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Antioxidant And Antiulcer Activity Of Phytoconstituents Isolated From *Cocinnia Grandis And Diplocyclos Palmatus* Fruit

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Abstract

*Cocinnia grandis and Diplocyclos pa*lmatus family (cucurbitacea) both contains Phytochemical compounds like carbohydrates, steroids, glycosides, flavonoids, alkaloids, andTannins. The secondary metabolites flavonoid and phenolic component.phytochemicals Scavange the free radical and confirm to their antioxidant activity by DPPH method, hydroxyl radical, ferric radical scavenging Method and To Determine higher the absobance higher is the potential of the Compound. The Isolated active chemicals using column chromatography utilizing the gradient elusion technique with the mobile phase n-hexane, ethyl acetate, and ethanol solvent . Four compounds with Rf values of 0.78, 0.69, 0.59, and 0.8 were recovered from Ethyle extract of *cocinnia grandis* (EECG) and given the names CG-1, CG-2, CG- 3, and CG-4.with mobile phases of n-hexane: chloroform and chloroform: methanol, phytoconstituents from Ethyle extract of *Diplocyclos palmatus* (EEDP) were successfully isolated Three compounds with Rf values of 0.86, 0.81 and 0.6. Acute oral toxicity was conducted in accordance with OECD Directive No. 425 (up and down technique). Using Aspirin plus pylorus ligationd ulcer model, and, isolated compounds from both plants were evaluated at the dosage levels of 20 mg/kg, 10 mg/kg, and 5 mg/kg for antiulcer action. Maximum antiulcer activity was seen for CG-3 and DP-3 at a dosage of 20 mg/kg. Rhamnocitrin, an isolated chemical from *Cocinniagrandis fruits,* and kaempferol, an isolated compound from *Diplocyclos palmatus* fruits, both showed notable in vivo antiulcer action by lowering ulcer index, increasing stomach pH, and decreasing ulcer volume. H+ K+ ATPase inhibitory action of CG-3(Rhamnocitrin) supported in vivo antiulcer research. From DP- 3 (kaempferol), which was evaluated using in vitro and molecular docking experiments.Using Molegro Virtual Docker (MVD) (MVD-201,6.0), computer assisted molecular docking simulation investigations were conducted. The results indicated that rhamnocitrin and kaempferol demonstrated excellent contact with low binding energy in the enzyme active pocket through non covalent interaction. As compared to the reference medication Omeprazole, it was shown that CG-3 (Rhamnocitrin) and DP-3 (Kaempferol) strongly inhibited the H+K+ ATPase in a dose-dependent manner.

Keyword: *Cocinnia grandis* , *Diplocyclos palmatus,* Antiulcer, antioxidant

1. Introduction

Numerous hollow organs that are part of the human digestive system are crucial to maintaining homoeostasis. With the use of physiological protective barriers like bicarbonate and prostaglandin secretions, various digestive system organs, notably the stomach, can endure various dangerous and damaging chemicals including bile salts, hydrochloric acid, and noxious compounds. However, when the

balance between aggressive and defensive elements is off, the gastrointestinal mucosa is damaged, which results in ulcers (1). The Latin term for sore or wound, ulcers, is where the word ulcer first appeared. The lesion of the stomach or duodenal mucosa known as a peptic ulcer develops when the mucosal epithelium is exposed to acid and pepsin (2). A peptic ulcer's mucosal damage results from an imbalance between aggressive and protective forces, which breaks the continuous epithelial lining (3). People today experience more stress as a result of modern lifestyles, work cultures, and fast food intake. These elements contribute to a number of gastrointestinal illnesses, including peptic ulcer. Additionally, Helicobacter pylori infection raises the risk of developing peptic ulcer disease (10). A dangerous and often fatal consequence of peptic ulcer disease is a perforated peptic ulcer (11). Out of the 4 million persons afflicted worldwide, 10–20% experience complications, of which 2% get perforated ulcers (12). Currently, surgical innervation is used to treat perforated peptic ulcers, which raises the expense of care. Peptic ulcer disease has no permanent treatment. To lessen the production of stomach acid, synthetic medications including proton pump inhibitors and H2 receptor blockers are employed. Anti-acid medications are used to counteract stomach acid. To strengthen the mucosal defence, cytoprotective drugs are used. Inflammation is reduced and symptoms are suppressed by corticosteroids. In India, herbal medications have been used in therapeutic settings for ages. Being a tried-and-true system, it has an advantage over other health management systems now in use, particularly when it comes to treating peptic ulcers, which complicated occurrences. Only because of the crucial role performed by the plant kingdom has life on our planet been able to survive. Since the beginning of civilisation, man has grown herbs for medical purposes in addition to food crops (13) despite the lack of scientific support, traditional remedies and alternative systems of medicine across the world have detailed

Anti-ulcer activity by pylorus ligation induced ulcer model

For the study, rats were given an overnight fast while having unlimited access to water. Animals were given care according to the groups stated above, and an hour later a pylorus ligation was performed using surgical sutures under ether anaesthesia at a body weight-based dose of 35 mg/kg by making a 1 cm-long incision immediately below the sternum on the belly without harming any blood vessels. In order to prevent cannibalism and coprophagy, ligated rats were kept in individual cages with elevated bottoms and allowed to recuperate. Animals were denied access to drink after surgery. Animals were killed by ether overdose 4 hours after pyloric ligation, and the stomach's oesophageal end was tied after the abdomen was opened. To evaluate gastric juice volume, gastric pH, free acidity, and total acidity, a small cut was made right above the knot at the pyloric area. To do ulcer scoring, the stomach was sliced open along the larger curvature. (14).

Determination of free acidity and total acidity

GI contents were centrifuged at 1000 rpm for 10 minutes. There was a volume. Pipette 1 ml of supernatant liquid and dilute with 10 ml of distilled water. A pH metre was used to measure pH. Sodium hydroxide 0.01N was titrated into the solution using Topfer's reagent as an indicator. Up to the point at which the solution becomes orange, the solution is titrated. Free acidity and sodium hydroxide volume are related. The solution was adjusted further till the pink colour returned. Total sodium hydroxide volume equals total acidity. (15).

Acidity (mEq/1/100g) = Vol. of NaOH X Normality X 1000

Aspirin induced ulcer

Animals were fasted for 36 hours before to treatment for aspirin-induced ulcer experiments, according to the groups specified. One hour had passed since the last treatment before the aspirin was given. By giving aspirin orally at a dosage of 200 mg/kg, ulcers were produced. Animals were killed 6 hours after aspirin treatment, and the stomach's larger curvature was opened to score for ulcers. (16).

Histopathological evaluation

Following evaluation of the ulcer score, stomachs were submerged in a 10% formalin solution. The ulcerated tissue's centre was divided in two along a lengthy diameter. If the stomach was shielded from harm, the basal portion was used to cut off the segment. The moist tissue was processed as usual and then sliced into 5 m thick sections using a rotary micrometer (17). Haematoxylin-eosin staining and Canada

balsam mounting were done to the sections. These were analysed for histological alterations such oedema, inflammation, infiltration, and erosion under the microscope. (18 n).

4.8 Antioxidant activity

The DPPH radical scavenging activity, Nitric oxide scavenging activity, Superoxide anion scavenging activity, Hydroxyl radical scavenging activity, and Reducing power assay were used to test the antioxidant activity of the ethanolic extract of Cocinnia grandis fruits and Diplocyclos palmatus fruits(.19)

DPPH radical scavenging activity

By evaluating its effectiveness in scavenging free radicals, the DPPH (1, 1- diphenyl-2-picryl hydrazyl) technique was used to test the antioxidant properties of isolated substances. ethanol was used to dissolve DPPH to a concentration of 0.1mM. Three millilitres of the test solution—an isolated chemical dissolved in ethanol—were mixed with one millilitre of this solution at various concentrations of 100–1000 g/ml. The solution was briskly agitated and let to stand at room temperature in the dark for 30 minutes. As a baseline, quercetin was used. A UV visible spectrophotometer was used to detect the absorbance at 517 nm. Estimates of DPPH inhibition percentage were made (20).

Reducing power

The Fe3+ to Fe2+ conversion was measured by monitoring the appearance of Perl's Prussian blue at 700 nm as part of the reducing power test. Test compound solution was made at concentrations of 40, 80, 100, 120, 140, 160, 180, and 200 g/ml. Each of these solutions was given one millilitre in a separate test tube. Potassium ferricyanide was added in an equal amount along with 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated for 20 minutes at 500C. 2.5 ml of 10% trichloroacetic acid was added after 20 minutes of incubation, and the mixture was centrifuged for 10 minutes at 3000 rpm. To 2.5 ml of supernatant liquid, 2.5 ml of distilled water and 0.5 ml of newly made 0.1% ferric chloride solution were added. At 700nm, absorbance was measured. As a benchmark, ascorbic acid solutions at concentrations of 5, 10, 20, 30, 40, 50, 60, 80, and 100 g/ml were utilised. (21).

1.1 Plant Material

Fruits from the plants *Cocinnia grandis and Diplocyclos palmatus were* gathered from near the village of Burhanpur in satpura range. The plants were verified and identified by submited voucher specimen in botanical college of PO Nahata college Bhusawal District Jalgaon under the supervision of head of the Department Dr S.V Patil.

1.2 Qualitative preliminary phytochemical screening

The presence of several chemical components such as carbohydrates, alkaloids, glycosides, flavonoids, steroids, and triterpenoids was examined in all of the fruit extracts from Fruits of *Cocinnia grandis* were extracted with ethanol, revealing the presence of alkaloids, flavonoids, glycosides, and carbohydrates When compared to the petroleum ether and chloroform extracts of the same fruit. The ethanolic extract of *Diplocyclos palmatus* fruits showed the presence of the majority of phytoconstituents. Fruits from *Diplocyclos palmatus* were found to include phenolic chemicals, tannins, steroids, glycosides, flavonoids, and triterpenes.

1.3 Estimation of total flavonoid and Phenolic content :Aluminum chloride colorimetric method.

The Folin-Ciocalteu technique was used to assess the total phenolic and flavonoid content of the fruits of *Cocinnia grandis and Diplocyclos palmatus* in petroleum extract, chloroform extract, and ethanolic extract. The phenolic and Flavanoids content of ethanolic extracts of *Cocinnia grandis and Diplocyclos palmatus* fruits was found to be higher than that of corresponding petroleum ether and chloroform extracts.

Table : 1 Total Phenolic and flavonoid content in different solvent system

Plant	Extract	TPC (Conc. µg/mg)	TFC (Conc. µg/mg)
Cocinnia grandis fruits	Petroleum ether	1.36	3.27
	Chloroform	6.24	7.14
	Ethanol	118.81	188.76
Diplocyclos palmatus fruits	Petroleum ether	1.16	2.15
	Chloroform	4.54	10.45
	Ethanol	98.85	174.61

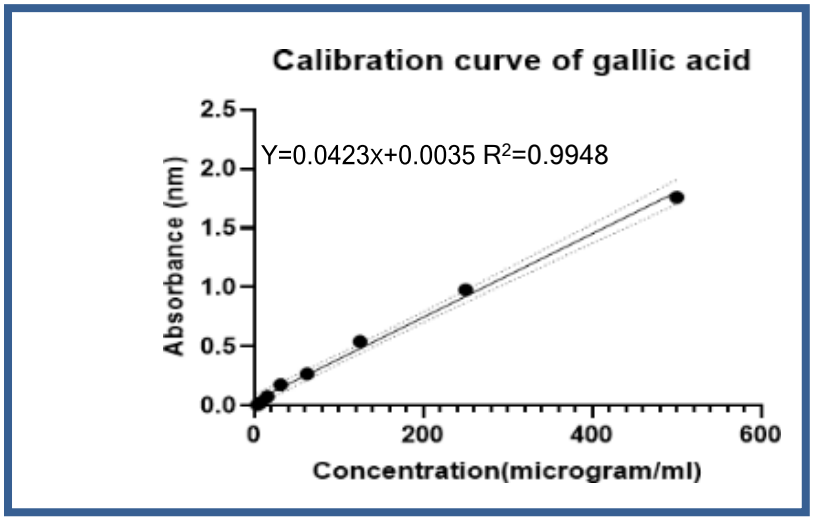


Figure : 1 Calibration curve of Gallic acid

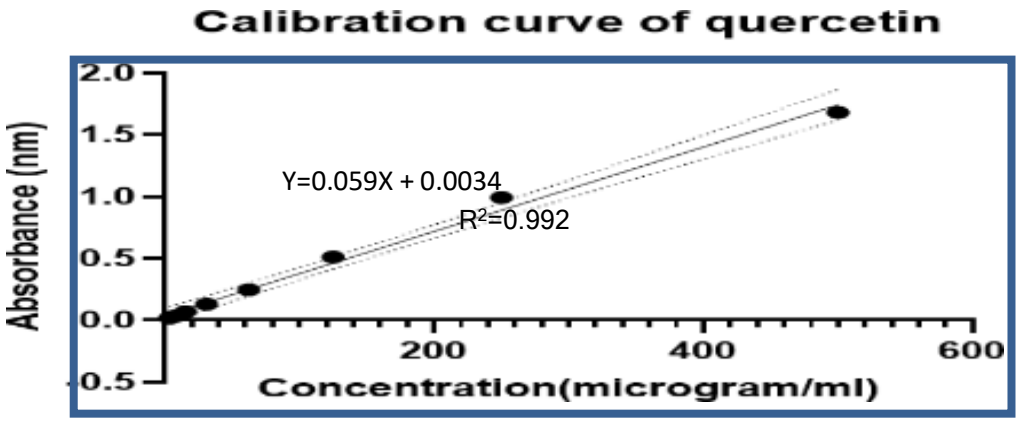


Figure : 2 Calibration curve of Quercetin

1.4 Pharmacological Screening

Acute toxicity studies:

Determination of acute oral toxicity of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* extracts in mice.Acute toxicity studies of all the plant extracts were performed according OECD guideline using up and down method. Both the plants did not demonstrate any sign and symptoms of evident toxicity, with no behavioural alteration or changes, and it did not cause animal deaths within 72 h of the treatment.

