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Antioxidant activity and antiulcer activity of phytoconstituents of *Coccinia grandis,and Diplocyclos palmatus fruit (Reaserch aticle)*

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Abstract

Coccinia grandis Linn (Common name- Kundari) is a very common plant and *Diplocyclos palmatus (*shivlngi) *f*amily Cucurbitaceae) distributed throughout India and it well known for its medicinal uses It is used as anti-inflammatory, laxative, antidiabetic , hepatoprotective , antibacterial, hypolipidaemic, Steroidal activity, antiinlammatory, analgesic activity . The plant mainly contains secondary metabolites flavonoid, β-sitosterol, β-carotene and linoleic and palmitic acids. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases . In the present investigation we have investigate antioxidant activity and antiulcer activity by using pylorus ligation method in rat model by using phytoconstituents of *Coccinia grandis,and Diplocyclos palmatus* fruit extracts.

Keyword: ***Coccinia grandis, Diplocyclos palmatus, Antiulcer* activity, antioxidant activity**

Introduction

Ulcers are deep lesions penetrating through entire thickness of gastrointestinal tract mucosa and muscularis mucosa. There are so many types of ulcers and most common are peptic ulcers.(2) Peptic ulcers are defect of the gastrointestinal mucosa that extend through the muscularis mucosa because of the presence of acid and pepsin.(3) Helicobacter pylori infection is responsible for nearly 80% of gastric ulcers and for over 90% of duodenal ulcers. Nonsteroidal anti-inflammatory drugs and aspirin use are other major causes of peptic ulcer, especially for gastric ulcers.(4) Stress either psychological or physical leads to oxidative stress in stomach i.e. production of reactive oxygen species.Oxidative stress involve in pathogenesis of gastric inflammation,ulcerogenesis and carcinogenesis in H.pyloriinfection. Helicobacter pylori (HP) is a frequent gastro-intestinal infectious agent having worldwide distribution. It is a Gram-negative, microaerophilic, spiral bacterium that shows particular tropism for the gastric mucosa, and induces a strong inflammatory response with release of various bacterial and host-dependent cytotoxic substances. In 1984, Marshall and Warren(5).

The host, and environmental and bacterial factors are important in the clinical manifestations of infections with this bacillus.(6)Apart from its well-demonstrated role in gastroduodenal diseases, some authors have suggested a potential role of HP infection in several extra-intestinal pathologies including haematological, cardiovascular,

neurological, metabolic, autoimmune,andskindiseases.(7)(8)(9).Helicobacter pylori infection has been considered a potential inducer of several immune-mediated skin disorders.These disorders can be manifestations of systemic vasculitides (Behçet's disease [BD]) or may be related to skin disorders with presumed autoimmune origin (urticaria, psoriasis, alopecia areata [AA], lichen planus, etc).(10)Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease (11) Oxygen radical absorbance capacity assay (ORAC), is one of the most popular and best There is, thus, a need to search for natural alternatives having anti-ulcer properties. This has been the basis for the development of new anti-ulcer standardized chemical in vitro antioxidant assay(12-15). It is widely used for evaluation and comparison of the antioxidant capacity in natural products (16).Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re enforcing gastric mucosal protection.(17,18) Although a number of anti-ulcer drugs such as H2 receptor antagonists, proton pump inhibitors and cytoprotectants are available, all these drugs have side effects and limitations(19). agents, which include herbal substances have been used to treat gastrointestinal disorders, including gastric ulcers(20).

1.*Coccinia grandis* fruits

Coccinia grandis L. of the family Cucurbitaceous is distributed in tropical Asia, Africa and is commonly found in Pakistan, India and Srilanka(21).Coccina is a climber and trailer (22). The fruit of *Coccinia grandis* is used as vegetable when green and eaten fresh when ripened into bright scarlet colour(23).Every part of this plant is valuable in medicine and various preparations have been mentioned in indigenous system of medicine for various skin diseases, bronchitis and Unani systems of medicine for ring worm, psoriasis small pox, scabies (24).and other itchy skin eruptions and ulcers(25). Oil of this plant is used as an injection into chronic sinuses. The plant is used in decoction for gonnorhoeae(26),diabetes and also useful in dropsically condition, pyelitis, cystitis, strangury, snake bite, urinary gravel and calculi(27).It is also useful to induce perspiration in fever and cures sores in the tongue(28).Ithas antilithic(29) hypolipidimic(30),antimutagenic(31) and hypoglycemic activities (32), (33), (34), (35).

2. *Diplocyclos palmatus* fruits

*Diplocyclospalmatu*s (L.) C. Jefrey is a slender-stemmed tendril climber commonly called as Shivlingi. Traditionally, this plant has been used in the folk medicine and possesses several activities such as gynaecological, anti-asthmatic, anti-convulsant, anti-venom, anti-infammatory, androgenic and Antioxidant(36),(37),(38). Phytochemical studies of *Diplocyclos palmatus* shows the presence of alkaloids, flavonoids, triterpinoid, saponins, steroids and proteins, resins with, Sugars, starch. The seeds have been reported to contain 12% oil, protein also contains goniothalamin, bryonin, punicicacid and lipids(39-40). In traditional system of medicine, different parts (Leaves, stem, flower, seeds and even whole plant) of *Diplocyclos palmatus* have been used to treat various diseases. It has considerable reputation as a potent adjunct in the treatment of various ailments such as jaundice, inflammation and fever. Shivalingi (*Diplocyclos palmatus* Linn.) is a lesser heard medicinal plant of Ayurveda with the fruits having important use in the area of reproductive medicine (female infertility, aphrodisiac, tonic).(41)

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 (42).

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(43)

Synthetic antiulcer medications have side effects that include impotence, gynecomastia, blood dyscriasis, hypertension, nephritis, impaired sexual drive, hepatitis, pancreatitis, increased liver enzyme activity and triglycerides, leucocytopenia, thrombocytopenia, electrolyte imbalance, arrhythmias, and hematopoietic changes 44

Due to the negative side effects of synthetic pharmaceuticals, herbal medicines are becoming increasingly important in the management and treatment of peptic ulcers (45). Due to their efficacy and safety profile, In the Ayurvedic book "Bhavprakashnighantu" chapter "Guduchyadivarg," Cocinnia grandis and Diplocyclos palmatus fruits are described as having the ability to lessen burning and inflammation ("shit and dahashaman") and to aid in wound healing ("Vranaropan"), both of which may be helpful in treating ulcers(46). Thus, the current research aims to identify the phytoconstituents that are responsible for the ulcer-healing potential of the fruits of the plants *Cocinnia grandis and Diplocyclos palmatus*.

Authentication of Plants

The plants were identified and authenticated by. Head of botony department .Arts and science commerce P .o nahata college of Bhusawal , Distric jalgaon Maharashtra.

Table 1. Authentication of plant materials

Name of Plant	Family
Cocinnia grandis fruits	Cucurbitacea .
Diplocyclos palmatus fruits	Cucurbitacea .

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Physico-chemical evaluation of plant material

Physico-chemical evaluation of dried powdered plant material of Cocinnia Grandis fruits and Diplocyclos palmatus fruits were evaluated using several evaluation criteria.

We looked at plant extractive values, drying loss, and Ash values. Ash values reveal the quality and purity of the medicine, whereas loss on drying establishes the presence of moisture. Alcohol (Ethanol) has higher extractive values for Cocinnia grandis and Diplocyclos palmatus fruits than water, chloroform, or petroleum ether. Yields from a series of solvent extractions utilising various solvents were reported. Cocinnia grandis and Diplocyclos palmatus fruit ethanolic extracts had higher percentage yields than their chloroform and petroleum ether counterparts. The outcome of the physico-chemical assessment is displayed in Table No. 5.2.

Table 2 Physico-chemical parameter of *Cocinnia grandis fruits* and*Diplocyclos palmatus fruits*

Sr.No	Parameters	*Cocinnia grandis fruits*	*Diplocyclos palmatus fruits*
1.Extractive Value			
a.	Alcohol soluble extractiveValue	13.26%w/w	15.09%w/w
b.	Water soluble extractive Value	8.8%w/w	7.6%w/w
c.	Chloroform soluble extractive Value	6.25%w/w	9.09%w/w
d.	Petroleum ether soluble extractive Value	3.25%w/w	3.80%w/w
2.Determination of moisture content			
a.	Loss on drying	5.72%w/w	8.5%w/w
3. Ash values			
a.	Total ash value	9.31%w/w	7.65%w/w
b.	Water soluble ash value	3.68%w/w	3.75%w/w
c.	Acid insoluble ash value	1.58%w/w	1.32%w/w
4. Percentage yield of extract			
a.	Petroleum ether extract	4.80%w/w	3.76%w/w
b.	Chloroform extract	7.01%w/w	4.50%w/w
c.	Ethanolic extract	14.62%w/w	16.23%w/w

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 Cocinnia grandis and Diplocyclos palmatus. Fruits from 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.

Table 3 Preliminary phytochemical screening of *Cocinnia grandis fruits* .

Phytoconstituents	**Chemical tests**	***Cocinnia grandis fruits***		
		Petroleum ether extract	**Chloroform extract**	**Ethanolic extract**
Carbohydrates	Molisch's test	-	-	+
Reducing sugars	Fehlings Test	-	-	+
	Benedicts test	-	-	+
Test for monosacchride	Barfoed's test	-	-	+
Test for non-reducing polysacchrides	Tannic acid test for starch	-	-	+
Proteins and amino	Biurets test	-	-	-

Acids	Millon's Test	-	-	-
	Xanthoprotein test	-	-	-
	Ninhydrin test	-	-	-
Steroids	Salkowski test	+	+	+
	Liebermann-Burchardt tests	+	+	+
Glycoside	Baljet's test	+	+	+
	Legal's test	-	+	+
	Keller killani test	-	-	-
Anthraquinone glycosides	Borntrager's test	-	-	+
	Modified Borntrager's test	-	-	+
Saponin glycoside	Foam test	-	-	+
Cyanogenetic glycosides	Grignards reaction or sodium picrate test	-	-	-
Coumarins glycoside	Sodium hydroxide test	-	-	-
Flavonoids	Shinoda test	-	+	+
	Lead acetate test	-	-	+
	Alkaline reagent test	-	+	+
Alkaloids	Dragendroff's test	-	+	+
	Mayer's test	-	+	+
	Hager's test	-	+	+
	Wagner's test	-	+	+
Tannins and phenolic compound	Ferric chloride test	-	-	+
	Gelatin test	-	-	+
	Bromine water	-	-	+
	Dilute iodine test	-	-	-
Triterpenes	Tschugajens test	+	+	-

Table 4 Preliminary phytochemical screening of *Diplocyclos palmatus fruits*

Phytoconstituents	Chemical tests	*Diplocyclos palmatus fruits*		
		Petroleum ether extract	Chloroform extract	Ethanolic extract
Carbohydrates	Molisch's test	-	+	+
Reducing sugars	Fehlings Test	-	+	+
	Benedicts test	-	+	+
Test for monosacchride	Barfoed's test	-	-	-
Test for non-reducing polysacchrides	Tannic acid test	-	-	-
Proteins and amino acids	Biurets test	-	-	-
	Millon's Test	-	-	-
	Xanthoprotein test	-	-	-
	Ninhydrin test	-	-	-
Steroids	Salkowski test	+	+	+
	Liebermann-Burchardt tests	+	+	+
Glycoside	Baljet's test	+	+	+
	Legal's test	+	+	+
	Keller killani test	+	+	+
Anthraquinone glycosides	Borntrager's test	+	+	+
	Modified Borntrager's test	+	+	+
Saponin glycoside	Foam test	+	+	-
Cyanogenetic glycosides	Grignards reaction or sodium picrate test	-	-	-
Coumarins glycoside	Sodium hydroxide test	-	-	-
Flavonoids	Shinoda test	-	+	+
	Lead acetate test	-	-	+
	Alkaline reagent test	-	+	+
Alkaloids	Dragendroff's test	-	-	-
	Mayer's test	-	-	-
	Hager's test	-	-	-
	Wagner's test	-	-	-

Tannins and phenolic compound	Ferric chloride test	+	+	+
	Gelatin test	+	+	+
	Bromine water	+	+	+
	Dilute iodine test	+	+	+
Triterpenes	Tschugajens test	-	+	+

Quantitative phytochemical screening

Estimation of total phenolic content: Folin–Ciocalteu's method

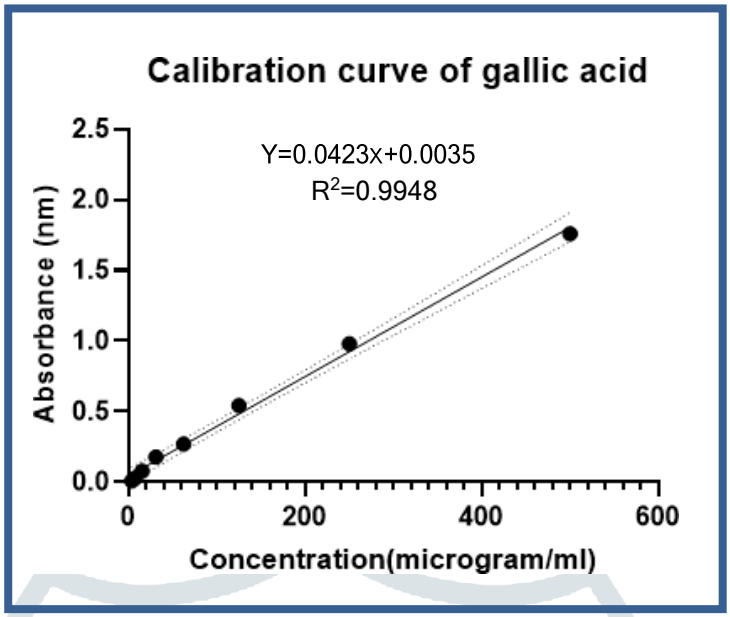
By using the Folin-Ciocalteu technique, the total phenolic content of fruit extracts from Cocinnia grandis and Diplocyclos palmatus in petroleum, chloroform, and ethanol was measured. It was discovered that the ethanolic extracts of Cocinnia grandis and Diplocyclos palmatus fruits had higher phenolic contents than the corresponding petroleum ether and chloroform extracts.

