study has been performed using 100, 200 and 300 mg/kg of ethanol extract of Cucurbita pepo fruit against aspirin + Pylorus ligation gastric ulcer models. The ethanol extract were administered to various groups, orally, twice a day as described

earlier. The result indicated a dose-dependent antiulcerogenic activity of extract EOCP. The best effect observed was at dose of 300 mg/kg onwards with EOCP. So for further studies on other biochemical parameters of gastric secretion or mucosal studies, a dose of 300 mg/kg was selected[24-30].

Table 25: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on aspirin + Pylorus ligation induced ulcer

Group	Treatment Dose (mg/kg)	Ulcer index (mm2/rat)	% Protection
I	Control	15.1 ± 2.4	-
II	standard	2.7 ± 1.5	83.78
III	EOCP (100 mg/kg)	6.9 ± 2.2	53.29
IV	EOCP (200 mg/kg)	4.4 ± 2.2*	73.18
V	EOCP (300 mg/kg)	3.1 ± 1.7**	80.21

Values are mean ± SEM for 6 rats

* P < 0.05, **P < 0.01, compared to control group

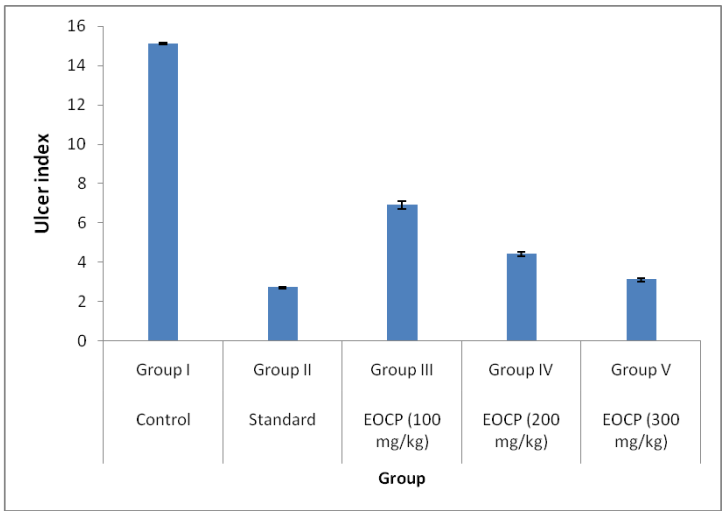

Figure 19: Effect of ethanolic extract of Cucurbita pepo fruit (EOCP) on ulcer index in aspirin + Pylorus ligation induced ulcer

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