1.5 Preliminary antiulcer activity

Preliminary anti-ulcer activity of extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* was carried out using ulcer inducing models such as pylorus ligation induced ulcer, , Aspirin induced ulcer model.

1.6 Pylorus ligation induced ulcer model

In this method contents of the stomach was carefully taken out and estimated for volume, pH, free acidity

and total acidity. Different parameters such as spot ulcer, haemorrhagic streak, ulcers and their numbers been observed and scoring weredone for all the groups.Ethanolic extract of *Cocinnia grandis fruits* (*EECG*) showed significant antiulcer activity as compared to petroleum ether and chloroform extract of same plant. *EECG* showeda significant reduction in ulcer index (0.93 ± 0.045***) as compared to control group (2.03 ± 0.03). Gastric pH was raised by *EECG* (4.55 ±0.02***) as compared to controlgroup (3.63 ± 0.19) but was less than standard group treated with Omeprazole (5.73± 0.09***). Significant reduction in free acidity and total acidity was observed in group treated with *EECG*. Ethanol induced ulcer Oral administration of *EECG* reduced the ulcer index induced by ethanol in rats (1.52± 0.04***) as compared to control (2.36 ± 0.06). *EECG*, *PECG* and *CECG* inhibited ulcer index by 35.72%, 20.65% and 4.78% respectively as tabulated Ulcer protective action of *EECG* was less then but comparable to group treated by standard drug omeprazole as shown in table

1.7 Aspirin induced ulcer

EECG at dose of 500mg/kg showed a significant ulcer protection at 43.15% comparable to standard group treated by omeprazole (75.79%). Ulcer index in *EECG* treated group was 1.35 ± 0.21***whereas *PECG* and *CECG* exhibited ulcer index 1.71 ± 0.36^{ns}, 2.19 ± 0.16 ^{ns}.

Table: 2 Effect of *Cocinnia grandis fruits* extracts on various parameters in pylorus ligated induced ulcer model

Treatment groups	Dose	Mean ulcer index	Gastric volume	Gastric pH	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)
Control (Normal Saline)	2ml/kg	2.13±0.04	3.25 ± 0.16	3.63±0.19	36.44±0.89	56.52± 1.14
Standard (Omeprazole)	20mg/kg	0.89±0.02***	1.92±0.19***	5.73±0.09***	15.16±0.51**	23.24±0.91**
PECG	500mg/kg	1.53±0.30^{ns}	3.05±0.09^{ns}	3.90±0.34^{ns}	32.26±1.79^{ns}	52.38±1.83^{ns}
CECG	500mg/kg	2.21±0.15^{ns}	2.72±0.25^{ns}	3.02±0.22^{ns}	26.31±4.79^{ns}	46.73±4.51^{ns}
EECG	500mg/kg	1.03±0.050**	2.58 ± 0.29*	4.55±0.02**	23.35±0.62**	35.78± 0.89**

Table : 3 Effect of *Cocinnia grandis fruits* extracts on mean ulcer index in aspirin induced ulcer model

Treatmentgroups	Dose	Aspirin inducedulcer
Control (NormalSaline)	2ml/kg	2.47± 0.07
Standard (Omeprazole)	20mg/kg	0.67± 0.06***
PECG	500mg/kg	1.81± 0.46 ^{ns}
CECG	500mg/kg	2.29± 0.26 ^{ns}
EECG	500mg/kg	1.25± 0.31***

All values are expressed as a mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 as compared to control group (One Way Analysis of Variance (ANOVA) followed by Dunnet test

1.8 Preliminary anti-ulcer activity of *Diplocyclos palmatus fruits*

Pylorus ligation induced ulcer model

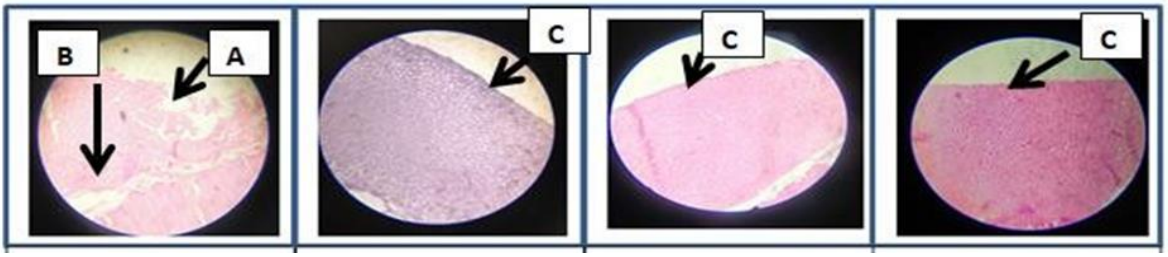
Treatment with *EEDP* at a dose of 500mg/kg showed substantial protective activity against the ulcer induced in rats by pyloric ligation. Ulcer index of the *EEDP* treated group was 0.90 ± 0.02**, which was significantly less than the control group 2.03 ± 0.30. *EEDP* also raised the gastric pH which was 4.90 ± 0.12*** as compared to control group (3.63 ± 0.19) thus reducing the gastric acidity. Reduction in gastric volume was observed in a group treated with *EEDP* (1.93± 0.24**). *PEDP* and *CEDP* did not show antiulcer activ

Table: 4 Effect of *Diplocyclos palmatus fruits* extracts on various parameters in pylorus ligated induced ulcer model

Treatment groups	Dose	Mean ulcer index	Gastric volume	Gastric pH	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)
Control (Normal Saline)	2ml/kg	2.23±0.40	3.15 ± 0.15	4.13± 0.29	34.74±0.99	54.52±1.14
Standard (Omeprazol)	20mg/kg	0.89±0.11***	1.82±0.20***	5.63 ± 0.10***	15.06±0.71***	23.24±0.91**
PEDP	500mg/kg	1.88 ± 0.20^{ns}	2.82 ± 0.04^{ns}	3.32±0.25^{ns}	32.61 ±0.70^{ns}	54.81±1.70^{ns}
CEDP	500mg/kg	1.81 ± 0.08^{ns}	3.08 ± 0.06^{ns}	3.26±0.14^{ns}	33.45±1.15^{ns}	52.10±1.85^{ns}
EEDP	500mg/kg	1.01± 0.03**	2.13 ± 0.32**	5.10±0.13***	22.03±0.94***	27.55±1.14***

All values are expressed as a mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 as compared to control group (One Way Analysisof Variance (ANOVA) followed by multiple comparison Dunnet's test).

Figure: 3 Rat stomach treated with treatment groups in pylorus ligatedinduced ulcer model



Control **Standard** **EECG** **EEDP**

Figure:4 Histopathological evaluation of rat stomach treated with treatmentgroups in pylorus ligated induced ulcer model

1.9 Antioxidant activity

On the basis of qualitative preliminary phytochemical screening and quantitative phytochemical screening such as total phenolic content and total flavonoid content ethanolic extract of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* was selected for *in vitro* antioxidant activity. *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* was carried out using DPPH radical scavenging activity, reducing power assay, Nitric oxide scavenging activity, Superoxide anion scavenging activity and Hydroxyl radical scavenging activity.

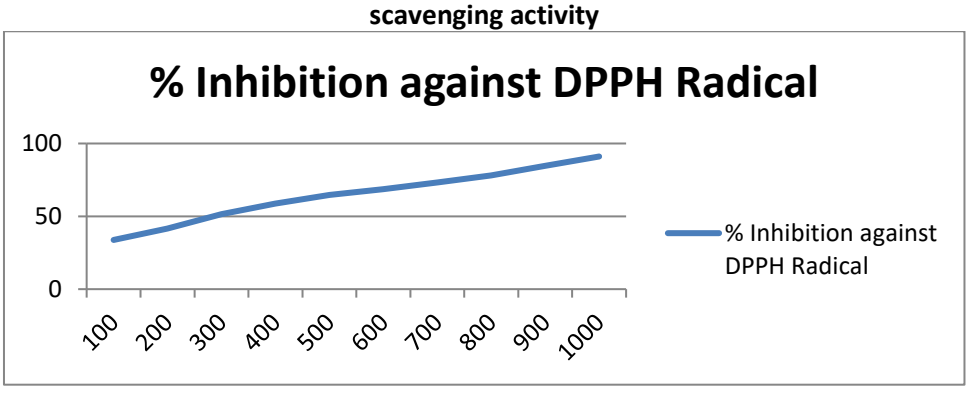
1.10 DPPH radical scavenging activity

Ethanolic extract of *Cocinnia grandis fruits* (*EECG*) showed significant antioxidant activity In a dose dependent manner. *EECG* showed IC_{50} value of 285.83 ± 28.77 (µg/mL) Ethanolic Extract of *Diplocyclos palmatus fruits* (EEDP) exhibited scavenging activity with IC50 value of 435.36±24.96 (µg/mL).