Estimation of total flavonoid content: Aluminum chloridecolorimetric method.

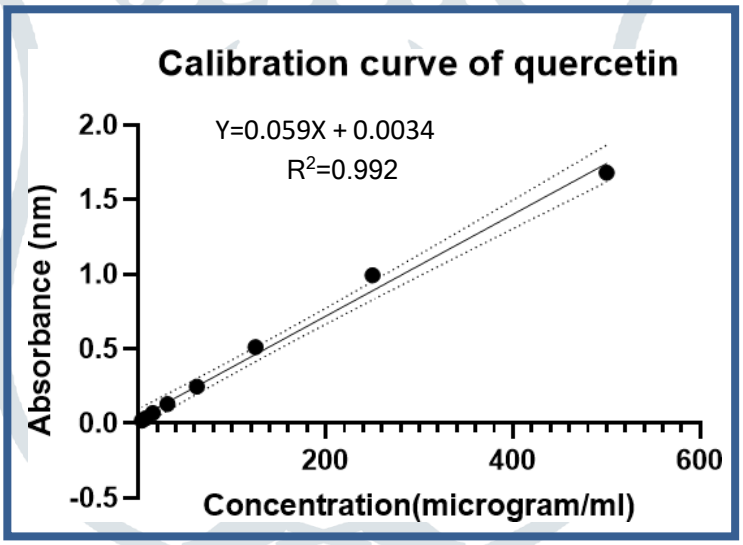
The Folin-Ciocalteu technique was used to assess the total phenolic content of the fruits of Cocinnia grandis and Diplocyclos palmatus in petroleum extract, chloroform extract, and ethanolic extract. The phenolic content of ethanolic extracts of Cocinnia grandis and Diplocyclos palmatus fruits was found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

Table 5 Total phenolic and total flavonoid content

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



Graph 1 Calibration curve of Gallic acid



Graph 2 Calibration curve of Quercetin

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. Results are tabulated in table

Table 6 Acute toxicity study of *Cocinnia grandis fruits* extracts

Treatment	Dose mg/kg	Number of Animals	Mortality			Toxicity Profile
			After 3h	After 6h	After 24h	
PECG	100	5	0	0	0	Safe
	500	5	0	0	0	Safe
	1000	5	0	0	0	Safe
	2000	5	0	0	0	Safe
	4000	5	0	0	0	Safe
	5000	5	0	0	0	Safe
CECG	100	5	0	0	0	Safe
	500	5	0	0	0	Safe
	1000	5	0	0	0	Safe
	2000	5	0	0	0	Safe
	4000	5	0	0	0	Safe
	5000	5	0	0	0	Safe
EECG	100	5	0	0	0	Safe
	500	5	0	0	0	Safe
	1000	5	0	0	0	Safe
	2000	5	0	0	0	Safe
	4000	5	0	0	0	Safe
	5000	5	0	0	0	Safe

Table 7Acute toxicity study of *Diplocyclos palmatus fruits* extracts

Treatment	Dose mg/kg	Number of Animals	Mortality			Toxicity Profile
			After 3h	After 6h	After 24h	
PEDP	100	5	0	0	0	Safe
	500	5	0	0	0	Safe
	1000	5	0	0	0	Safe
	2000	5	0	0	0	Safe
	4000	5	0	0	0	Safe
	5000	5	0	0	0	Safe
CEDP	100	5	0	0	0	Safe
	500	5	0	0	0	Safe
	1000	5	0	0	0	Safe
	2000	5	0	0	0	Safe
	4000	5	0	0	0	Safe
	5000	5	0	0	0	Safe
EEDP	100	5	0	0	0	Safe
	500	5	0	0	0	Safe
	1000	5	0	0	0	Safe
	2000	5	0	0	0	Safe
	4000	5	0	0	0	Safe
	5000	5	0	0	0	Safe

Table 8 Irwin Table for Acute toxicity

Treatment	Parameter observed																			
	Behavioural response									Neurological response							Autonomic response				
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	1	2	3	4	5
	Alertness	Stereotype	Irritability	Fearfulness	Touch	Pain response	Grooming	Restlessness	Righting reflex	Limb tone	Grip strength	Pinna reflex	Corneal reflex	Straub tail	Convulsions	Writhing	Defecation	Urination	Piloerection	Pupil size	Skin colour
PECG	N	N	-	-	N	N	-	N	N	N	-	-	N	Ab	Ab	Ab	-	N	Ab	N	N
CECG	N	N	-	-	N	N	-	N	N	N	-	-	N	Ab	Ab	Ab	-	N	Ab	N	N
EECG	N	N	-	-	N	N	-	N	N	N	-	-	N	Ab	Ab	Ab	-	N	Ab	N	N
PEDP	N	N	-	-	N	N	-	N	N	N	-	-	N	Ab	Ab	Ab	-	N	Ab	N	N
CEDP	N	N	-	-	N	N	-	N	N	N	-	-	N	Ab	Ab	Ab	-	N	Ab	N	N
EEDP	N	N	-	-	N	N	-	N	N	N	-	-	N	Ab	Ab	Ab	-	N	Ab	N	N

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 ligationinduced ulcer, ethanol induced ulcer, cold restraint stress induced ulcer and Aspirin induced ulcer model.

Preliminary anti-ulcer activity of *Cocinnia grandis fruits*

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 were done 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* showed a 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*. Results are tabulated in Table

Table 9 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**

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).

Preliminary anti-ulcer activity of *Diplocyclos palmatus fruits*

Pylorus ligation 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 (Omeprazole)	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***

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 activity. Results are Table 11 Effect of *Diplocyclos palmatus fruits* extracts on various parameters in pylorus ligated induced ulcer model

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).

Table 12 *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* compared to standard Omeprazole

Drug	
	Pylorus ligated induced ulcer
PECG	29.85
CECG	1.37
EECG	53.55
PEDP	2.80
CEDP	1.23
EEDP	54.83
Standard	61.07

Ulcer protection percentage by different extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruit in* aspirin induced ulcer model

Figure 1 Rat stomach treated with treatment groups in pylorus ligatedinduced ulcer model

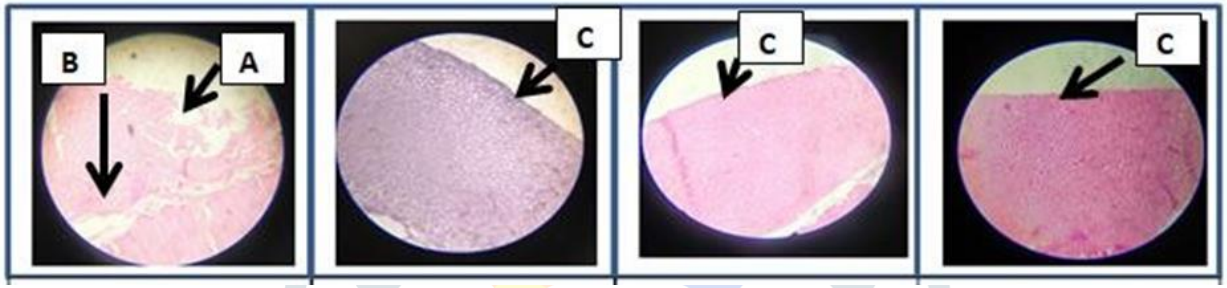


Figure 2 Histopathological evaluation of rat stoma

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.

DPPH radical scavenging activity

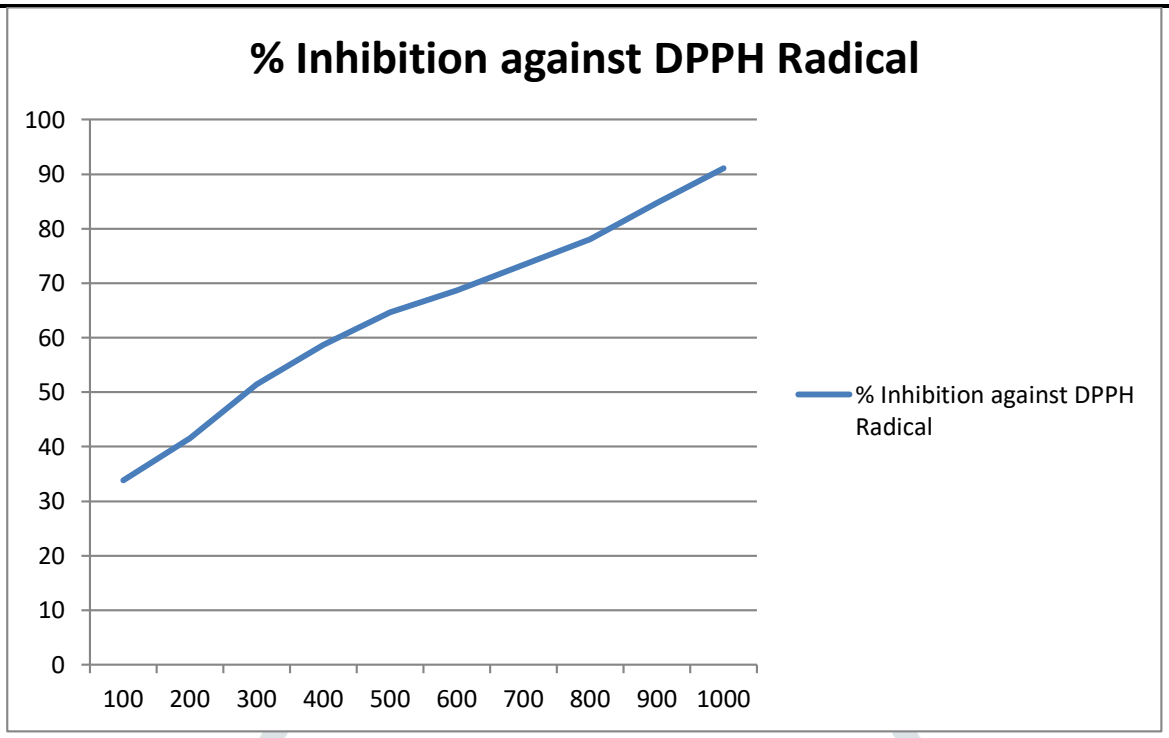
Ethanolic extract of *Cocinnia grandis fruits* (*EECG*) showed significant antioxidant activity in a dose dependent manner. *EECG* showed IC_{50} value of 285.83 ± 28.77 (µg/mL) Ethanolic extract of *Diplocyclos palmatus fruits* (*EEDP*) exhibited scavenging activity with IC_{50} value of 435.36 ± 24.96 (µg/mL). Results are shown in Table: 5.14

Table : 13 *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* using DPPH radical scavenging activity

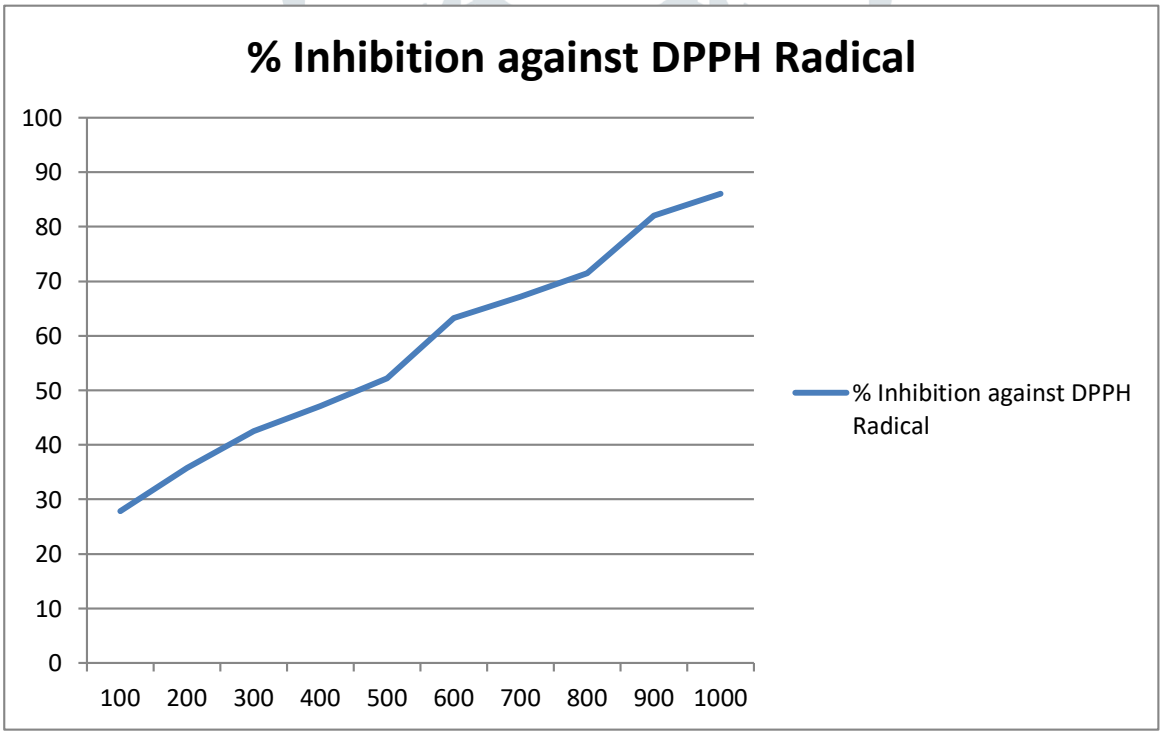
Drug	Concentration (µg/mL)	% Inhibition against DPPH radical	IC_{50} (µg/mL)
Ethanolic extract of *Cocinnia grandis fruits*	100	33.79 ±1.43	285.83 ± 28.77
	200	41.52± 3.19	
	300	51.46 ± 6.45	
	400	58.73 ±2.78	
	500	64.70 ±3.32	
	600	68.72±2.44	
	700	73.34 ±2.40	
	800	78.10 ±1.88	
	900	84.76±3.42	
	1000	91.06 ±1.32	
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	
Standard Quercetin	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	