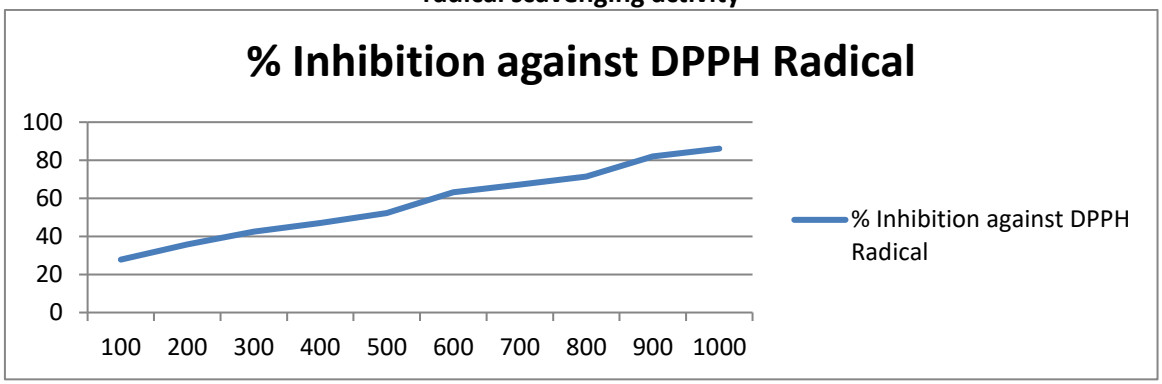
Table 5 : *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* using DPPH radical scavenging activity

Drug	Concentration (µg/mL)	% Inhibition against DPPHradical	IC_{50} (µg/mL)
Ethanolic extract of *Cocinnia grandis fruits*	100	33.79 ±1.43	285.83 ±28.77
	200	41.52± 3.19	
	300	51.46 ± 6.45	
	400	58.73 ±2.78	
	500	64.70 ±3.32	
	600	68.72±2.44	
	700	73.34 ±2.40	
	800	78.10 ±1.88	
	900	84.76±3.42	
	1000	91.06 ±1.32	
sad			
Ethanolic extract of *Diplocyclos palmatus fruits*	100	27.80 ± 3.75	435.36 ± 24.96
	200	35.80 ±4.10	
	300	42.55 ±2.89	
	400	47.07 ±2.18	
	500	52.24 ± 0.60	
	600	63.25 ±1.53	
	700	67.15 ±2.86	
	800	71.46 ±1.08	
	900	82.06 ±2.11	
	1000	86.09 ±1.87	
StandardQuercetin	2	43.21±1.42	3.31 ± 0.34
	4	51.43±1.71	
	8	62.23±3.85	
	16	65.54± 5.83	
	32	67.39±10.10	
	62.5	77.23± 0.68	
	125	83.70±1.98	

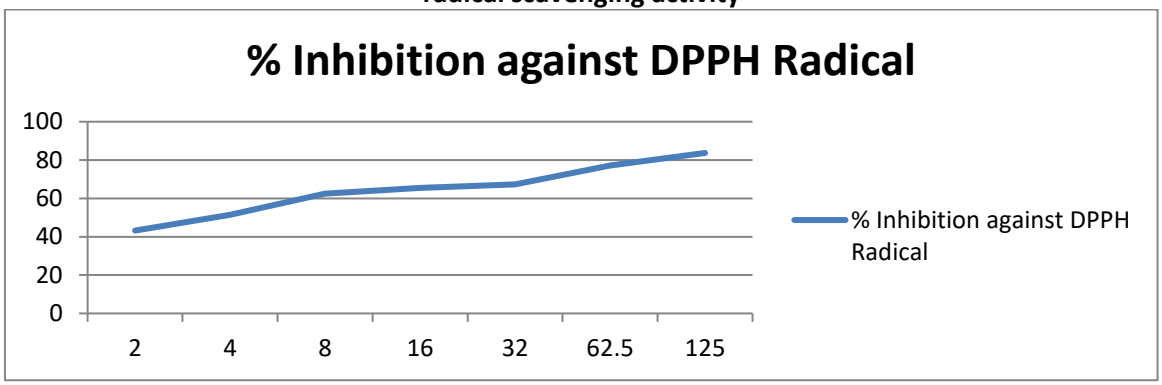
Graph :1 : *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* using DPPH radical scavenging activity



Graph : 2 *In vitro* antioxidant activity of ethanolic extracts of *Diplocyclos palmatus fruits* using DPPH radical scavenging activity



Graph : 3 *In vitro* antioxidant activity of ethanolic extracts of standard drug (Quercetin) using DPPH radical scavenging activity



1.13 Reducing power

Reducing power assay is based on conversion of Fe^{3+} to Fe^{2+} by the sample. Test sample which have more reduction potential reacts with potassium ferricyanide (Fe^{3+}) to from potassium ferrocyanide (Fe^{2+}) which then reacts with ferric chloride to form ferric ferrous complex that has absorption maximum at 700nm. The reducing power of ethanolic extract of *Cocinnia grandis fruits*, ethanolic extract of *Adiantum lunulatum* and standard solution Ascorbic acid increases with increase in amount ofsample concentration which is in good linear relation as shown in Table

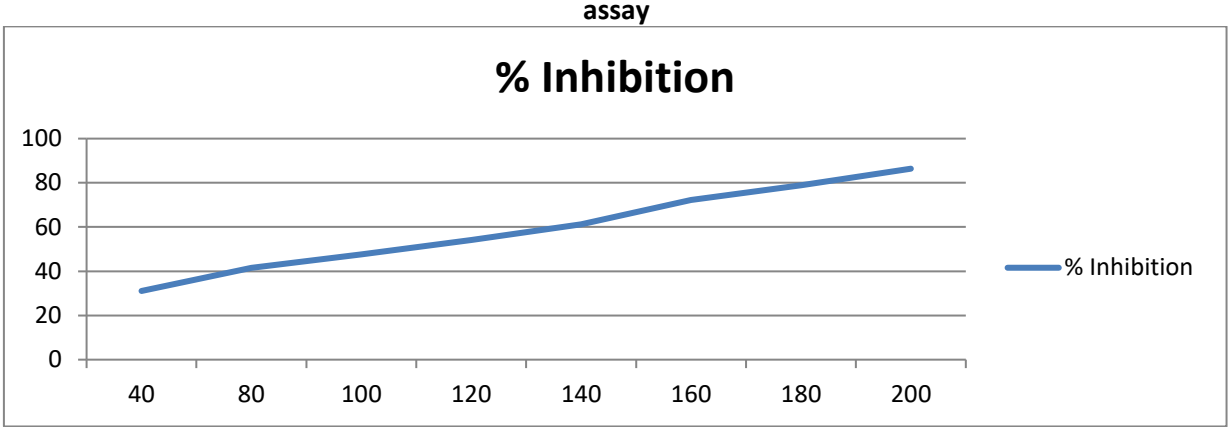
Table: 6 *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* using reducing power assay

Drug	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Ethanolic extract of *Cocinnia grandis fruits*	40	31.07 ± 2.48	107.23 ± 5.88
	80	41.54 ± 3.48	
	100	47.68 ± 2.25	
	120	54.10 ± 1.15	
	140	61.24 ± 1.19	
	160	72.20 ± 4.89	
	180	78.82 ± 4.24	
	200	86.39 ± 2.79	
Ethanolic extractof *Diplocyclos palmatus fruits*	40	24.80 ± 3.27	120.60 ± 8.07
	80	35.70 ± 4.72	
	100	39.15 ± 9.26	
	120	43.81 ± 4.70	
	140	61.11 ± 0.72	
	160	68.05 ± 1.80	
	180	71.72 ± 2.51	
	200	82.05 ± 2.63	
	5	48.71± 0.87	

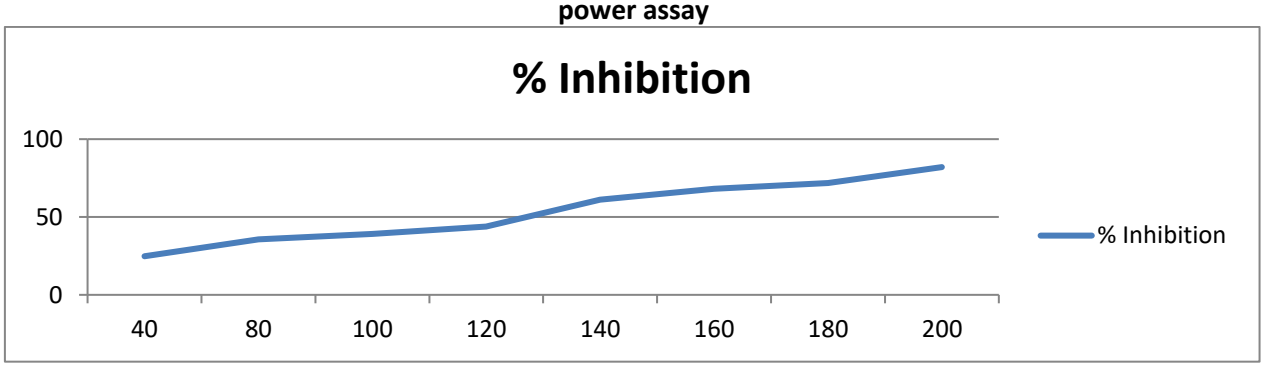
Standard Ascorbic acid	10	52.42 ±2.39	8.10 ± 3.52
	20	55.63 ± 3.26	
	30	57.50 ± 1.10	
	40	61.89 ± 1.51	
	50	64.20 ± 0.82	
	60	67.61 ± 1.36	
	80	72.32 ± 0.50	
	100	74.34 ± 0.31	

All Values are expressed as mean ± S.D (n=3).

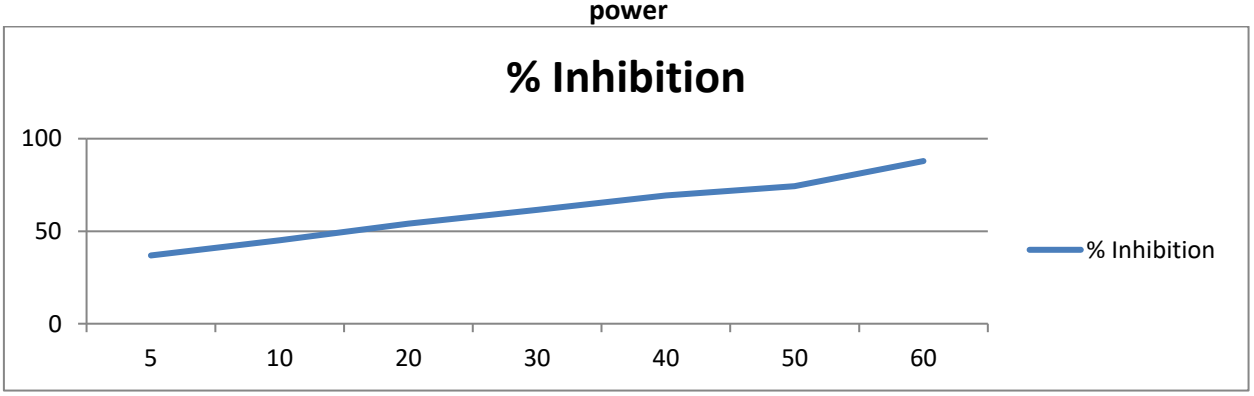
Graph : 4 ***In vitro*** **antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* using reducing power assay**



Graph : 5 ***In vitro*** **antioxidant activity of ethanolic extracts of *Diplocyclos palmatus fruits* using reducing power assay**



Graph: 6 ***In vitro*** **antioxidant activity of ethanolic extracts of standard drug (Ascorbic Acid) using reducing power**



1.14 Isolation of Phytoconstituents

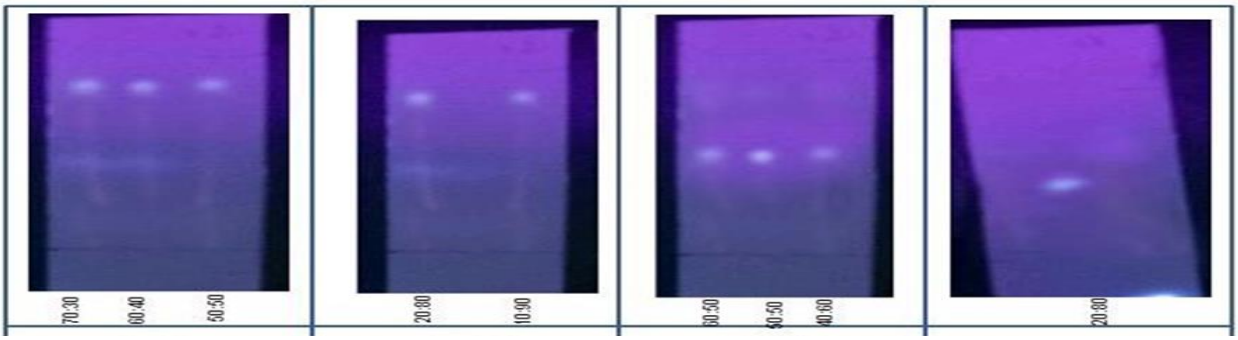
On the basis of results obtained of phytochemical investigation and preliminary pharmacological screening it was found that ethanolic extract of *Cocinnia grandis fruits* and ethanolic extract of *Diplocyclos palmatus fruits* was more potent and hence was further selected for isolation of phytoconstituents.

1.15 Isolation of phytoconstituents from ethanolic extract of *Cocinnia grandis fruits*

Ethanolic extract of *Cocinnia grandis fruits* was subjected to isolation of active constituents Using column chromatography by gradient elusion technique. Hexane: ethyl acetate and Ethyl acetate: ethanol was used in different concentration as mobile phase as given in Table Below All the fractions were obtained by gradient elusion technique. Fractions were subjected to thin layer chromatography and fractions with similar R*f* value were pooled together.

Table :7 Characteristic of isolated compound from *Cocinnia grandis fruits*

Isolated Compound	Rf Value	Colour	State
Compound 1 (CG-1)	0.78	White	Solid
Compound 2(CG-2)	0.69	Yellow to brownish	Solid
Compound 3(CG-3)	0.59	Yellow	Solid
Compound 4 (CG-4)	0.38	White	Solid



PU1 ***PU2*** ***PU3*** ***PU4***

Figure: 5 Thin layer chromatography of different fractions obtained from column chromatography of ethanol extract of *Cocinnia grandis fruits* eluted with hexane, followed by combinations of hexane: ethyl acetate and ethyl acetate: ethanol**.**

1.**16 Isolation of phytoconstituents from ethanolic extract of *Diplocyclospalmatus***

Ethanolic extract of *Diplocyclos palmatus fruits* was subjected to separation of phytoconstituents using column chromatography by gradient elusion technique. Hexane: chloroform and chloroform: methanol was used in different concentration as mobile phase. All the fractions were obtained by gradient elusion technique. Fractions were subjected to thin layer chromatography and fractions with similar R*f* value were pooled together

Table : 8 Various fractions obtained from column chromatography ofethanolic extract of *Diplocyclos palmatus* fruits from various proportion from 100 percentage n hexane solvent to chloroform and 100%methanol

n-hexane:chloroform	50:50	51-60	1	Compound 1	0.86
n-hexane:chloroform	40:60	61-70	0	---	---
n-hexane: chloroform	30:70	71-80	1	Compound 2	0.81
chloroform:methanol	70:30	131-140	1	Compound 3	0.62
chloroform: methanol	60:40	141-150	1	Compound 3	0.62
chloroform:methanol	50:50	151-160	1	Compound 3	0.62

Table: 9 Characteristic of isolated compound from *Diplocyclos palmatus fruits*

Isolated Compound	R*f* Value	Colour	State
Compound 1 (DP-1)	0.86	Brown	solid
Compound 2 (DP-2)	0.81	White	solid
Compound 3 (DP-3)	0.62	Yellow	solid

Figure Thin layer chromatography of different fractions obtained from column chromatography of ethanol extract of *Diplocyclos palmatus fruits* eluted with hexane, followed by combinations of hexane: chloroform and chloroform: methanol.
Yield of CG-1 was very less hence it was discarded for further studies

1.17 Pharmacological screening for antiulcer activity of isolated compounds
1.18 Antiulcer activity of isolated compound of *Cocinnia grandis fruits*
1.19 Aspirin plus pylorus ligation induced ulcer model
In Aspirin plus pylorus ligation ulcer induced model isolated compound from *Cocinnia grandis fruits* named as CG-3 showed significant ulcer protective activity in dose dependent manner. CG-3 was administered at dose level of 5mg/kg, 10mg/kg and 20mg/kg. CG-3 at dose level of 20mg/kg showed a significant reduction in ulcer index (0.97± 0.05***) as compared to control group (2.92 ± 0.04) and was equivalent to standard group treated with omeprazole (0.83± 0.04***). Gastric volume was considerably reduced by CG-3 (1.99±0.29**) as compared to control (3.17± 0.18). Gastric volume observed in standard group was (1.27 ± 0.16***). pH recorded in group treated with CG-3 at a dose of 20mg/kg (4.07± 0.44***) was significantly increased then in control group (2.58 ± 0.14). Group treated with CG-2 and CG-4 did not show any reduction in ulcer index, gastric volume and had no impact on gastric pH. Results are tabulated in Table below

Table : 10 Effect of CG-3 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

Parameters	Control	Standard (Omeprazole) 20mg/kg	CG-3 5mg/kg	CG-3 10mg/kg	CG-3 20mg/kg
ULCER INDEX	2.62±0.14	0.73±0.04**	2.78±0.23^{ns}	1.77±0.26**	0.67±0.06**
GASTRCVOLUE	3.15±0.08	1.37±0.16**	2.71±0.42^{ns}	2.82±0.20^{ns}	1.89±0.39**
pH	2.38±0.24	5.81±0.14**	3.27±0.30^{ns}	3.31±0.28**	4.27±0.54***
FREE ACIDITY	38.49±1.74	14.31±0.72*	35.43±1.11^{ns}	31.79±2.70**	24.33±0.49***
TOTDP ACIDITY	57.89±1.46	25.13±0.79*	56.79±0.72^{ns}	44.23±3.83**	24.48±1.12***

All values are expressed as a mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 as compared to control group (One Way Analysisof Variance (ANOVA) followed by multiple comparison Dunnet's test).

Graph :6 Effect of CG-3 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

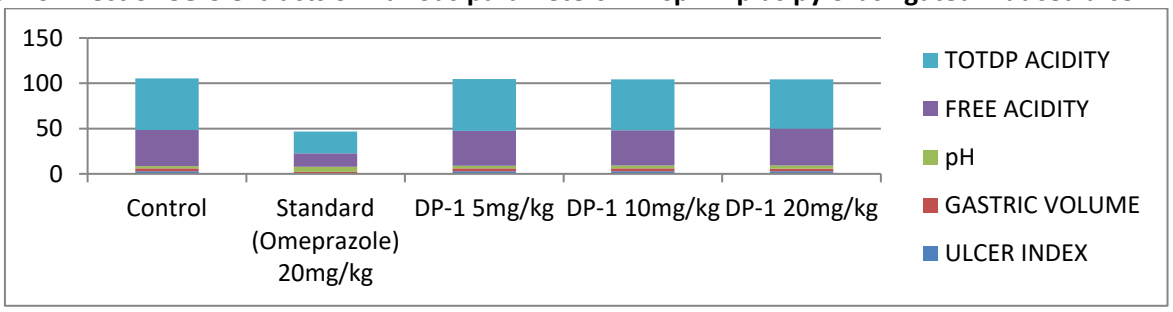


Table:11 Percentage ulcer protection by different treatment groups at The dose level 20mg/kg

Treatment group	Ulcer protection
CG-2	NS
CG-3	66.72
CG-4	NS
DP-1	NS
DP-2	NS
DP-3	69.06
Standard	71.63

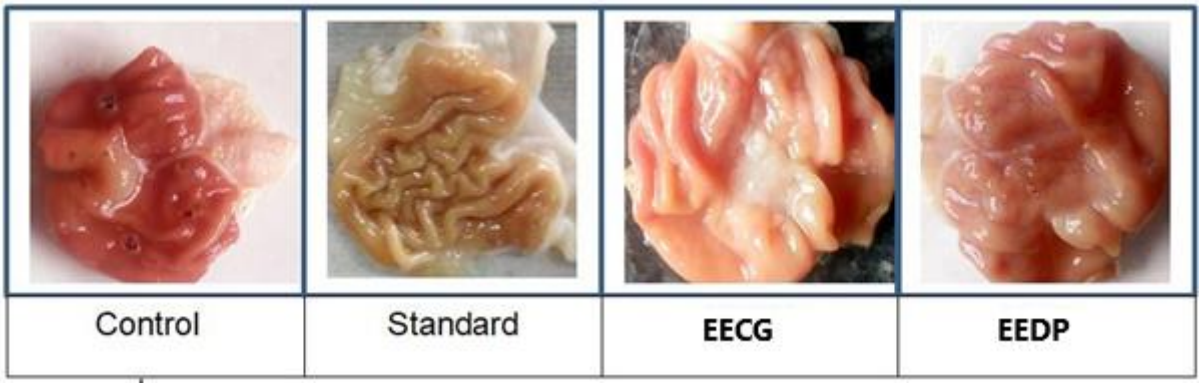


Figure :7 Rat stomach treated with treatment groups in pylorus ligated ligatedinduced ulcer model

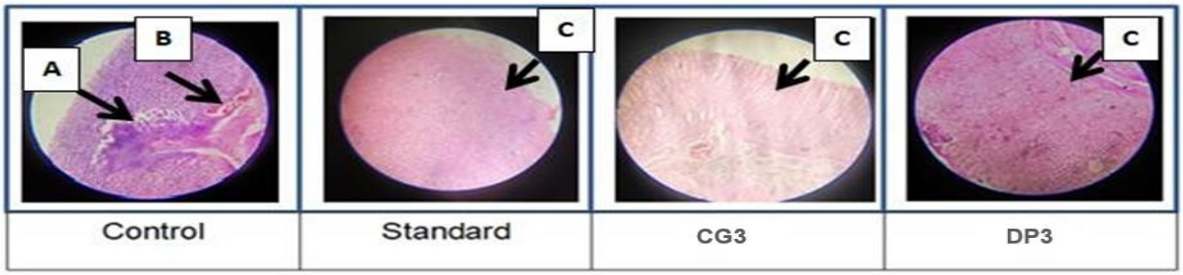


Figure: 8 Histopathological evaluation of rat stomach treated with isolated compounds of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* in aspirin plus pylorus ligated induced ulcermodel

1.20Structural elucidation of pharmacologically active isolated compound (CG-3) from *Cocinnia grandis fruits*

1.21 Melting point determination of CG-3

Melting point of pharmacologically active isolated compound CG-3 from *EECG* was determined using Galen Kamp melting point apparatus and was found to be 224-227^{o}C

1.22 Ultraviolet absorbance of CG-3

CG-3 was scanned through UV range of 200-400nm and showed maximum absorbance at 270nm and 363nm.

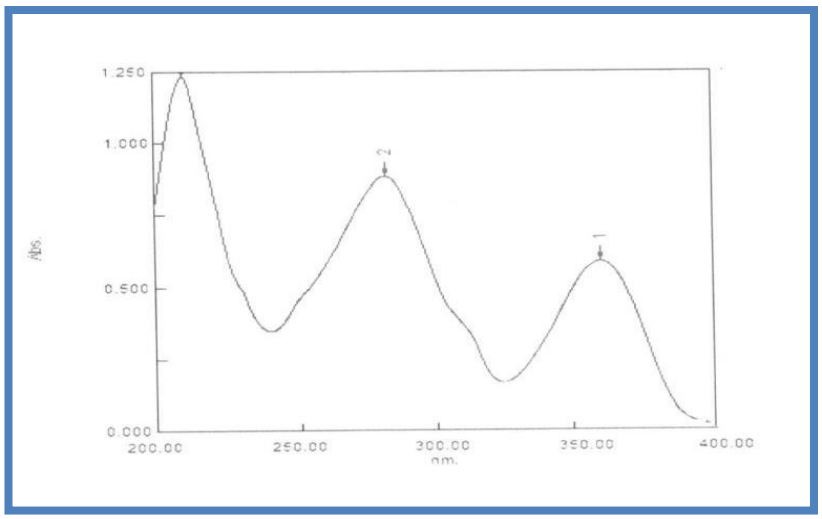


Figure :9 Ultraviolet absorbance spectra of compound CG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

A 10ppm solution of isolated drug CG-3 showed the λ_{max} to be at 270 nm. The drug solution obeys Beer-Lambert's law in the concentration range of 2-12 µg/ml with a correlation coefficient of 0.9993 indicating good linearity in this concentration range as depicted in the standard calibration curve in figure 10

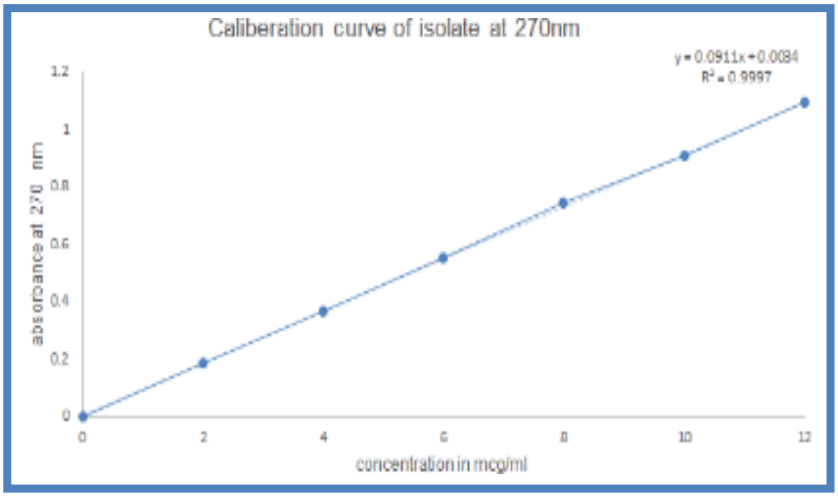


Figure :10 Ultraviolet absorbance caliberation cur

Figure :10 Ultraviolet absorbance caliberation curve at 270nm of compound CG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

Table :14 Absorbance of various concentrations isolate in methanol at270 nm

Concentration (µg/ml)	Absorbance at 270
2	0.187
4	0.365
6	0.552
8	0.747
10	0.907
12	1.094

1.23 Fourier Transform Infrared spectroscopy of CG-3

IR spectrum of sample CG-3 showed the presence of a band at 3375.43 cm^{-1} due to presence of hydroxyl group. The absorption bands at 3140.11 and 2818 cm^{-1} indicates the presence of aromatic C-H and aliphatic C-H stretching respectively. A strong absorption band at 1662.64 cm^{-1} showed the presence of carbonyl group. The presence of bands at 1627.92 and 1612.49 indicates presence of C=C aromatic bonds. Band at 1174.65 cm^{-1} is due to C-O bond stretching. IR spectrum of sample CG-3 showed the presence of hydroxyl group, aromatic carbons, aliphatic carbon and presence of carbonyl carbon.