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

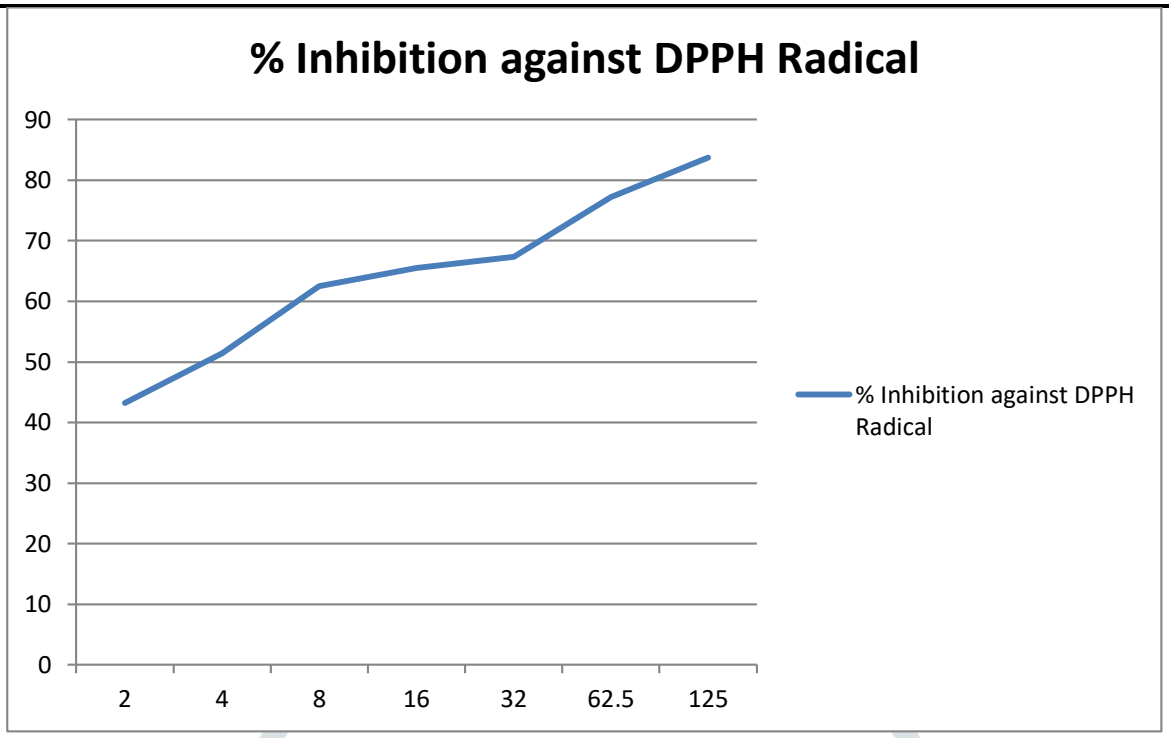
Graph 3 : *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* using DPPH radical scavenging activity



Graph 4 : *In vitro* antioxidant activity of ethanolic extracts of *Diplocyclos palmatus fruit* using DPPH radical scavenging activity



Graph 5: *In vitro* antioxidant activity of ethanolic extracts of standard drug (Quercetin) using DPPH radical scavenging activity



Reducing power

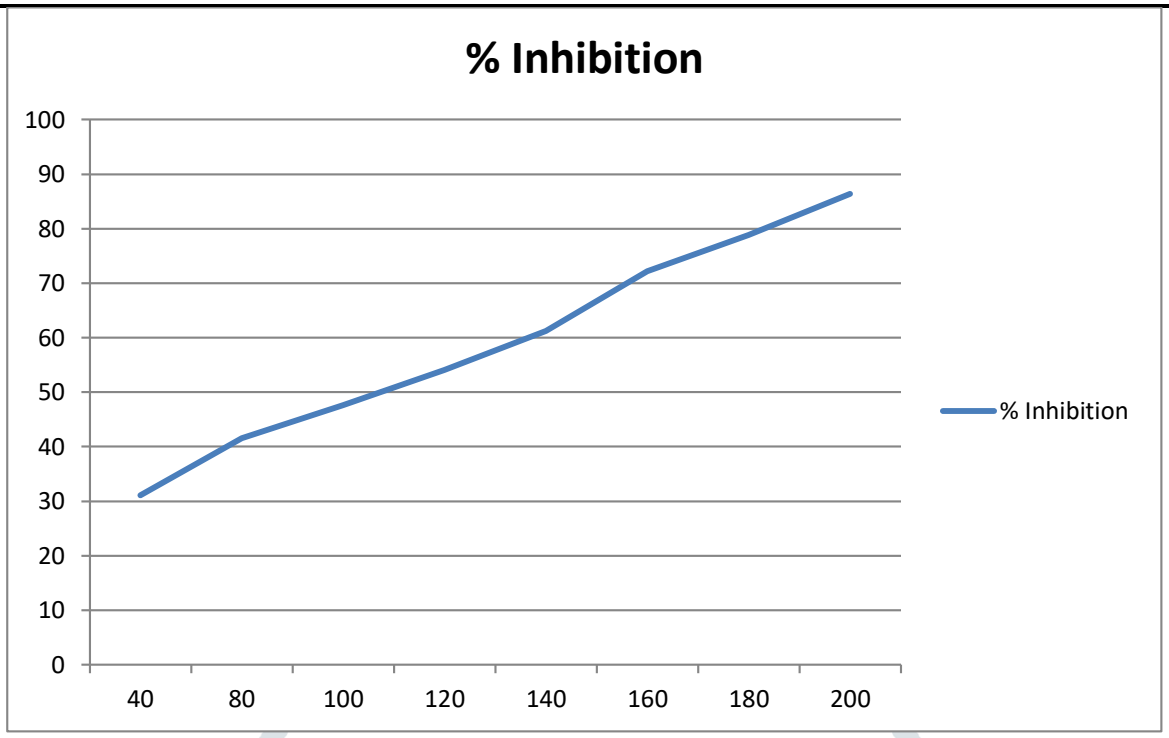
Reducing power assay is based on conversion of Fe^{3+} to Fe^{2+} by the sample. Test sample which have more reduction potential reacts with potassium ferricyanide (Fe^{3+}) to from potassium ferrocyanide (Fe^{2+}) which then reacts with ferric chloride to form ferric ferrous complex that has absorption 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 5.15

Table 14 *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* using reducing power assay

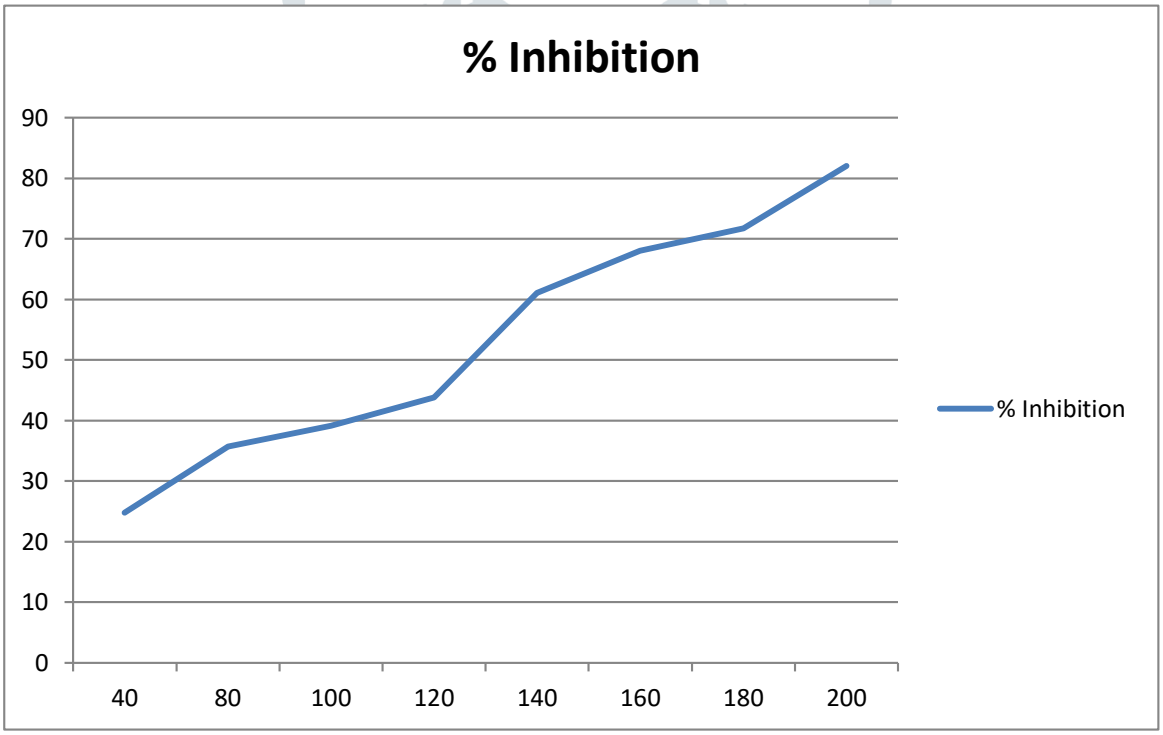
Drug	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Ethanolic extract of *Cocinnia grandis fruits*	40	31.07 ± 2.48	107.23 ± 5.88
	80	41.54 ± 3.48	
	100	47.68 ± 2.25	
	120	54.10 ± 1.15	
	140	61.24 ± 1.19	
	160	72.20 ± 4.89	
	180	78.82 ± 4.24	
	200	86.39 ± 2.79	
Ethanolic extract of *Diplocyclos palmatus 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	
Standard Ascorbic acid	5	48.71± 0.87	8.10 ± 3.52
	10	52.42 ±2.39	
	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 6 : *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* using reducing power assay



Graph 7 : *In vitro* antioxidant activity of ethanolic extracts of *Diplocyclos palmatus fruits* using reducing power assay



Graph 8 *In vitro* antioxidant activity of ethanolic extracts of standard drug (Ascorbic Acid) using reducing power assay

Hydroxyl radical scavenging activity

Ethanolic extract of *Cocinnia grandis fruits* and ethanolic extract of *Diplocyclos palmatus fruits* showed does dependent increase in the capacity to quench hydroxyl radicals as tabulated in Table 5.18. IC_{50} of *Cocinnia grandis fruits* was found to be 55.37 ± 8.79, *Diplocyclos palmatus fruits* 72.00 ± 11.87. Ascorbic acid was used a reference standard.

Table 15 *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* using Hydroxyl radical scavenging activity

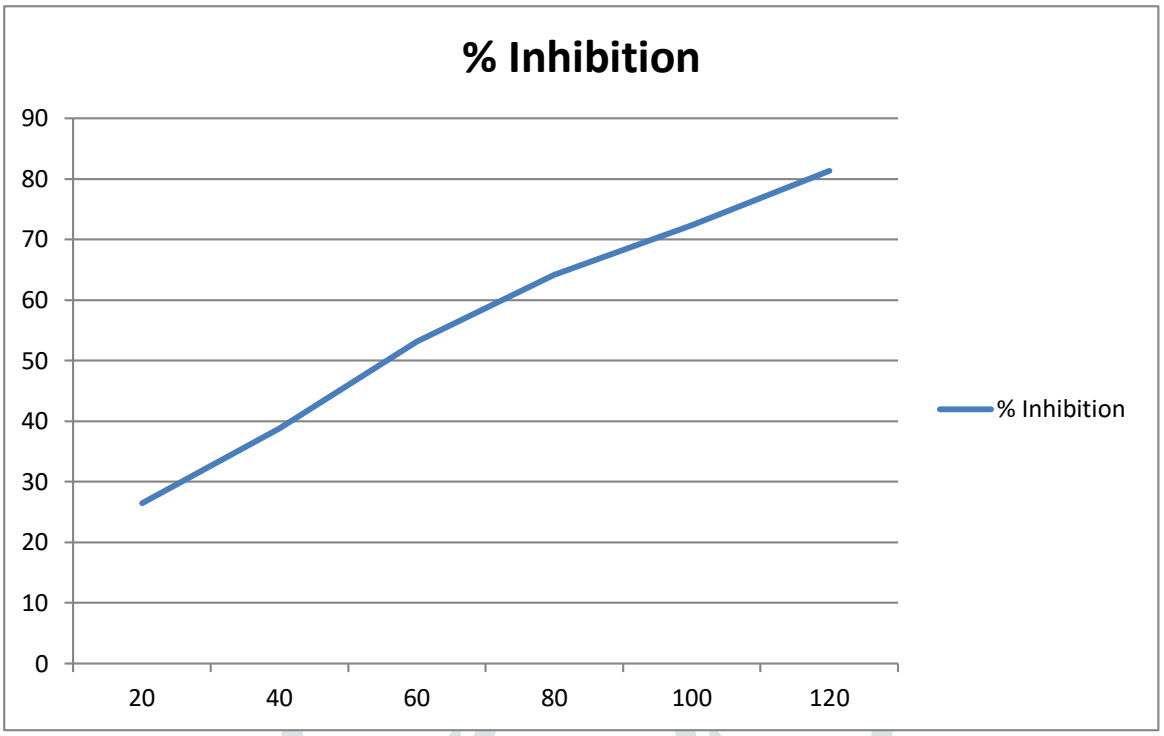
Drug	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Ethanolic extract of *Cocinnia grandis fruits*	20	26.44 ± 4.44	55.37 ± 8.79
	40	38.79 ± 5.14	
	60	53.12 ± 7.25	
	80	64.15 ± 2.55	
	100	72.32 ± 1.17	
	120	81.36 ± 1.32	
Ethanolic extract of *Diplocyclos palmatus fruits*	20	25.33 ± 12.56	72.00 ± 11.87
	40	31.24 ± 12.64	
	60	37.12 ± 13.07	
	80	55.66 ± 10.11	
	100	70.24 ± 5.28	
	120	80.96 ± 3.20	
Standard Ascorbic acid	10	47.23 ± 1.12	15.39± 1.40
	20	52.82 ± 1.98	
	40	60.08 ± 3.59	
	60	67.62 ± 2.62	
	80	74.37 ± 2.54	
	100	83.88 ± 1.96	