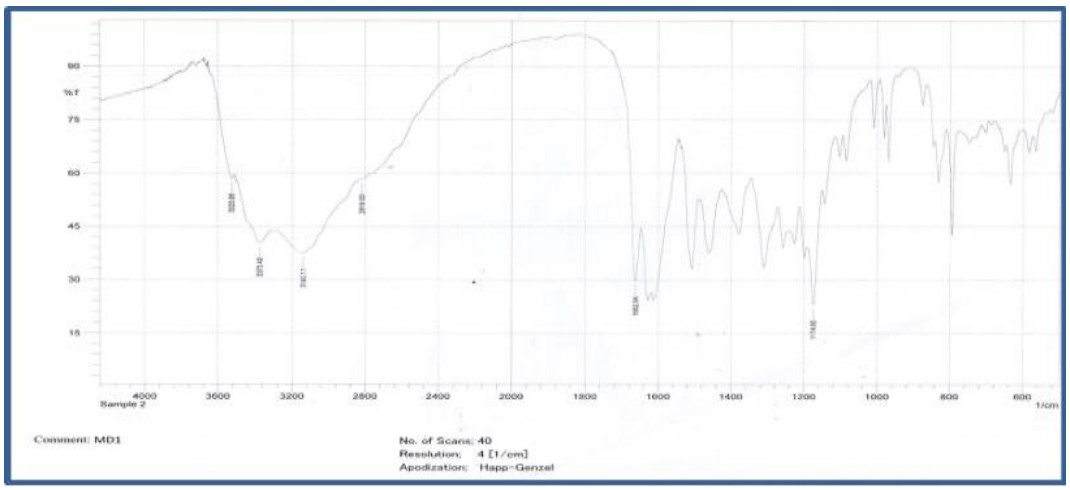


Figure :10 FTIR – Spectroscopy

Table:15 FTIR – Spectroscopy interpretation of compound CG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

Table No. 5.32 FTIR – Spectroscopy interpretation of compound CG-3 isolatedfrom ethanolic extract of *Cocinnia grandis fruits*

Sr. No	Wave number (cm^{-1})	Group assigned
1	3375.43	-O-H Stretching
2	3140.11	-CH aromatic stretching
3	2818.00	-CH aliphatic stretching
4	1662.64	-C=O stretching
5	1627.92	-C=C-aromatic stretch
6	1612.49	
7	1462.04	$-CH_3$ (bending)
8	1390.67	-C-O Stretch
9	1174.65	

1.24 ^{1}HNMR and ^{13}CNMR of compound CG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

^{1}HNMR

^{1}H NMR spectrum of sample CG-3 showed a singlet at δ 3.897 for three aliphatic protons; a singlet at δ 6.051 for one aromatic proton; a singlet at δ 6.453 for one aromatic proton; a doublet at δ 6.850- 6.893 for two aromatic protons; a doublet at δ 8.151-8.201 for two aromatic protons; a singlet at δ 9.951 for one proton; a singlet atδ 10.732 for one proton and a singlet at δ 12.430 for one proton. ^{1}H NMR showed the presence of methyl group, presence of six aromatic protons and three hydroxylgroups.

^{13}CNMR

^{13}C NMR spectrum of CG-3 showed a singlet at δ 56.00 for one aliphatic carbon; the fourteen aromatic carbons appeared at δ 80.43 (s); 92.00 (s); 97.40 (s); 105.00 (s); 115.00 (d); 121.00 (s); 133.80 (d); 137.20 (s), 156.00 (s); 160.00 (s); 162.70 (s); 166.00 (s) and a singlet at δ 175.50 shows a presence of carbonyl carbon. ^{13}C NMR showed presence of one aliphatic carbon, fourteen aromatic carbons and a carbonyl carbon.

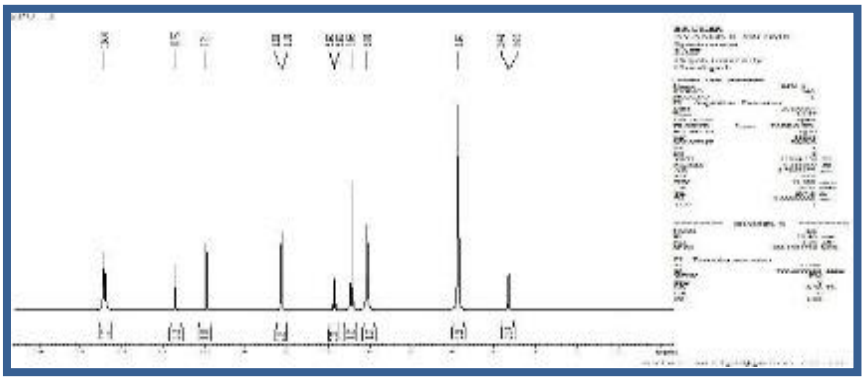


Figure:11 ^{1}H NMR spectrum of compound CG-3 isolated from ethanolicextract of *Cocinnia grandis fruits*

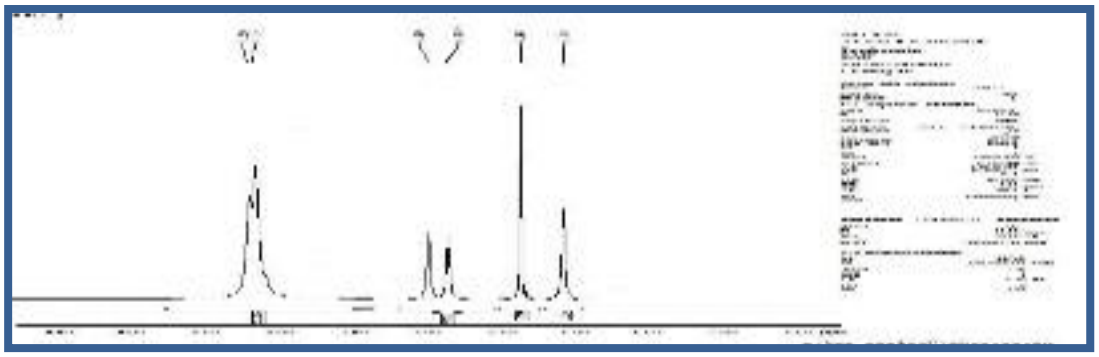


Figure:12 ^{1}H NMR spectrum of compound CG-3 isolated from ethanolicextract of *Cocinnia grandis fruits*

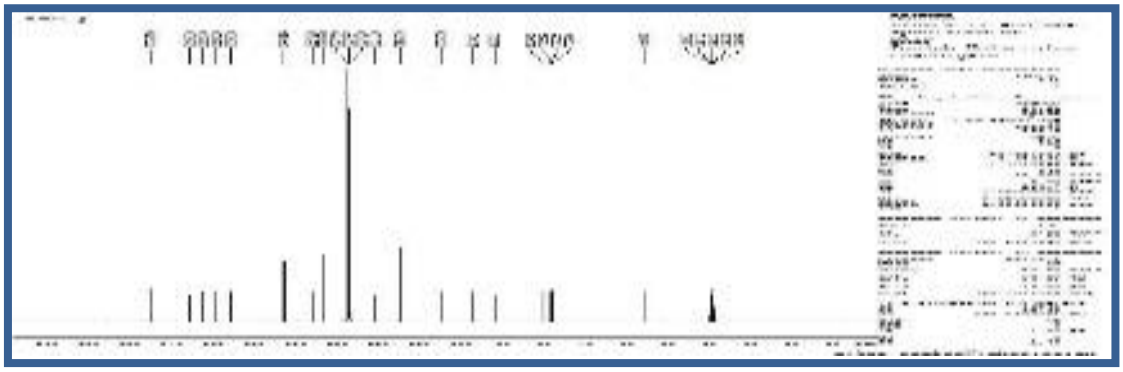


Figure:13 ^{13}C NMR spectrum of compound CG-3 isolated from ethanolicextract of *Cocinnia grandis frui*

1.25 Mass Spectra

The mass spectrum of sample CG-3 showed fragments at m/z 299, 284, 271, 255, 243, 227, 185, 183, 157, 141, 129 and 113. The molecular weight of the compound is found to be 300.

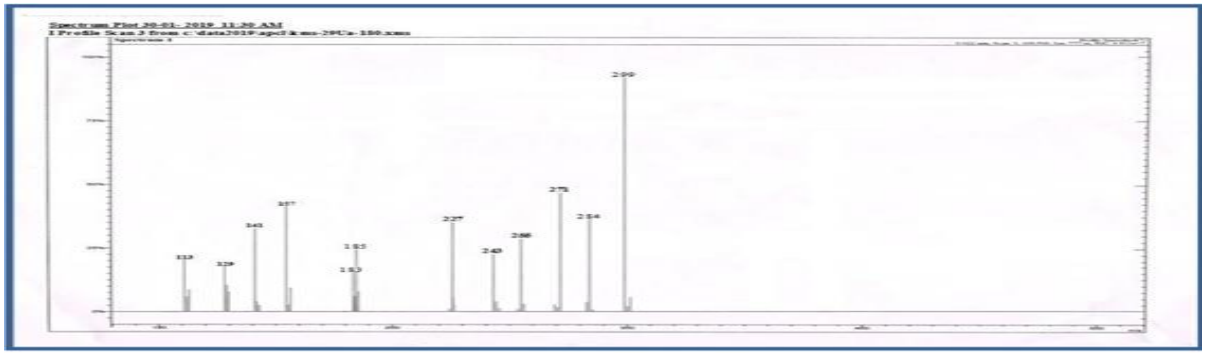


Figure :14 Mass Spectra of compound CG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

1.26 Structural elucidation of pharmacologically active isolated compound (DP-3) from *Diplocyclos palmatus fruits*

1.27 Melting point determination of DP-3

Melting point of pharmacologically active compound DP-3 isolated from *EEDP* was determined using Galen Kamp melting point apparatus and was found to be 277-279°C

1.28 Ultraviolet absorbance of DP-3

DP-3 was scanned through UV range of 200-400nm and showed maximum absorbance at 265nm and 365nm.