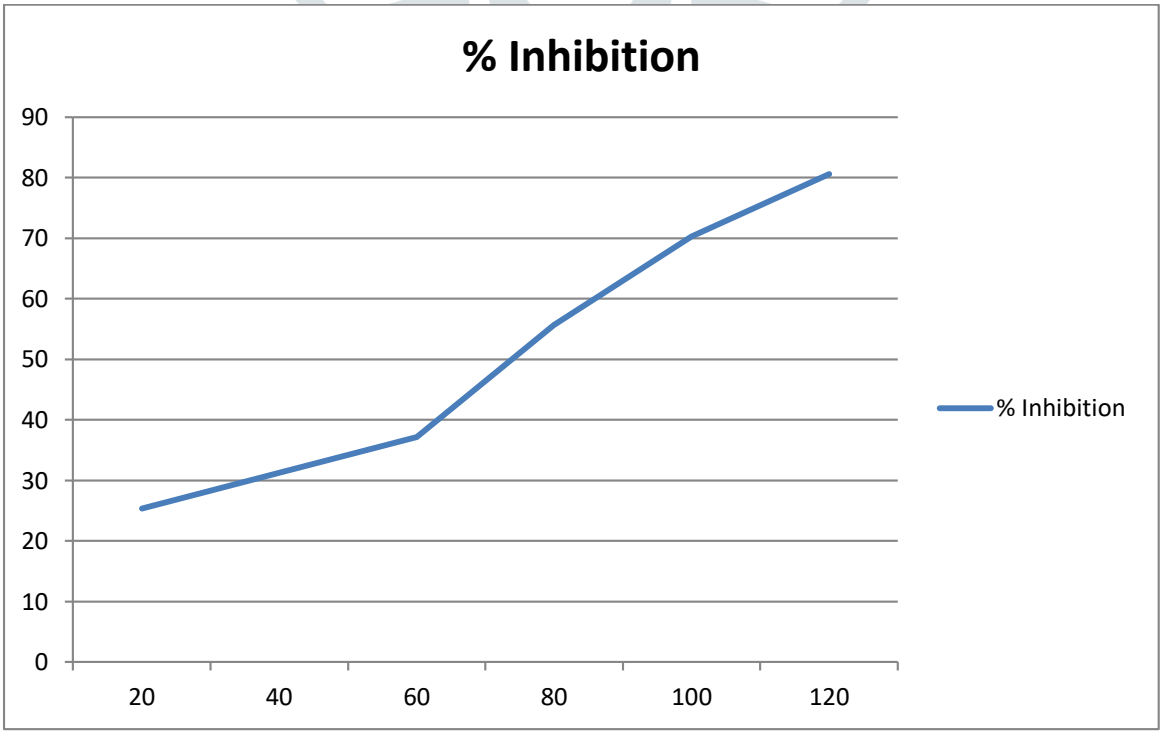
All Values are expressed as mean ± S.D (n=3).

Isolation of Phytoconstituents

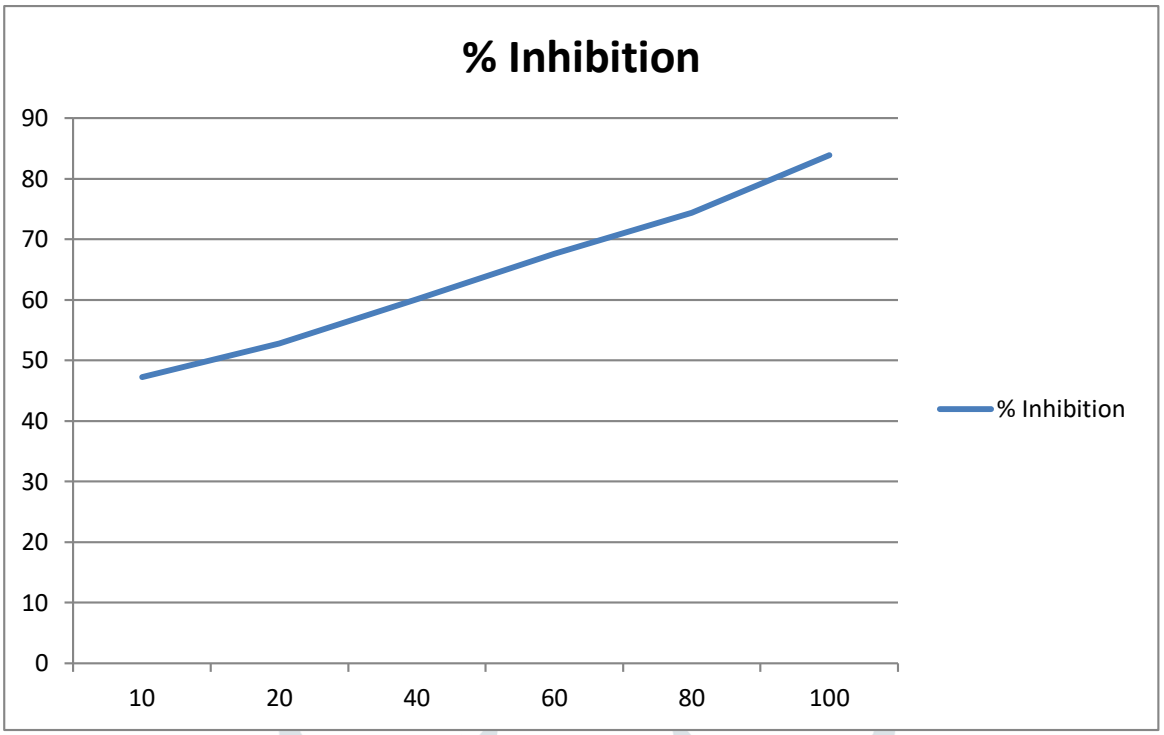
On the basis of results obtained of phytochemical investigation and preliminary pharmacological screening it was found that ethanolic extract of *Cocinnia grandis fruits* and ethanolic extract of *Diplocyclos palmatus fruits* was more potent and hence was further selected for isolation of phytoconstituents.

Graph 7: *In vitro* antioxidant activity of ethanolic extracts of *Cocinnia grandis fruits* using Hydroxyl radical scavenging activity





Graph:8 *In vitro* antioxidant activity of ethanolic extracts of standard drug (Ascorbic Acid) using Hydroxyl radical scavenging activity



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 5.19. 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.

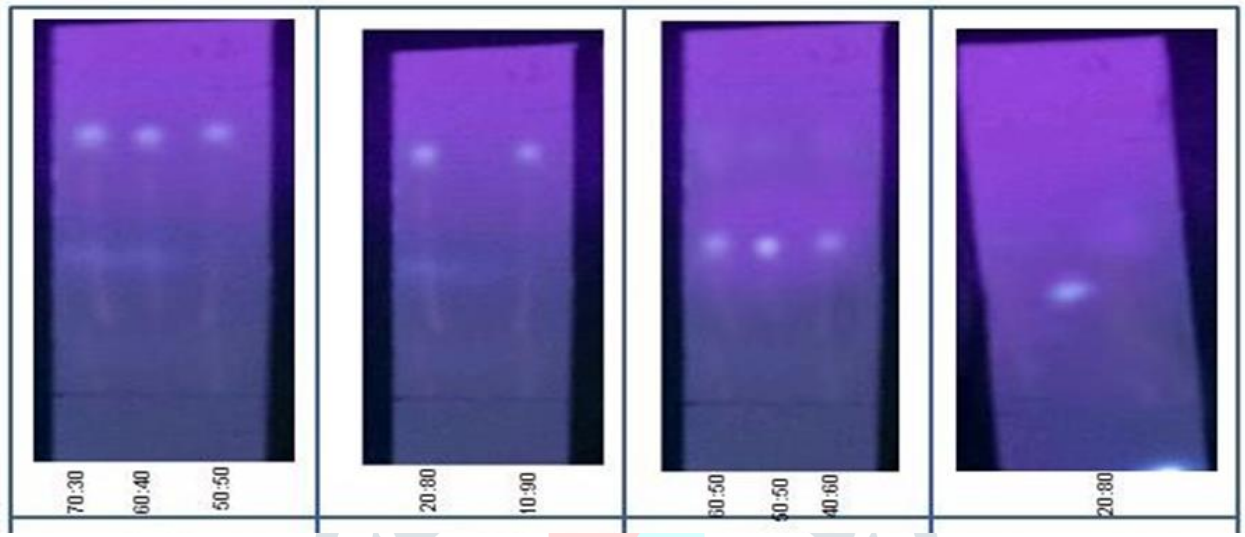
Table 15 Various fractions obtained from column chromatography ofethanolic extract of *Cocinnia grandis fruits*

Mobile phase(Eluted solvent)	Concen -tration	Fraction No	Number of spot on TLC plate	Fraction	Rf Value
n-hexane	100	1-10	0	---	---
n-hexane: ethyl acetate	90:10	11-20	0	---	---
n-hexane: ethyl acetate	80:20	21-30	0	---	---
n-hexane: ethyl acetate	70:30	31-40	1	Compound 1	0.78
n-hexane: ethyl acetate	60:40	41-50	1	Compound 1	0.78
n-hexane: ethyl acetate	50:50	51-60	1	Compound 1	0.78

n-hexane: ethyl acetate	40:60	61-70	0	---	---
n-hexane: ethyl acetate	30:70	71-80	0	---	---
n-hexane: ethyl acetate	20:80	81-90	1	Compound 2	0.69
n-hexane: ethyl acetate	10:90	91-100	1	Compound 2	0.69
ethyl acetate	100	101-110	0	---	---
ethyl acetate:ethanol	90:10	111-120	0	---	---
ethyl acetate:ethanol	80:20	121-130	0	---	---
ethyl acetate:ethanol	70:30	131-140	0	---	---
ethyl acetate:ethanol	60:40	141-150	1	Compound 3	0.59
ethyl acetate:ethanol	50:50	151-160	1	Compound 3	0.59
ethyl acetate:ethanol	40:60	161-170	1	Compound 3	0.59
ethyl acetate:ethanol	30:70	171-180	0	---	---
ethyl acetate:ethanol	20:80	181-190	1	Compound 4	0.38
ethyl acetate:ethanol	10:90	191-200	0	---	---
ethanol	100	201-220	0	---	---

Table 16 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 8.2 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.

Isolation of phytoconstituents from ethanolic extract of *Diplocyclos palmatus fruits*

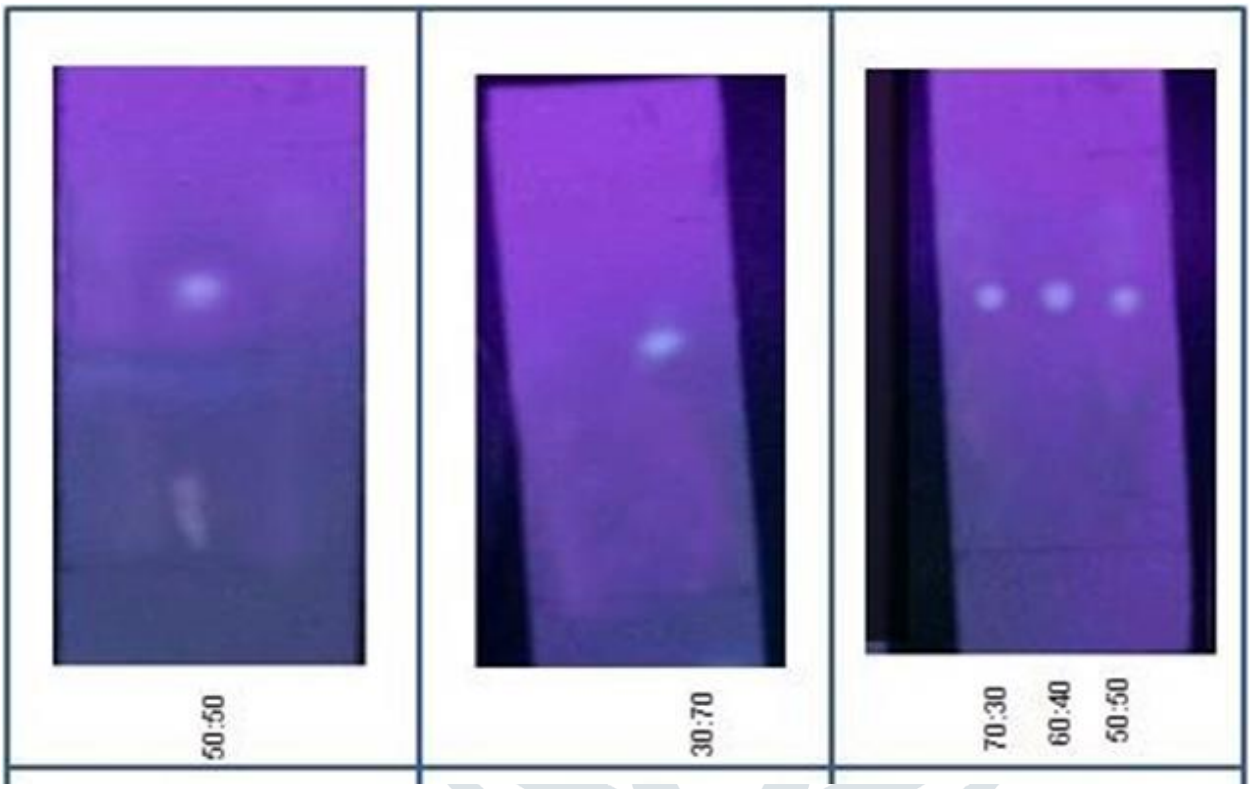
Ethanolic extract of *Diplocyclos palmatus fruits* was subjected to separation of phytoconstituents using column chromatography by gradient elusion technique. Hexane: chloroform and chloroform: methanol was used in different concentration as mobile phase. All the fractions were obtained by gradient elusion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

Table 1 7 Various fractions obtained from column chromatography ofethanolic extract of *Diplocyclos palmatus fruits*

Mobile phase (Eluted solvent)	Concentration	Fraction No	Number of spot on TLC Plate	Fraction	R*f* Value
n-hexane	100	1-10	0	---	---
n-hexane: chloroform	90:10	11-20	0	---	---
n-hexane: chloroform	80:20	21-30	0	---	---
n-hexane: chloroform	70:30	31-40	0	---	---
n-hexane: chloroform	60:40	41-50	0	---	---
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
n-hexane: chloroform	20:80	81-90	0	---	---
n-hexane: chloroform	10:90	91-100	0	---	---
chloroform	100	101-110	0	---	---
chloroform: methanol	90:10	111-120	0	---	---
chloroform: methanol	80:20	121-130	0	---	---
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
chloroform: methanol	40:60	161-170	0	---	---
chloroform: methanol	30:70	171-180	0	---	---
chloroform: methanol	20:80	181-190	0	---	---
chloroform: methanol	10:90	191-200	0	---	---
methanol	100	201-210	0	---	---

Table 18 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



DL1 **DL2** **DL3**

Figure 4 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

Pharmacological screening for antiulcer activity of isolated compounds

Antiulcer activity of isolated compound of *Cocinnia grandis fruits*

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 5.23-5.25.

Table 19 Effect of CG-2 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

Parameters	Control	Standard (Omeprazole) 20mg/kg	CG-2 5mg/kg	CG-2 10mg/kg	CG-2 20mg/kg
ULCER INDEX	2.82±0.14	0.73±0.14***	3.19±0.26 ns	2.69±0.21 ns	2.80±0.29ns
GASTRIC VOLUME	3.27±0.18	1.37±0.06***	2.73±0.24 ns	2.79±0.25ns	2.51±0.18ns
pH	2.48±0.14	6.81±0.14***	2.79±0.27 ns	2.59±0.11 ns	2.62±0.094 ns
FREE ACIDITY	38.79±1.84	15.61±0.52***	37.12±1.29 ns	38.13±0.41 ns	36.77±1.73 ns
TOTDP ACIDITY	57.69±1.36	23.23±0.79***	54.75±1.38 ns	53.07±1.77 ns	56.03±1.817 ns

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).

Table 20 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***
GASTRIC VOLUME	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 9 Effect of CG-3 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

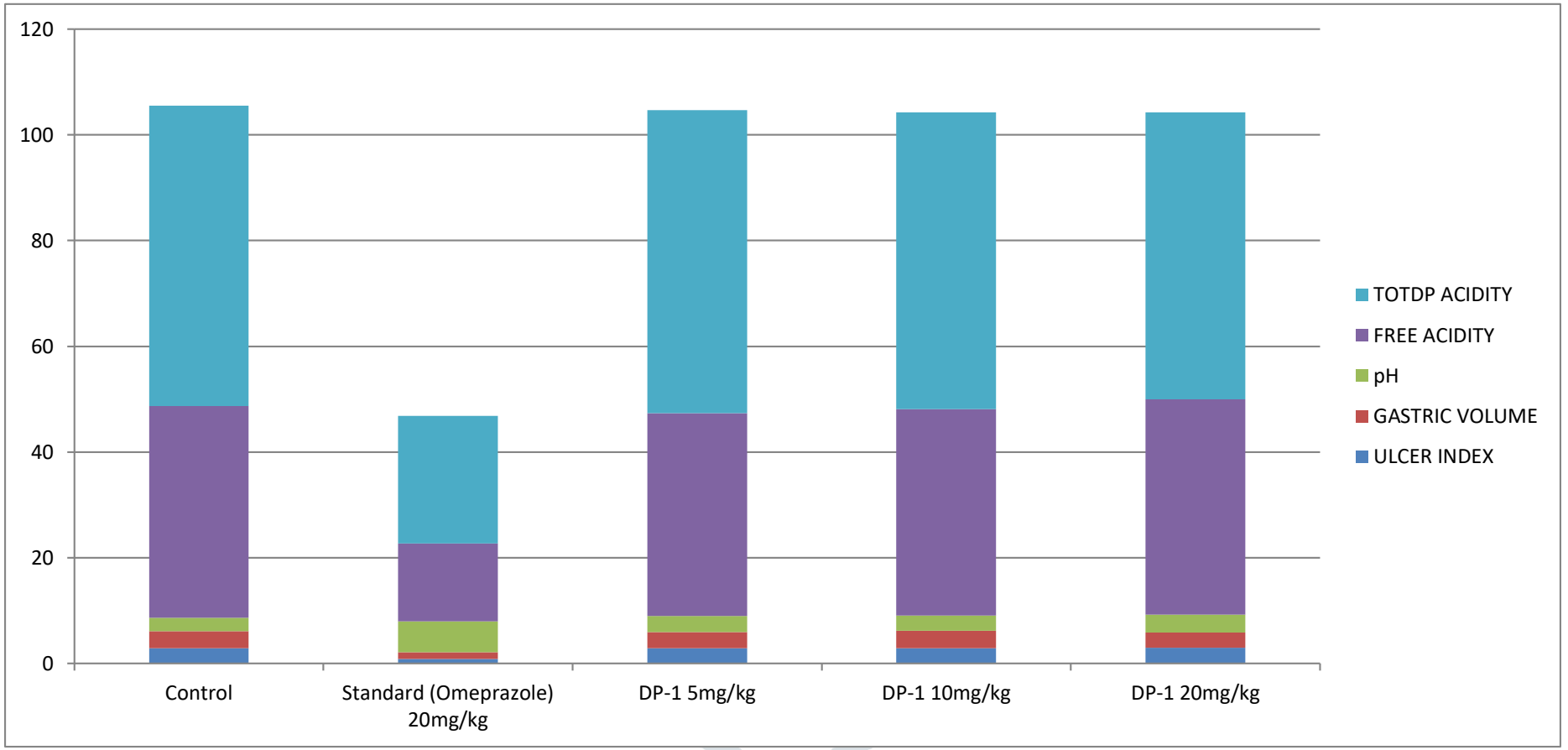


Table 21 Effect of CG-4 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

Parameters	Control	Standard (Omeprazole) 20mg/kg	CG-4 5mg/kg	CG-4 10mg/kg	CG-4 20mg/kg
ULCER INDEX	2.58±0.04	0.63±0.043***	2.88±0.13^{ns}	2.89±0.22^{ns}	2.98±0.08^{ns}
GASTRIC VOLUME	3.30±0.18	1.47±0.26***	3.09±0.17^{ns}	3.29±0.12^{ns}	3.03±0.07^{ns}
pH	2.51±0.24	5.71±0.14***	3.09±0.14^{ns}	2.91±0.07 ^{ns}	2.97±0.27 ^{ns}
FREE ACIDITY	37.78±1.74	13.81±0.72***	37.34±0.89^{ns}	36.32±1.53^{ns}	38.78±1.36
TOTDP ACIDITY	56.10±1.46	24.33±0.79***	52.49±2.16^{ns}	52.71±1.96 ^{ns}	50.70±2.99 ^{ns}

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).

Anti-ulcer activity of isolated compounds from *Diplocyclos palmatus fruits*

Isolated compounds from *Diplocyclos palmatus fruits* namely DP-1, DP-2 and DP-3 were screened for anti-ulcer activity using different ulcer inducing models.

Aspirin plus pylorus ligation induced ulcer model

In Aspirin plus pylorus ligation ulcer inducing model, group treated with DP-3 at dose level of 20mg/kg showed maximum ulcer inhibiting activity (0.90 ± 0.05***) then DP-1 (2.99±0.21^{ns}), DP-2 (2.88± 0.14 ^{ns}) and control group (2.92±0.04). DP-3 also reduced the gastric volume (1.90± 0.12***) which was equivalent to the reduction in gastric volume by standard omeprazole (1.27± 0.16***). DP-3 also raised the gastric pH and reduced the total acidity and free acidity as shown in Table 5.27-5.29.

Table 22 Effect of DP-1 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

Parameters	Control	Standard (Omeprazole) 20mg/kg	DP-1 5mg/kg	DP-1 10mg/kg	DP-1 20mg/kg
ULCER INDEX	2.92±0.04	0.83±0.043***	2.92±0.15 ns	2.89±0.29 ns	2.99±0.21 ns
GASTRIC VOLUME	3.17±0.18	1.27±0.16***	3.02±0.21 ns	3.29±0.15 ns	2.85±0.11 ns
pH	2.58±0.14	5.91±0.04***	3.02±0.18 ns	2.93±0.17 ns	3.36±0.16 ns
FREE ACIDITY	39.99±1.84	14.71±0.62***	38.36±1.00 ns	39.02±0.08 ns	40.80±2.19 ns
TOTDP ACIDITY	56.89±1.36	24.13±0.69***	57.36±1.52 ns	56.12±1.95 ns	54.22±2.46 ns

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 10 Effect of DP-1 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

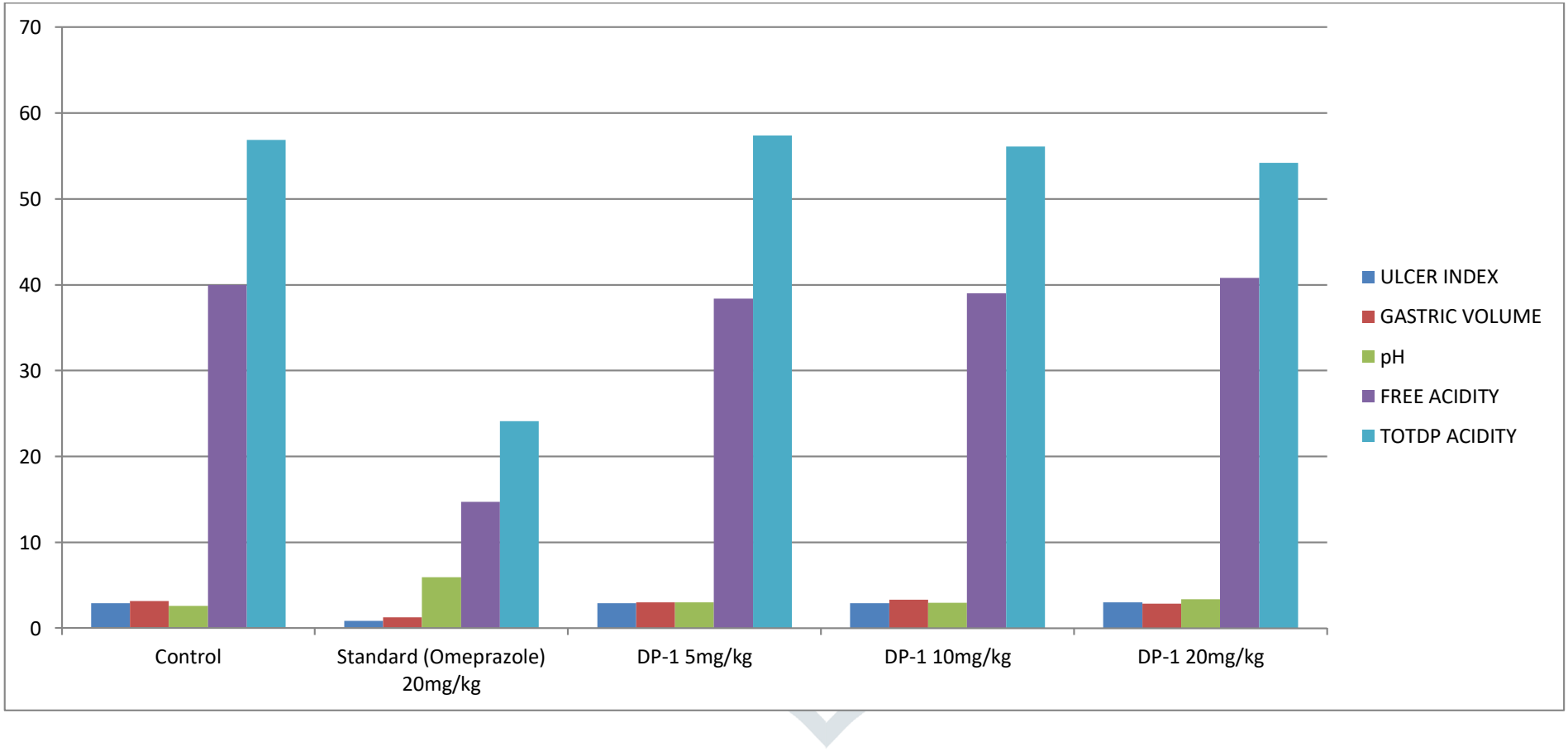


Table 23 Effect of DP-2 extracts on various parameters in Aspirin plus pylorus ligation induced ulcer model