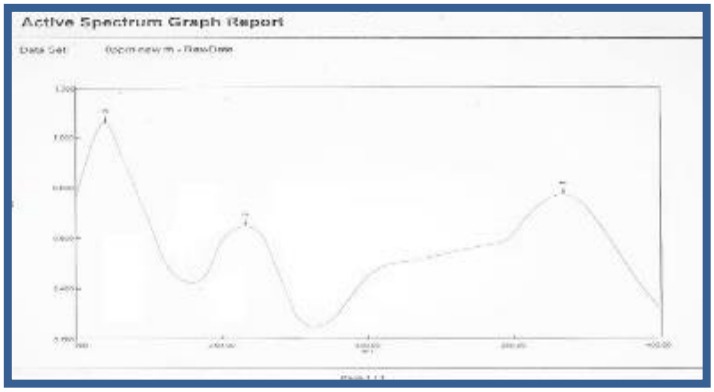


Figure:15 Ultraviolet absorbance spectra of compound DP-3 isolated from ethanolic extract of *Diplocyclos palmatus fruits*

A 10ppm solution of isolated drug showed the λ_{max} to be at 365 nm. The drug solution obeys Beer-Lambert's law in the concentration range of 2-12 µg/ml with a correlation coefficient of 0.9993 indicating good linearity in this concentration range as depicted in the standard calibration curve in

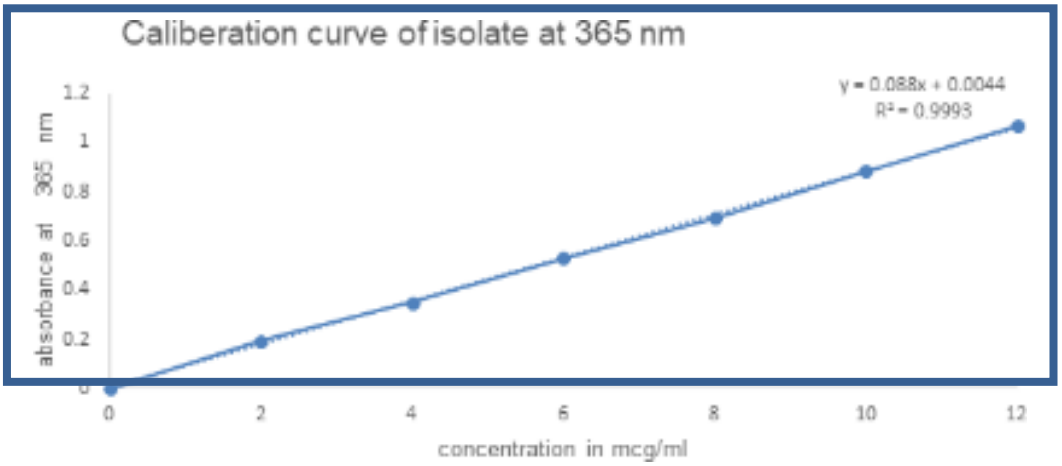


Figure :16 Ultraviolet absorbance calibration curve at 365nm of compound DP-3

Table:16 Absorbance of various concentrations isolate in methanol at 365nm

Concentration (µg/ml)	Absorbance atnm
2	0.196
4	0.351
6	0.532
8	0.693
9	0.883
10	1.07

1.27 Fourier Transform Infrared spectroscopy of DP-3

IR spectrum of compound DP-3 showed the presence of a band at 3460.30 cm^{-1} due to presence of hydroxyl group. The absorption bands at 3363.86 and 3230.77 cm^{-1} indicates the presence of aromatic C-H and aliphatic C-H stretching respectively. A strong absorption band at 1600.92 cm^{-1} showed the presence of carbonyl group anda band at 1290.38 cm^{-1} is due to C-O bond stretching. The IR spectrum of Compound DP-3 showed presence of hydroxyl group, aromatic carbons and presence of carbonyl carbon.

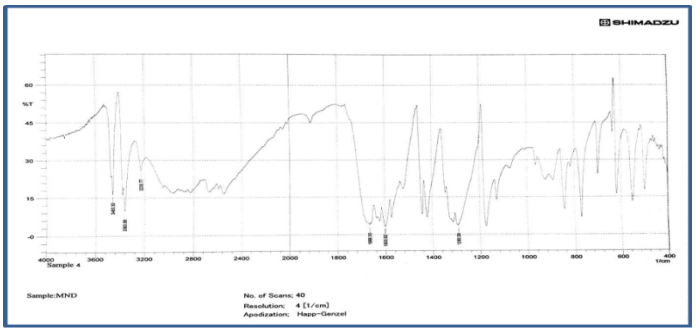


Figure:17 FTIR spectrum of compound DP-3 isolated from ethanolic extractof *Diplocyclos palmatus fruits*

Table :17 FTIR – Spectroscopy interpretation of compound DP-3 isolatedfrom ethanolic extract of *Diplocyclos palmatus fruits*

Sr. No	Wave number (cm^{-1})	Group assigned
1	3460.30	-O-H Stretching
2	3363.86	-CH aromatic stretching
3	3230.77	-CH aliphatic stretching
4	1600.92	-C=O stretching
5	1290.38	-C-O Stretch

1.28 ^{1}HNMR and ^{13}CNMR of isolated compound DP-3

^{1}HNMR

^{1}H NMR spectrum of compound DP-3 showed a multiplet at δ 6.182 — 6.202 for onearomatic proton; a doublet at δ 6.299 – 6.304 for one aromatic proton; a multiplet at δ
6.341 — 6.368 for one aromatic proton; a doublet at δ 6.406- 6.411 for one aromatic protons; a doublet at δ 7.222 - 7.243 for two aromatic protons; a singlet at δ 9.814 for two protons; a singlet at δ 10.749 for one proton and a singlet at δ 12.626 for one proton. ^{1}H NMR showed the presence of six aromatic protons and four hydroxyl groups.

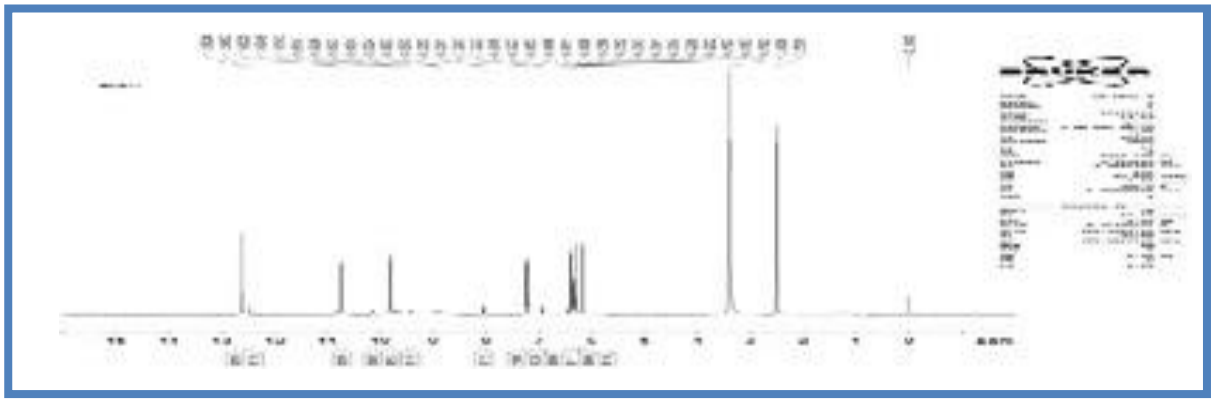


Figure:18 ^{1}HNMR spectrum of compound DP-3 isolated from ethanolicextract of *Diplocyclos palmatus fruits*

^{13}C NMR

^{13}C NMR spectrum of compound DP-3 showed presence of fifteen aromatic carbonsat δ 92.32 (s); 102.85 (s); 103.49 (s); 106.73 (s); 109.11 (d); 131.70 (d); 136.17 (s);
148.97 (s), 156.72 (s); 156.75 (s); 160.38 (s); 163.63 (s) and a singlet at δ 176.15
shows a presence of carbonyl carbon. ^{13}C NMR showed presence of fifteen aromaticcarbons and a carbonyl carbon

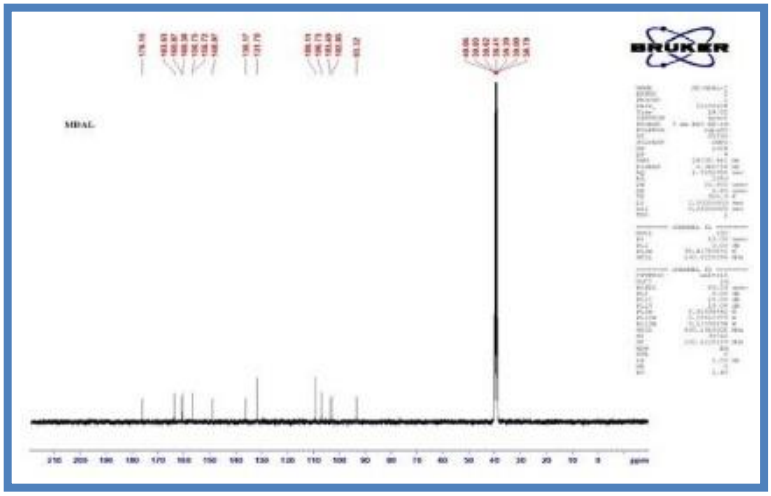


Figure :19 ^{13}C NMR spectrum of compound DP-3 isolated from ethanolicextract of *Diplocyclopalmatus fruit*

1.29 Mass Spectra

The mass spectrum of compound DP-3 isolated from ethanolic extract of *Diplocyclos palmatus fruits* showed fragments at m/z 285, 257, 256, 244, 228, 183, 157, 141, 129 and113. The molecular weight of the compound is found to be 286.

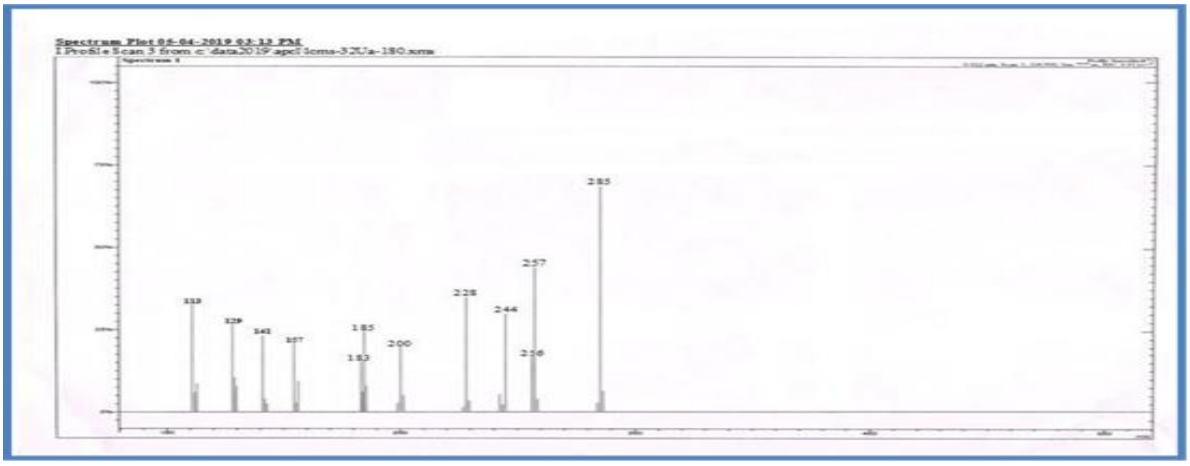


Figure:20 Mass Spectra of compound DP-3 isolated from ethanolic extract of *Diplocyclos palmatus fruits*

Table:18 Spectroscopic data of compound DP-3 isolated from ethanolicextract of *Diplocyclos palmatus fruits*

Sr.No	Spectroscopic technique	Data
1	UV	265nm, 365nm
2	IR FTIR	3460.30, 3363.86, 3230.77, 1600.92, 1290.38 cm^{-1}
3	MS spectroscopy	285, 257, 256, 244, 228,183,157,141,129,113
4	^{1}H NMR DMSO	δ:12.430(s,1H), 10.749(s,1H), 9.814(s,2H), 7.222- 7.243(d,2H), 6.406-6.411(d,1H,Ar-H), 6.341-6.368(m,1H, Ar-H), 6.299-6.304(d,1H,Ar-H), 6.182-6.202(m,1H,Ar-H)
5	^{13}C NMR DMSO	δ:92.32(s), 102.85 (s), 103.49(s), 106.73(s), 109.11(d), 131.70(d), 136.17(s), 148.97(s), 156.72(s), 156.75(s), 160.38(s), 163.63(s), 176.15(s)

30 Molecular Docking Study

H^+ K^+ ATPase secretes the acid in the stomach and is thus responsible for acidifying the gastric lumen. Any drugs capable of inhibiting proton pump have prospective of being acid suppressing drugs. The crystal structure of the target enzyme H^+ K^+ ATPase was determined at 6.5A resolution in the E2P state with a bound BYK99 which is a potent potassium competitive acid blocker BYK99 bound structure has common conformational change required for potassium competitive acid binding.The site at which the known H^+ K^+ ATPase inhibitor binds with the target protein was selected as active site Proton pump inhibitor Omeprazole was used as standard drug. Proton pump inhibitors are a weak base which get protonated and gets accumulated in the gastric lumen where they are converted to active sulfonamides which binds covalently with the Cys 813 which results in acid suppression .