Parameters	Control	Standard (Omeprazole) 20mg/kg	DP-2 5mg/kg	DP-2 10mg/kg	DP-2 20mg/kg
ULCER INDEX	2.72±0.04	0.73±0.04***	2.42±0.08 ns	3.04±0.19 ns	2.68±0.14 ns
GASTRIC VOLUME	3.07±0.18	1.57±0.16***	3.25±0.16 ns	3.13±0.18 ns	3.08±0.17 ns
pH	2.48±0.14	6.01±0.04***	3.15±0.17 ns	2.86±0.18 ns	3.15±0.16 ns
FREE ACIDITY	38.69±1.84	16.71±0.62***	37.78±0.61 ns	37.42±1.34 ns	38.19±1.71 ns
TOTDP ACIDITY	57.89±1.36	25.13±0.69***	53.27±2.08 ns	55.05±2.04 ns	54.72±1.75 ns

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 11 Effect of DP-2 extracts on various parameters in Aspirin plus pylorus ligation induced ulcer model

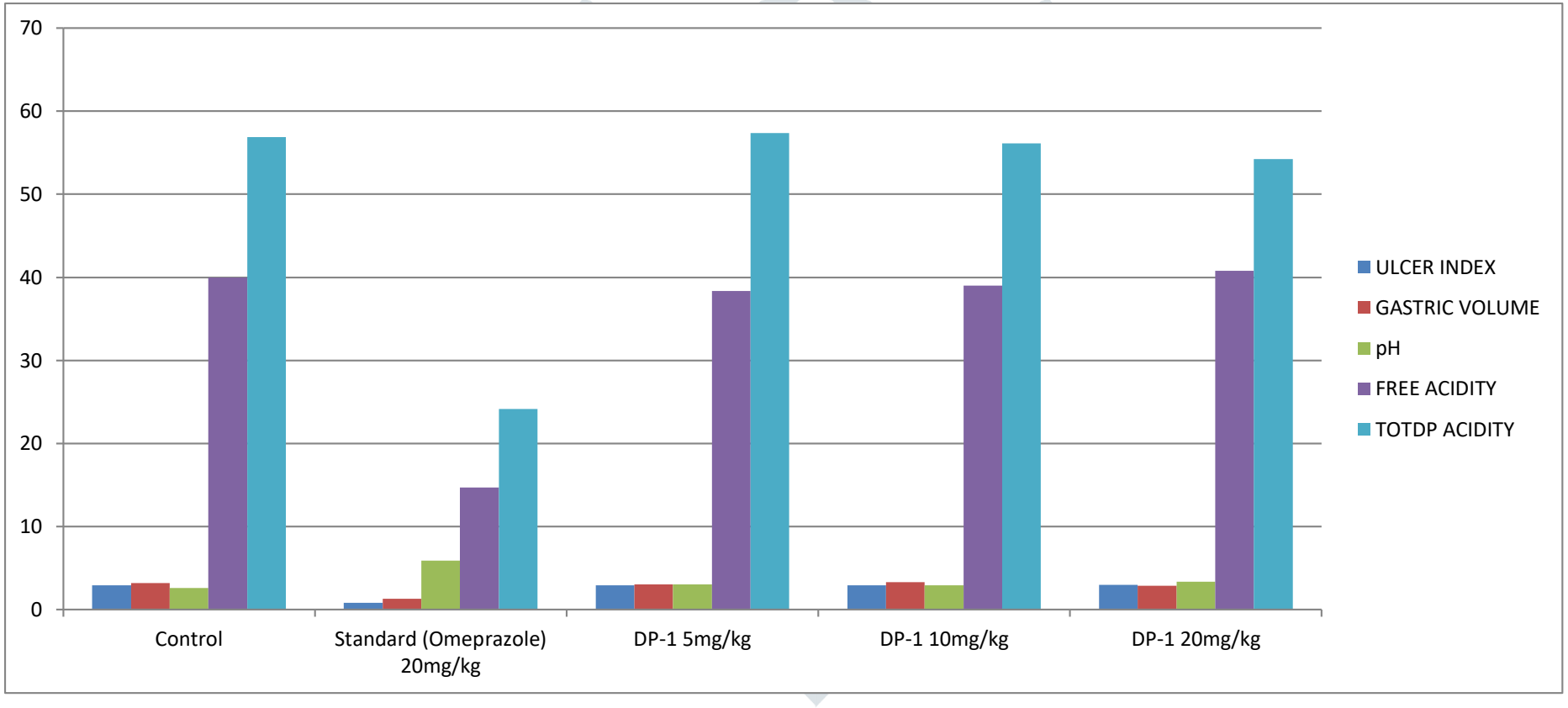


Table 24 Effect of DP-3 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

Parameters	Control	Standard (Omeprazole) 20mg/kg	DP-3 5mg/kg	DP-3 10mg/kg	DP-3 20mg/kg
ULCER INDEX	2.92±0.04	0.83±0.04***	2.7770±0.13^{ns}	2.04±0.28***	0.90±0.05***
GASTRIC VOLUME	3.17±0.18	1.27±0.16***	3.00±0.23 ^{ns}	2.38±0.20*	1.90±0.12***
pH	2.58±0.14	5.91±0.04***	3.02±0.14 ^{ns}	4.25±0.29**	5.42±0.18***
FREE ACIDITY	39.99±1.84	14.71±0.62***	38.20±1.26 ^{ns}	33.75±1.63*	20.80±1.02***
TOTDP ACIDITY	56.89±1.36	24.13±0.69***	58.00±0.63 ^{ns}	40.50±1.87***	25.88±0.58***

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 12 Effect of DP-3 extracts on various parameters in Aspirin plus pylorus ligated induced ulcer model

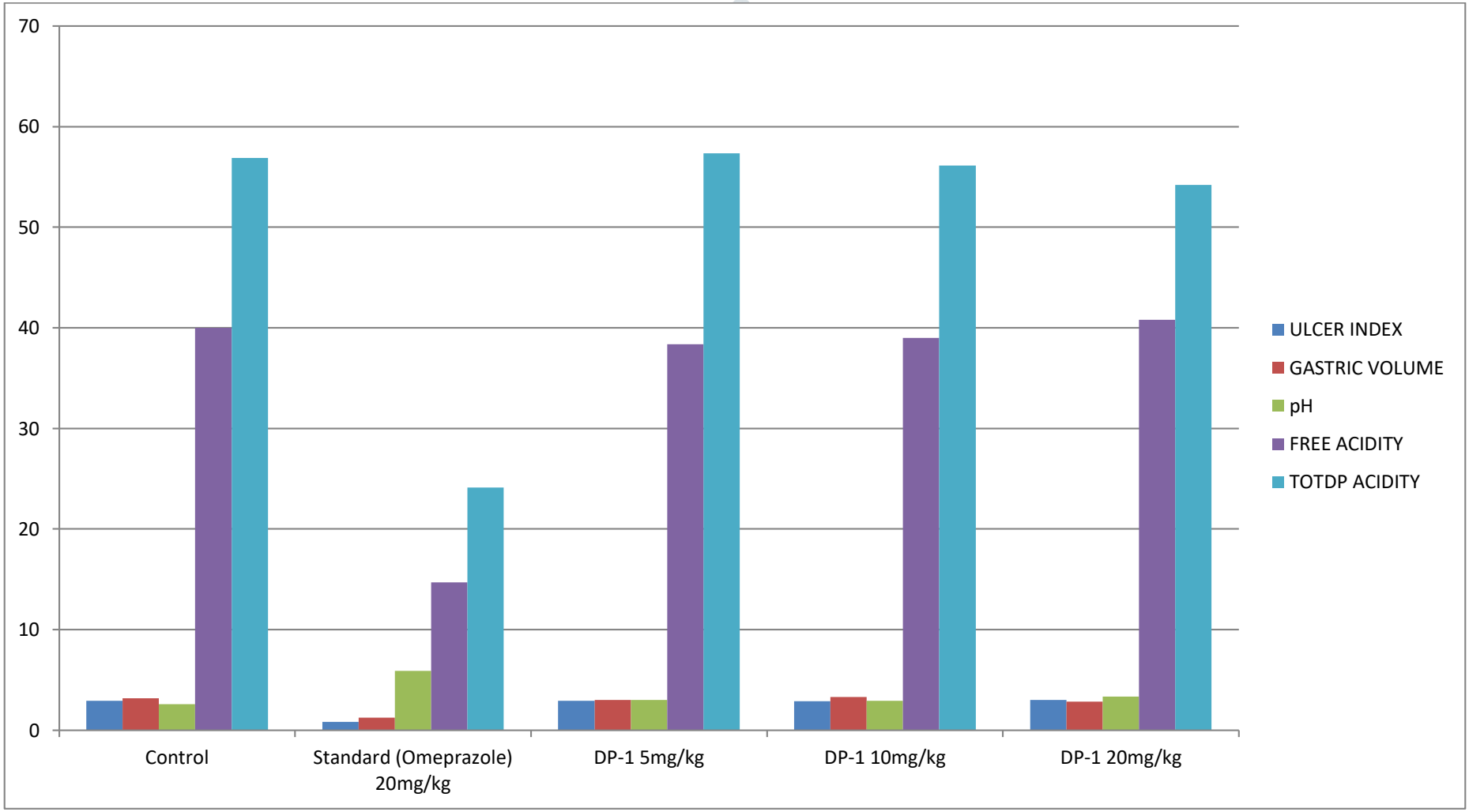
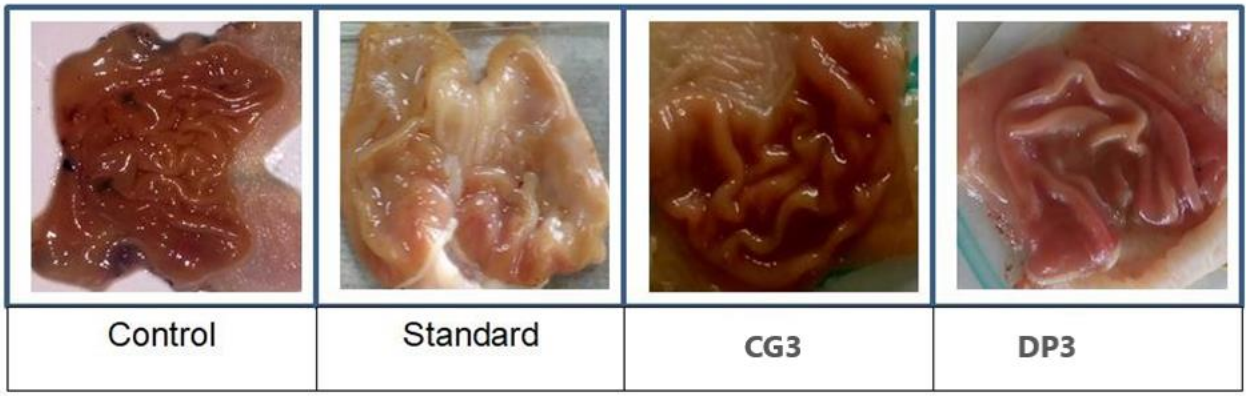


Table 25 Percentage ulcer protection by different treatment groups at thedose level 20mg/kg

Treatment group	Ulcer Protection (%)
	Aspirin plus Pylorus ligatedinduced ulcer
CG-2	NS
CG-3	66.72
CG-4	NS
DP-1	NS
DP-2	NS
DP-3	69.06
Standard	71.63

Figure 5 Rat stomach treated with isolated compounds of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* in aspirin plus pylorus ligated induced ulcer model



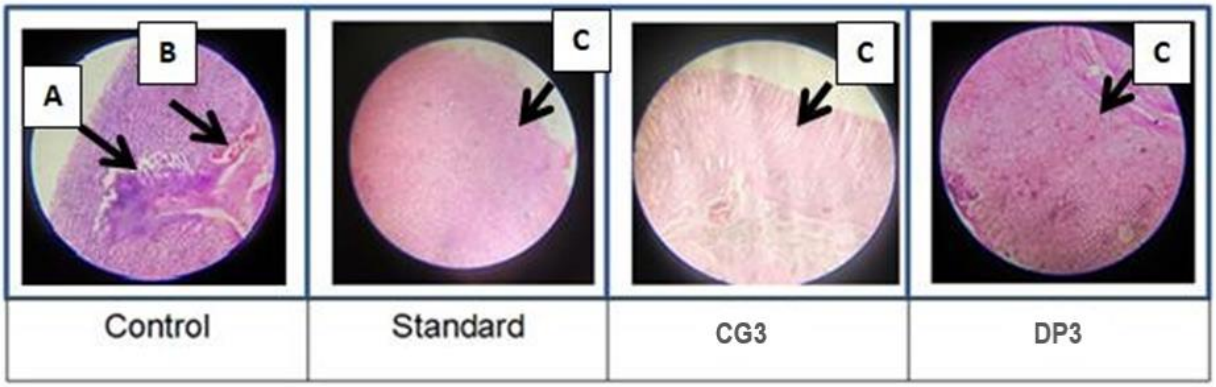


Figure : 7 Histopathological evaluation of rat stomach treated with isolated compounds of *Cocinnia grandis fruits* and *Diplocyclos palmatus fruits* in aspirin plus pylorus ligated inducedmodel ulcer

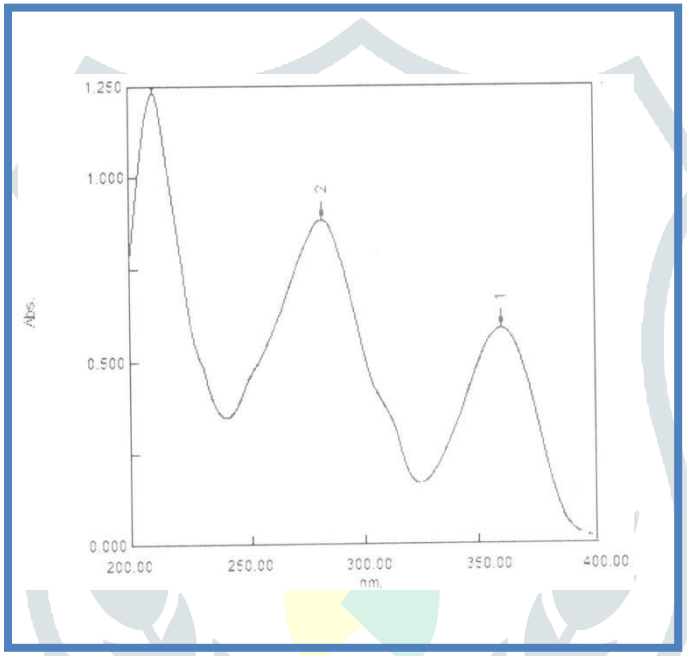
.Structural elucidation of pharmacologically active isolated compound(CG-3) from

Cocinnia grandis fruits

Melting point determination of CG-3

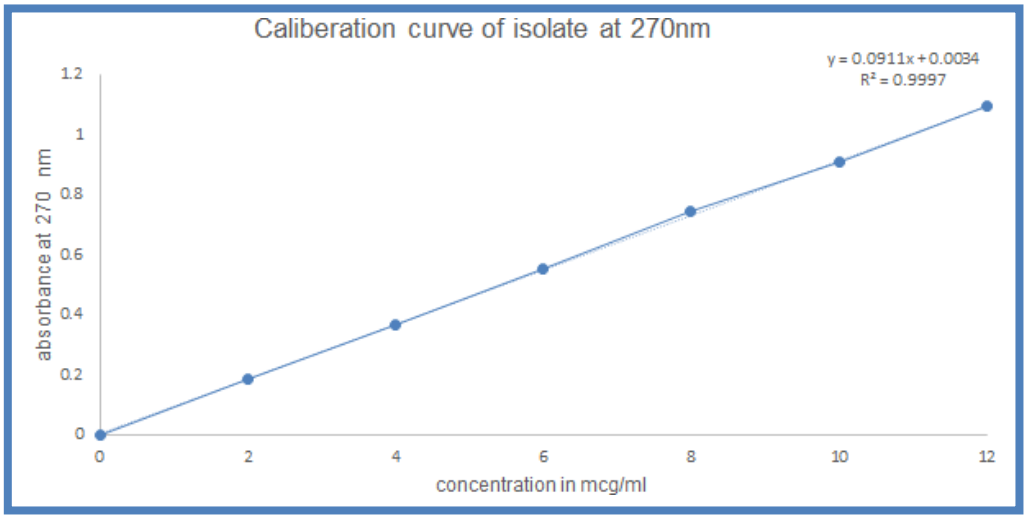
Melting point of pharmacologically active isolated compound CG-3 from *EECG* was determined using Galen Kamp melting point apparatus and was found to be 224- 227^{o} C11.2 Ultraviolet absorbance of CG-3

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



Graph 13 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 5.85.



Graph 14 Ultraviolet absorbance caliberation curve at 270nm of compoundCG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

Table 26 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

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.

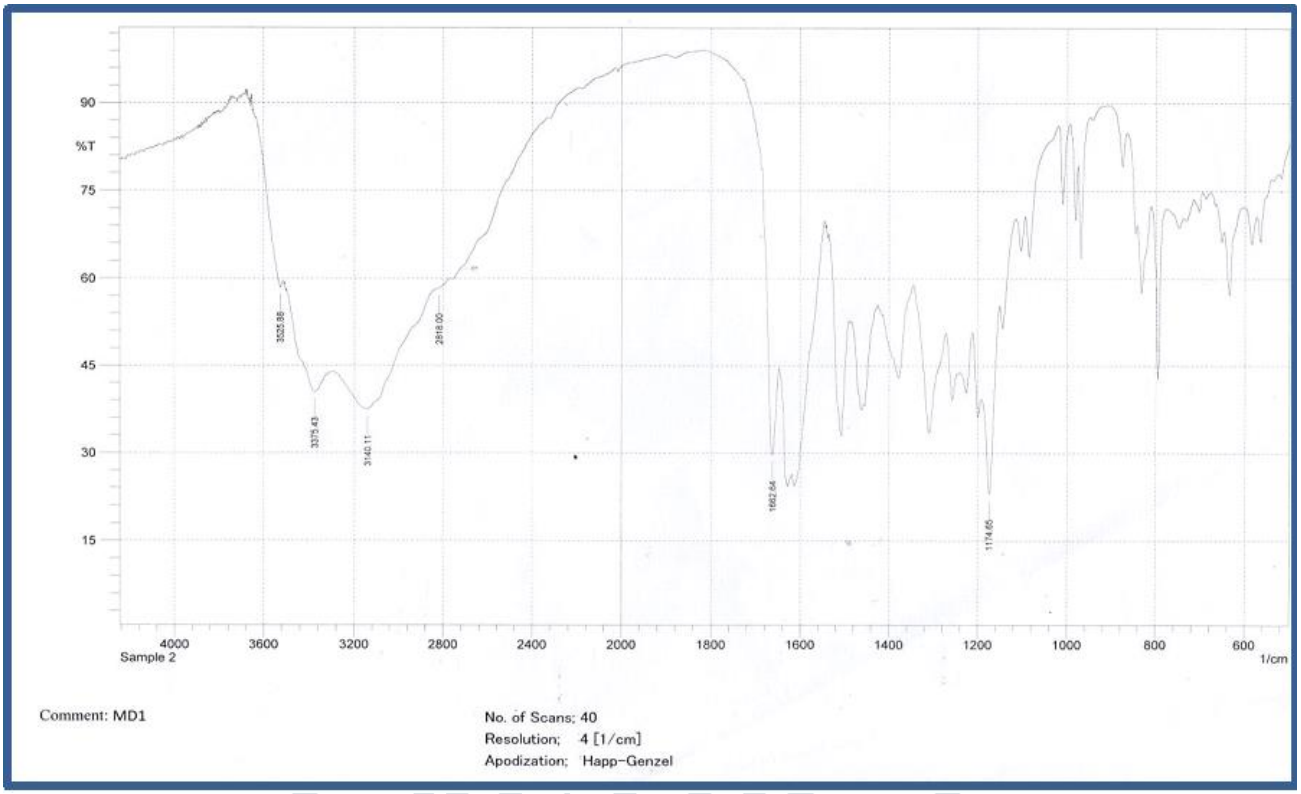


Figure 8 FTIR spectrum of compound CG-3 isolated from ethanolic extractof *Cocinnia grandis fruits*

Table No. 27 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}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 thepresence 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}CNMR showed presence of one aliphatic carbon, fourteen aromatic carbons and a carbonyl carbon.

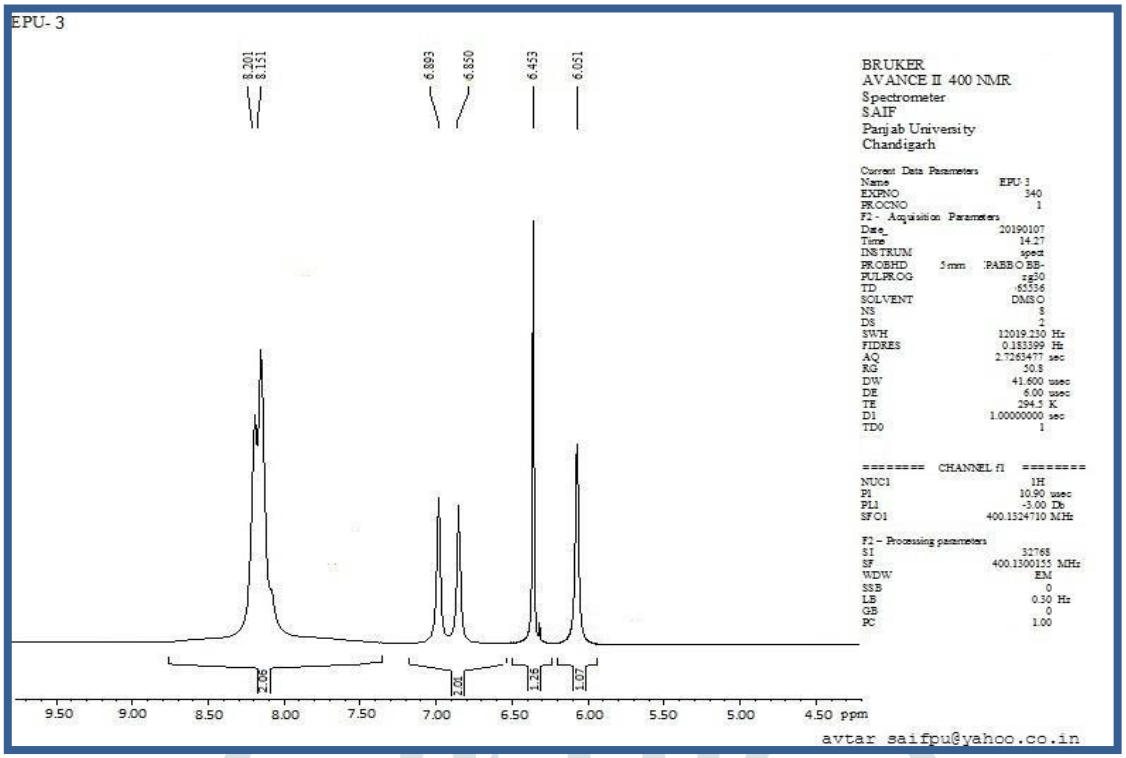


Figure 9 ^{1}H NMR spectrum of compound CG-3 isolated from ethanolicextract of *Cocinnia grandis fruits*

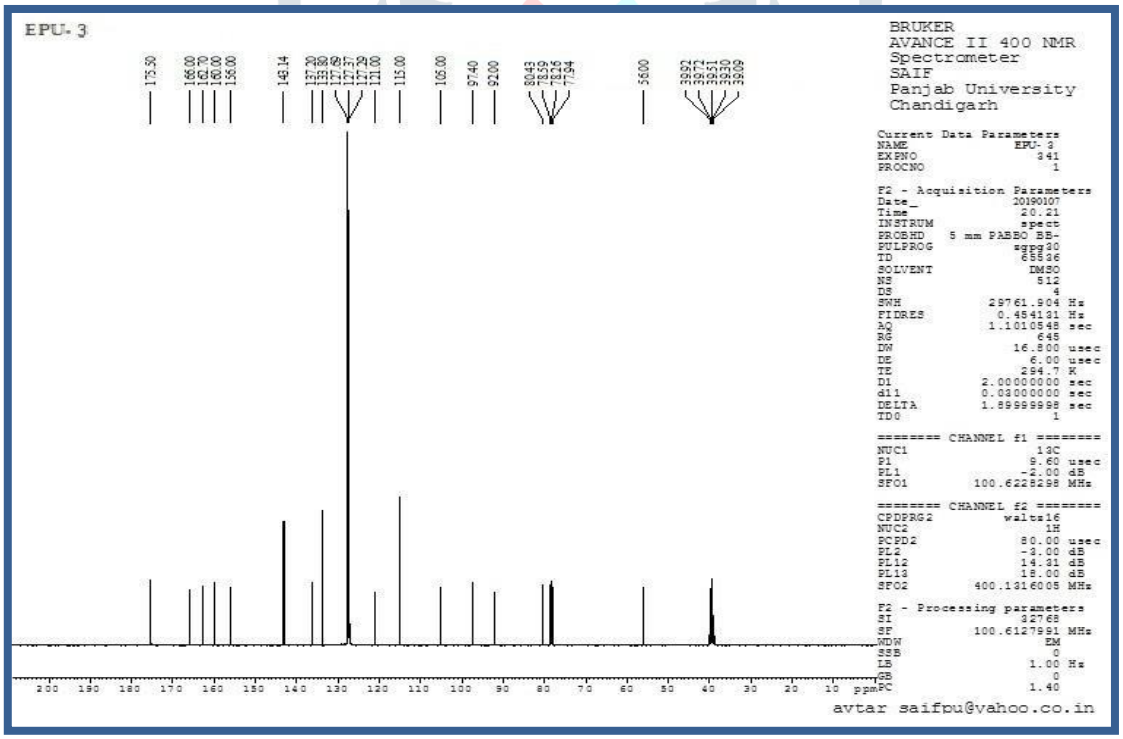


Figure 10 ^{13}C NMR spectrum of compound CG-3 isolated from ethanolicextract of *Cocinnia grandis fruits*

13 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.