The site at which Proton pump inhibitors like omeprazole binds with the target protein was selected as active site. Omeprazole forms the linkage with the amino acid residues such as Leu^{921}, Cys^{813}, Ile^{119}, His^{902}. Isolated compound CG-3 (Rhamnocitrin) forms the linkage with amino acid Cys^{813}, Gly812, Asn^{138}, Trp^{899},Gln^{924}. Isolated compound DP-3 (Kaempferol) forms the hydrogen bonding with amino acid residues His^{902},Asp^{132}, Leu^{133} Leu ^{921},Tyr ^{928}. The MolDock scores of the omeprazole was found to be -112.113 whereas MolDock score of Rhamnocitrin and Kaempferol -86.456 and 89.377 respectively. The best dock poses of the omeprazole, Rhamnocitrin and Kaempferol is depicted.

Table:19 Molecular docking data of Omeprazole, Rhamnocitrin and Kaempferol

Compound	MolDock Score	Rerank Score	H Bond
Omeprazole	-112.113	-27.5184	-5.69787
Rhamnocitrin	-89.3773	-63.3615	-18.7666
Kaempferol	-86.4568	-73.4343	-11.8466

1.30 Molecular Docking Study

H^+ K^+ ATPase secretes the acid in the stomach and is thus responsible for acidifying the gastric lumen. Any drugs capable of inhibiting proton pump have prospective of being acid suppressing drugs. The crystal structure of the target enzyme H^+ K^+ ATPase was determined at 6.5A resolution in the E2P state with a bound BYK99 which is a potent potassium competitive acid blocker BYK99 bound structure has common conformational change required for potassium competitive acid binding. The site at which the known H^+ K^+ ATPase inhibitor binds with the target protein was selected as active site Proton pump inhibitor Omeprazole was used as standard drug. Proton pump inhibitors are a weak base which get protonated and gets accumulated in the gastric lumen where they are converted to active sulfonamides which binds covalently with the Cys 813 which results in acid suppression .

The site at which Proton pump inhibitors like omeprazole binds with the target protein was selected as active site. Omeprazole forms the linkage with the amino acid residues such as Leu^{921}, Cys^{813}, Ile^{119}, His^{902}. Isolated compound CG-3 (Rhamnocitrin) forms the linkage with amino acid Cys^{813}, Gly812, Asn^{138}, Trp^{899},Gln^{924}. Isolated compound DP-3 (Kaempferol) forms the hydrogen bonding with amino acid residues His^{902},Asp^{132}, Leu^{133} Leu ^{921},Tyr ^{928}. The MolDock scores of the omeprazole was found to be -112.113 whereas MolDock score of Rhamnocitrin and Kaempferol -86.456 and 89.377 respectively. The best dock poses of the omeprazole, Rhamnocitrin and Kaempferol is depicted.

Table:19 Molecular docking data of Omeprazole, Rhamnocitrin and Kaempferol

Compound	MolDock Score	Rerank Score	H Bond
Omeprazole	-112.113	-27.5184	-5.69787
Rhamnocitrin	-89.3773	-63.3615	-18.7666
Kaempferol	-86.4568	-73.4343	-11.8466

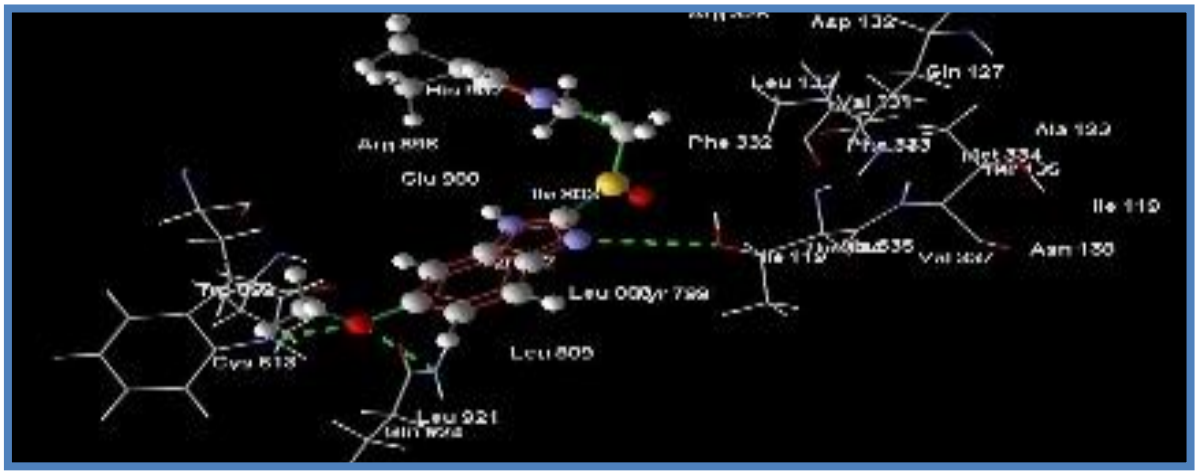


Figure: 21 The standard drug Omeprazole docked in best of its conformationinto the binding site of 5Y0B

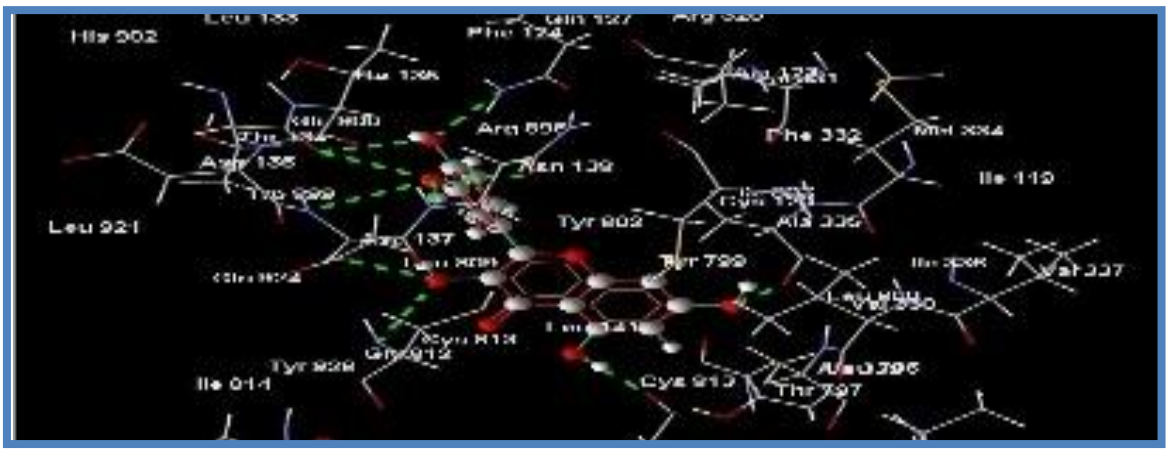


Figure: 22 The compound Rhamnocitrin (CG-3) docked in best of itsconformation into the binding site of 5Y0B

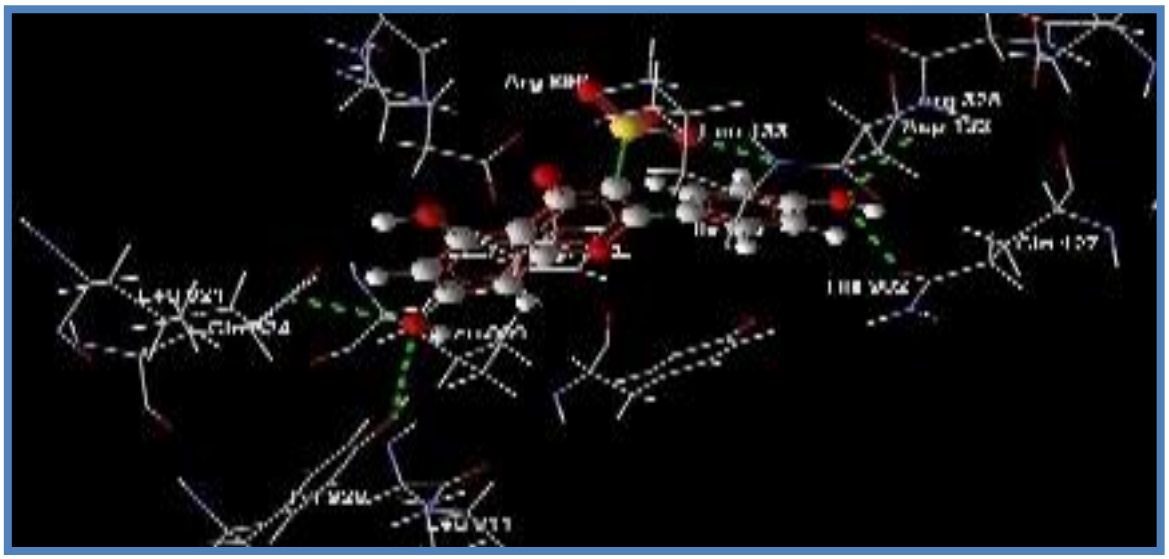


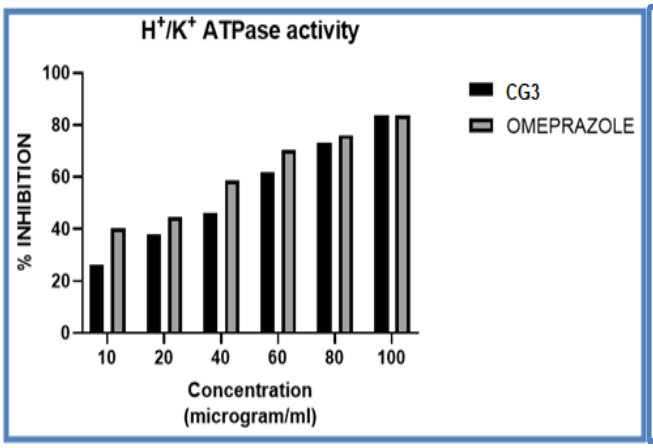
Figure: 2 3 The Compound Kaempferol (DP-3) docked in best of itsconformation into the binding site of 5Y0B 1.40 *In Vitro* H^+ K^+ ATP*ase* activity

Isolated compound from *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* namely CG-3 and DP-3 respectively showed a promising *in vivo* antiulcer activity with H^+K^+ ATPase inhibitory activity in docking studies and hence were accessed for determination of *invitro* H^+ K^+ ATP*ase* inhibitory activity. Omeprazole was used as a standard. Resultsof the activity is shown in table

Figure :23 shows that CG-3 significantly caused H^+ K^+ ATPase inhibition in dose dependent manner. IC_{50} of CG-3 was found to be 42.43 ±1.66. DP-3 depicted significant H^+K^+ ATPase inhibitory activity with IC $_{50}$ Value of 58.47 ± 5.27 as shown in table

Table :20 H^+K^+ ATPase inhibitory action of CG-3 and DP-3

	Treatment		
Concentration(µg/ml)	**CG-3**	**DP-3**	**Omeprazole**
10	26.33±5.06	15.59±2.57	40.24±3.67
20	37.81±1.68	29.16±1.48	44.71±4.28
40	46.22±2.85	38.33±2.48	58.54±3.23
60	61.77±1.93	57.36±2.06	70.32±4.93
80	73.04±1.62	67.59±3.88	76.02±1.86
100	83.59±2.88	75.64±0.90	83.74±1.76
IC_{50}	42.43±1.66	58.47±5.27	26.47±3.92



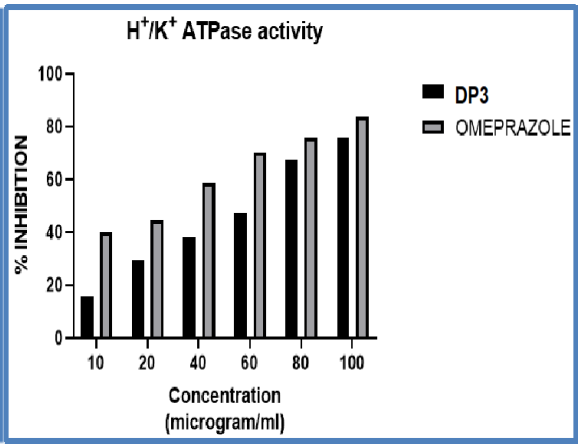


Figure :25 Percentage H^+ K^+ ATPase Figure CG-3 and Standard Omeprazole

Figure: 26 Percentage H^+ K^+ ATPase DP-3 and Standard Omeprazole

1.31Qualitative and quantitative analysis of phenolic compounds by RP-HPLC

Preparation of sample and standard solution

The dried powder of fruit (500 mg) with methanol (10 ml) was kept on orbital shaker incubator at 110±2 rpm (25 ˚C, 48 h). The further mixture was filtered through Whatman filter No. 1 and centrifuged at 10,000 rpm for 20 min at 4 ˚C. Supernatant was collected and stored at 4 ˚C until HPLC analysis. Phenolic compounds such as GA, CA, HBA, CLA, VA and coumaric acid (COA) (Sigma-Aldrich, St. Louis, Mo, USA) were weighed accurately and dissolved separately in methanol to obtain a standard stock solution (1 mg/ml). Further, working stock solution was prepared by diluting the stock solution with methanol to get five different concentrations for calibration curves. All the stock and working stock solutions were stored at 4 °C until further use.

RP-HPLC analysis

RP-HPLC analysis of phenolic compounds was performed on Jasco chromatographic system (Model no. LC-2000 Plus) equipped with a quaternary pump, autosampler, and UV detector (UV 2070). The separation was performed using Hiber C18 column (5 µm, 250-4, 6 mm). The built in ChromNAV software system was used for data processing. The mobile phase consisted of water: acetonitrile: glacial acetic acid (90:5:5) was used with 0.9 ml/min flow rate and 20 µl injection volume. The peaks were monitored at 280 nm with 50 min as run time. The identification of phenolic compounds in extracts was performed by comparing their retention times with those of standards. Identified phenolics were confirmed by spiking with known concentration of the respective standard. A standard curve of selected phenolics with four different concentrations (25–200 µg/ml) was prepared and expressed as micrograms per gram of dry weight (mg/g DW). The system suitability was assessed by triplicate injection of standard solutions and extracts. The peak areas of three replicate injections of standard solutions and extracts were considered to evaluate the repeatability of the method. All solutions (mixed standards, samples and spiked solutions) were filtered through 0.22 µm nylon syringe filter.

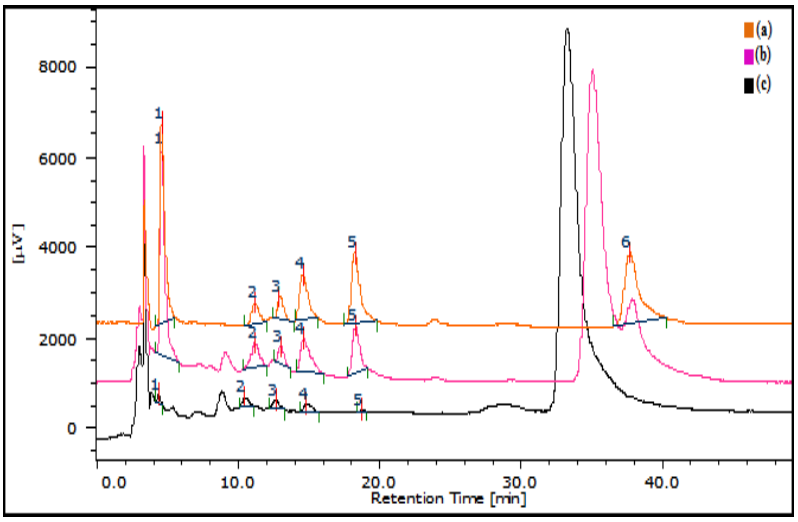


Figure 27 HPLC chromatograms a) Mixture of standard phenolics compounds 1) GA 2) CA, 3) HBA, 4) CLA, 5) VA; 6) COA; b) Crude extract of fruit of *D. palmatus* spiked with above mention standard compounds; C) Crude extract of fruit of *D. palmatus* where the peaks were identified as follows: 1) GA, 2) CA, 3) HBA, 4) CLA, 5) VA

1.32 DISCUSSION

Ethanolic extract of *Cocinnia grandis* fruits (EECG) demonstrated the presence of many phytoconstituents, including carbohydrates, steroids, glycosides, flavonoids, alkaloids, and tannins,.

Diplocyclos palmatus fruits' preliminary phytochemical screening revealed the presence of sugars, steroids, glycosides, flavonoids, tannins, and triterpenes in its ethanolic extract (EEDP). Compare to other solvent extract revealed the presence of less phytoconstituents. Glycosides, tannins, and steroids were all detected in PEDP.

In Total phenolic content in *cocinnia grandis* and flavonoids content in the *Diplocyclos palmatus* fruit extract is greater than PEDP and CEDP.

One of the main secondary metabolites, flavonoids, is in charge of the plant's antioxidant action. The quantity and location of free hydroxyl groups affect the antioxidant activity of flavonoids Flavonoids have been shown to have ulcer-healing and antiulcer properties the results showed that EECG and EEDP possess increased flavonoid concentration.

In toxicity tests, all three extracts from *Cocinnia grandis* and Diplocyclos palmatus fruits, namely PECG, CECG, EECG, PEDP, CEDP, and EEDP, were shown to be well tolerated. Since no animal died. Using several ulcer producing models, including pylorus ligation-induced ulcers, an aspirin-induced ulcer model, the antiulcer effectiveness of PECG, CECG, EECG, PEDP,CEDP, and EEDP was evaluated.

When compared to the control group, the ulcer index significantly decreased in the EECG and EEDP groups. The amount of pH elevation caused by EECG and EEDP was equivalent to that of the reference group receiving omeprazole treatment. Compared to the control group receiving vehicle treatment, EECG and EEDP significantly lowered both total acidity and free acidity. This strategy of ulcer reduction works well with substances that promote mucus secretion and/or decrease stomach acid secretion.

In isolation mobile phases of n-hexane: chloroform and chloroform: methanol, phytoconstituents from EEDP were successfully isolated. Three compounds with Rf values of 0.86 were identified from the fruits *of Diplocyclos palmatus* and given the names DP-1, DP-2, and DP-3.respectively 0.81 and 0.62.

To identify the active ingredient responsible for the ulcer-protective qualities isolated compounds from *Cocinnia grandis* fruits CG-2, CG-3, and CG-4 as well as isolated compounds from *Diplocyclos palmatus* fruits DP-1, DP-2 and DP-3 were tested for in vitro antiulcer activity.

During acute toxicity investigations, EECG and EEDP were determined to be safe up to a level of 5000mg/kg. Isolated chemicals amounted to roughly 200 mg from 30g of ethanolic extract. Therefore, assuming extract was safe up to 5000 mg/kg, the safe dose of the e؟quivalent isolate was determined to be about 10 mg/kg. Thus, the three dosages of 20 mg/kg, 10 mg/kg, and 5 mg/kg chosen for the research.Utilising the aspirin with pylorus ligation, anti-ulcer screening of isolated compounds was carried out.

In comparison to CG-2, CG-4, DP-1, DP-2, and the control group receiving vehicle treatment, CG-3 and DP-3 had greater antiulcer activity. The ulcer index, stomach volume, total acidity, free acidity, and gastric pH all decreased in a dose-dependent manner as a result of The epithelial lining of the rats in the control group's histopathology slides showed clear signs of disruption, whereas the group that received conventional treatment with Omeprazole, group treated with 20 mg/kg of CG-3, and group treated with 20 mg/kg of DP-3, both demonstrated intactness of the gastric epithelium and no mucosal ulcers.

According to the findings of the current research, it was important that the compounds CG-3 and DP-3, which were isolated from the fruits of *Cocinnia grandis and Diplocyclos palmatus*, respectively, both demonstrated strong antiulcer action and were thus chosen for future investigation.

CG-3 and DP-3 were characterised and structurally clarified using a variety of spectrum techniques, including UV, IR, NMR, and mass spectroscopy.

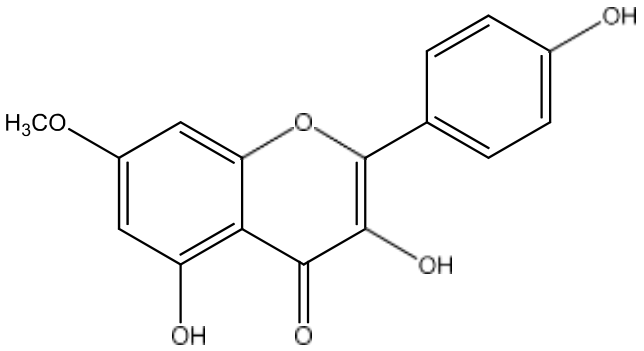
Compound CG-3, a yellow amorphous solid with a melting point of 224–2270 C, was isolated. Its greatest absorbance was observed at 270 and 363 nm when it was scanned over the UV spectrum.

Due to the presence of a hydroxyl group, the IR spectra of sample CG-3 revealed the presence of a band at 3525 and 3375 cm-1. The existence of aromatic C-H stretching and aliphatic C-H stretching, respectively, is shown by the absorption bands at 3140 and 2818 cm-1. A band at 1174 cm-1 is caused by the stretching of the C-O bond, while a significant absorption band at 1662 cm-1 indicated the existence of the carbonyl group. The compound CG-3's IR spectra revealed the presence of a hydroxyl group, aromatic carbons, aliphatic carbons, and carbonyl carbon.

The sample CG-3's 1H NMR spectrum included singlets at 3.897 for three aliphatic protons, 6.051 for one aromatic proton, 6.453 for one aromatic proton, 6.850 to 6.893 for two aromatic protons, 8.151 to 8.201 for two aromatic protons, 9.951 for one proton, 10.732 for one proton, and 12.430 for one proton. Three hydroxyl groups, six aromatic protons, and the presence of the methyl group were all detected by 1H NMR..

According to the sample's spectrum analysis, fused aromatic rings are present and are joined to carbonyl, methyl, and three hydroxyl groups. Shinoda test results for compound CG-3 showed the presence of flavonoids. The integral values obtained from the UV spectrum, IR spectrum, NMR spectrum, and mass spectrum and their correlation with published literature suggest that Compound CG-3 may be Rhamnocitrin

with the molecular formula C16 H12 O6. The spectral data of CG-3 were in agreement with the results reported earlier .



Rhamnocitrin (7-O-Methyl Kaempferol)

Yellow crystalline solid compound DP-3, having a melting point of 277–2790 C Methanol was used as a solvent for the UV scan of DP-3, which revealed highest absorbance at 265 and 365 nm.
Due to the presence of a hydroxyl group, the IR spectra of compound DP-3 revealed the presence of a band at 3460 cm-1. The existence of aromatic C-H and aliphatic C-H stretching are shown by the absorption bands at 3363 and 3230 cm-1, respectively. A band at 1290 cm-1 is caused by the stretching of the C-O bond, and a significant absorption band at 1600 cm-1 indicated the existence of the carbonyl group. Compound DP-3's IR spectra revealed the presence of a hydroxyl group, aromatic carbons, and carbonyl carbon.

A multiplet at 6.182 to 6.202 for one aromatic proton, a doublet at 6.299 to 6.304 for one aromatic proton, a multiplet at 6.341 to 6.368 for one aromatic proton, and a doublet at 6.406 to 6.411 for one aromatic proton were all visible in the chemical DP-3's 1H NMR spectrum. Singlet at 9.814 for two protons; a doublet at 7.222–7.243 for two aromatic protons; a singlet at 10.749 for one proton; and a singlet at 12.626 for one proton. Four hydroxyl groups and six aromatic protons were detected by 1H NMR.

Based on spectrum data, this molecule also has four hydroxyl groups and a flavonoid nucleus connected to a carbonyl group. In the shinoda test, compound DP-3 also demonstrated the presence of flavonoids. The spectrum values of compound DP-3 were similar to and agreed with those published in the literature, indicating that it may represent kaempferol . lowering ulcer index, increasing stomach pH, and decreasing ulcer volume. H+ K+ ATPase inhibitory action of CG-3 (Rhamnocitrin) supported in vivo antiulcer research. DP-3 (kaempferol), which was evaluated using in vitro and molecular docking experiments.

1.33 SUMMARY AND CONCLUSION

Isolated constituents from cocinnia grandis and Diplocyclos palmatus has effective in the given doses concentration could be attributed to a decrease in gastric acid secretion, (protection of the mucosal barrier and restoration of mucosal secretions, inhibition of free radical generation or prevention of lipid peroxidation,and free radical scavenging shows antioxidant activity

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