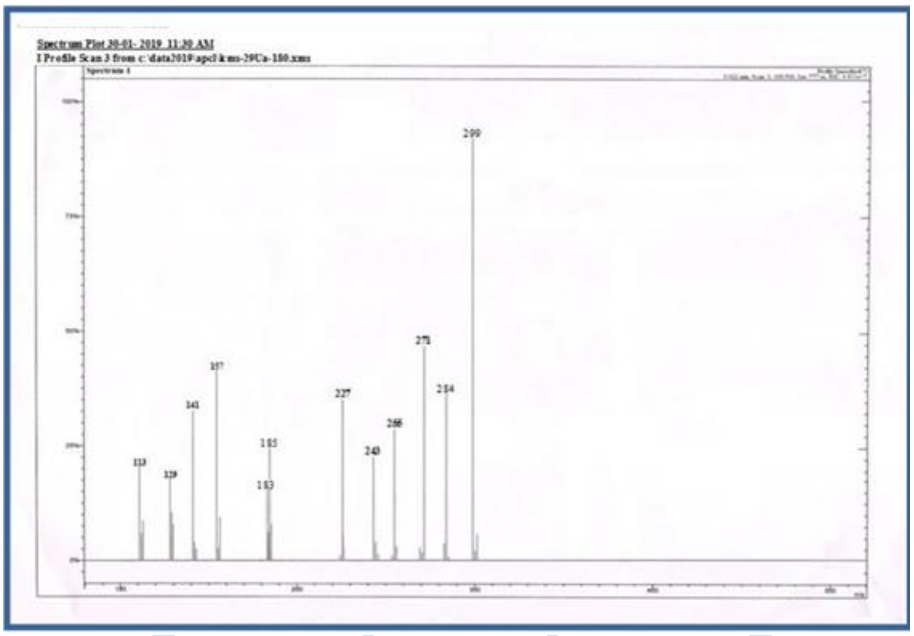


Figure 11 Mass Spectra of compound CG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

Table 29 Spectroscopic data of compound CG-3 isolated from ethanolic extract of *Cocinnia grandis fruits*

Sr. No	Spectroscopic technique	Data
1	UV	270nm, 363nm
2	IR FTIR	3375.43, 3140.11, 2818.00,1662.64,1627.92, 1612.49 1462.04,1309.67, 1174.65 cm^{-1}
3	MS spectroscopy	299, 284, 271, 255, 243, 227, 185, 183, 157, 141, 129, 113.
4	^{1}H NMR DMSO	δ:12.430(S,1H), 10.732(S,1H), 9.951(S,1H), 8.151-8.201(d,2Ar-H), 6.850-6.893(d,2Ar-H), 6.453(S,1Ar-H), 6.051(s,1H,Ar-H), 3.897(S,3H)
5	^{13}C NMR DMSO	δ:56.00,80.43(s), 92.00 (s), 97.40(s), 105.00(s), 115(d), 121.00(S), 133.80(d), 137.20(s), 156(s), 160(s), 162.70(s), 166.00(s), 175.50(s)

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

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^{o}C

Ultraviolet absorbance of DP-3

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

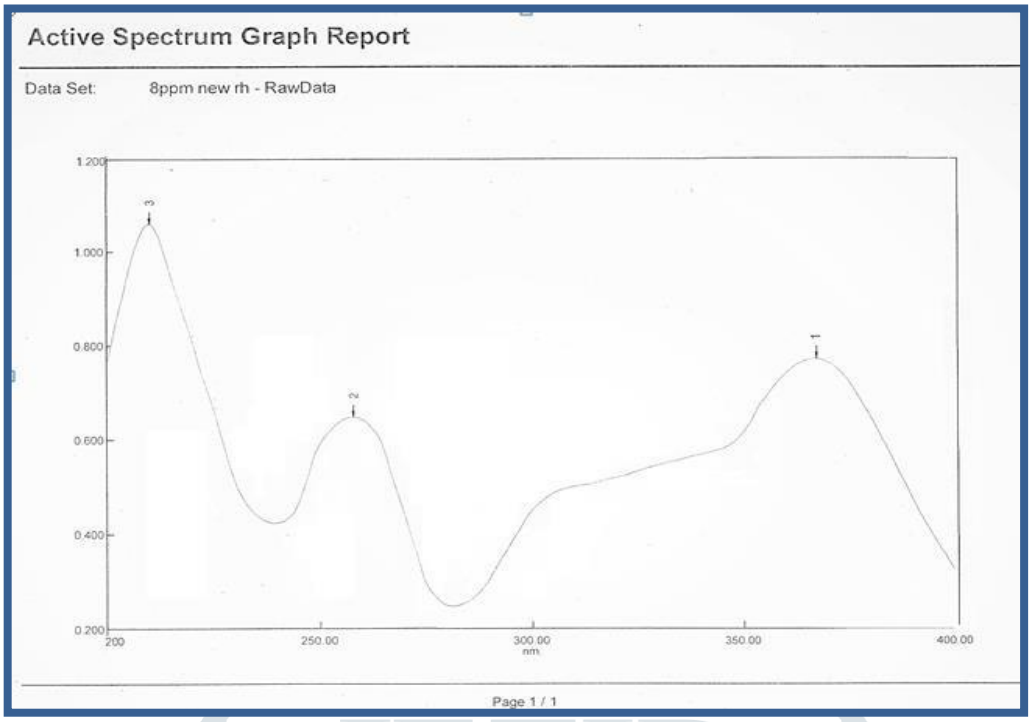


Figure 12 Ultraviolet absorbance spectra of compound DP-3 isolated from ethanolic extract of *Diplocyclos palmatus fruits*

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

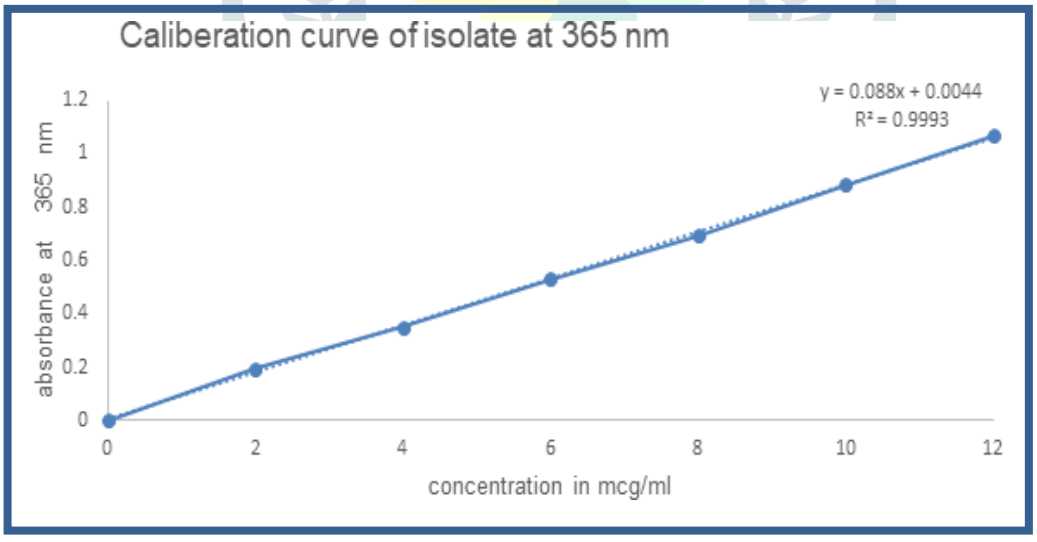


Figure 13 Ultraviolet absorbance calibration curve at 365nm of compound DP-3 isolated from ethanolic extract of *Diplocyclos palmatus fruits*

Table 30 Absorbance of various concentrations isolate in methanol at 365nm

Concentration (µg/ml)	Absorbance at nm
2	0.196
4	0.351
6	0.532
8	0.693
10	0.883
12	1.07

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.

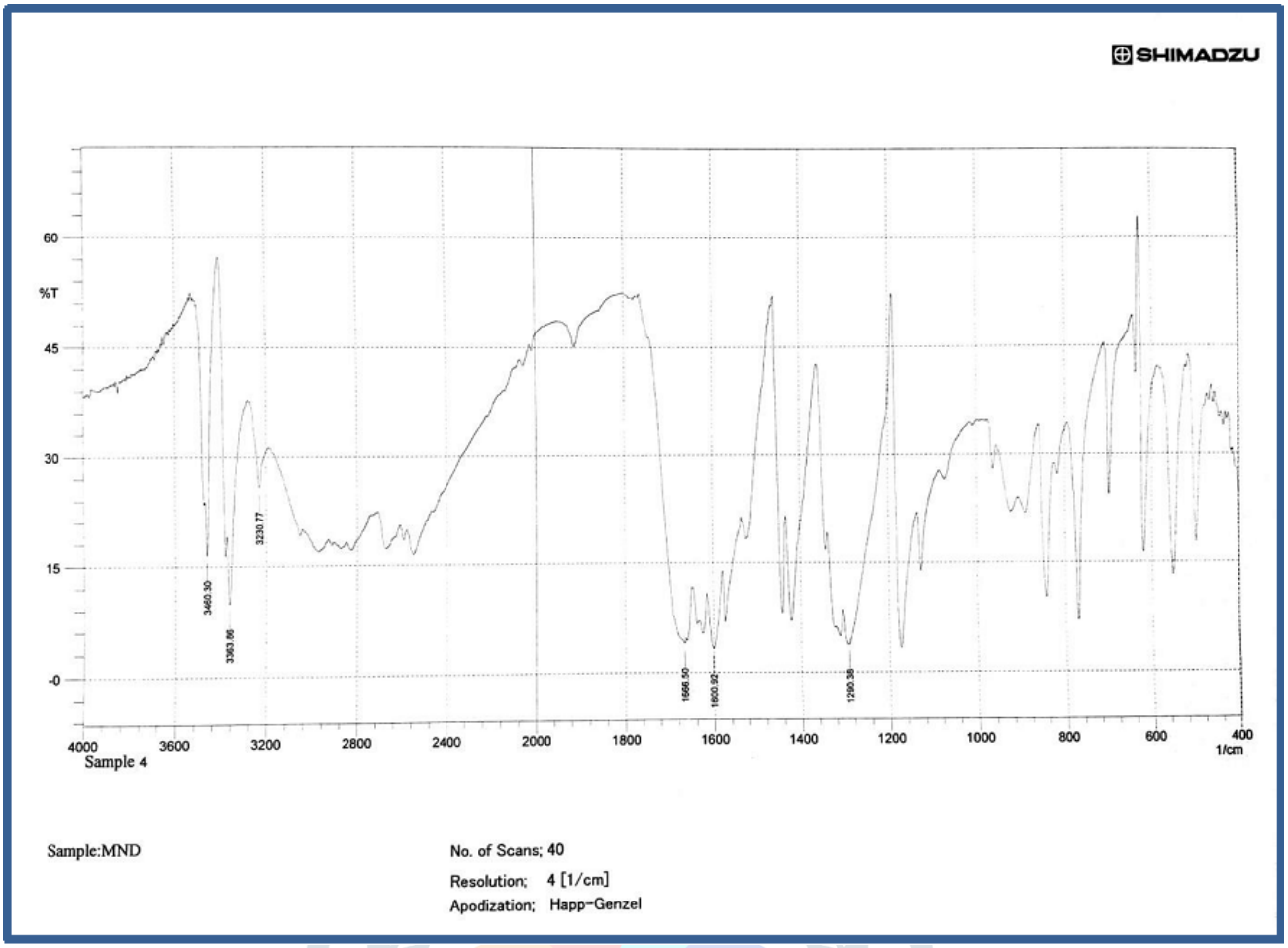


Figure 14 FTIR spectrum of compound DP-3 isolated from ethanolic extractof *Diplocyclos palmatus fruits*

Table 31 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}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 one aromatic 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.

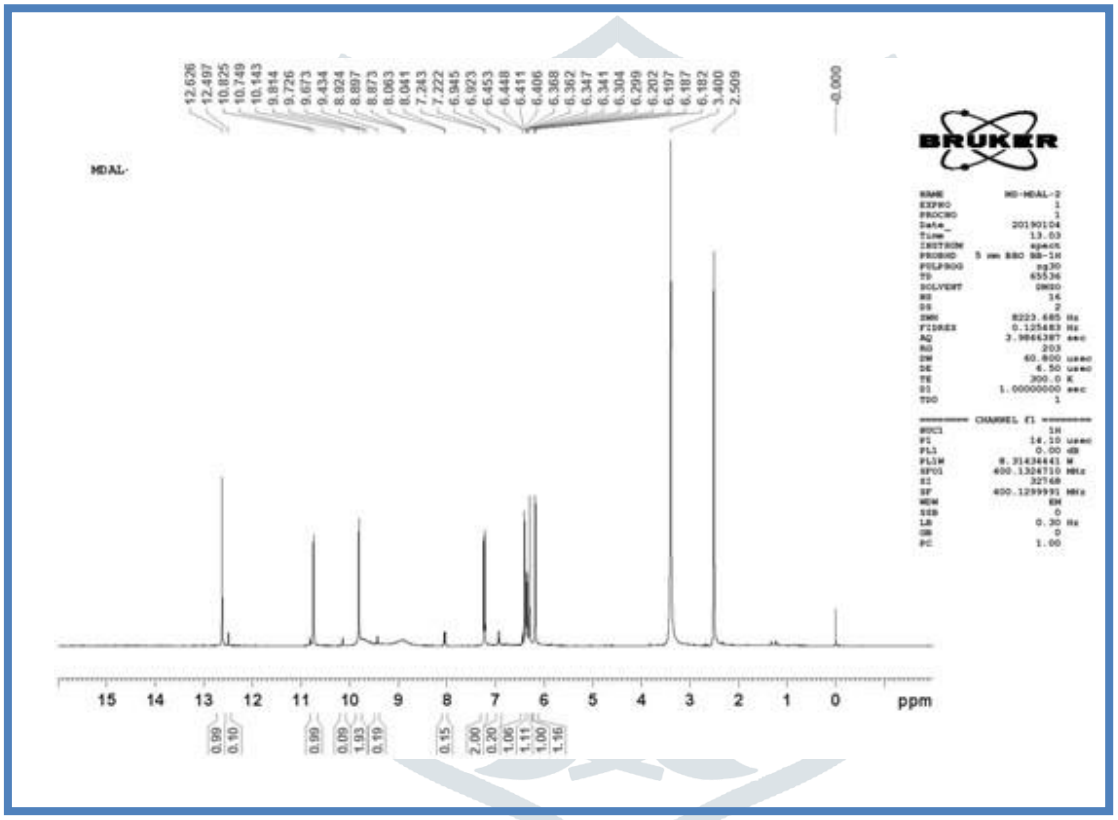


Figure ^{1}HNMR spectrum of compound DP-3 isolated from ethanolicextract of *Diplocyclos palmatus fruits*

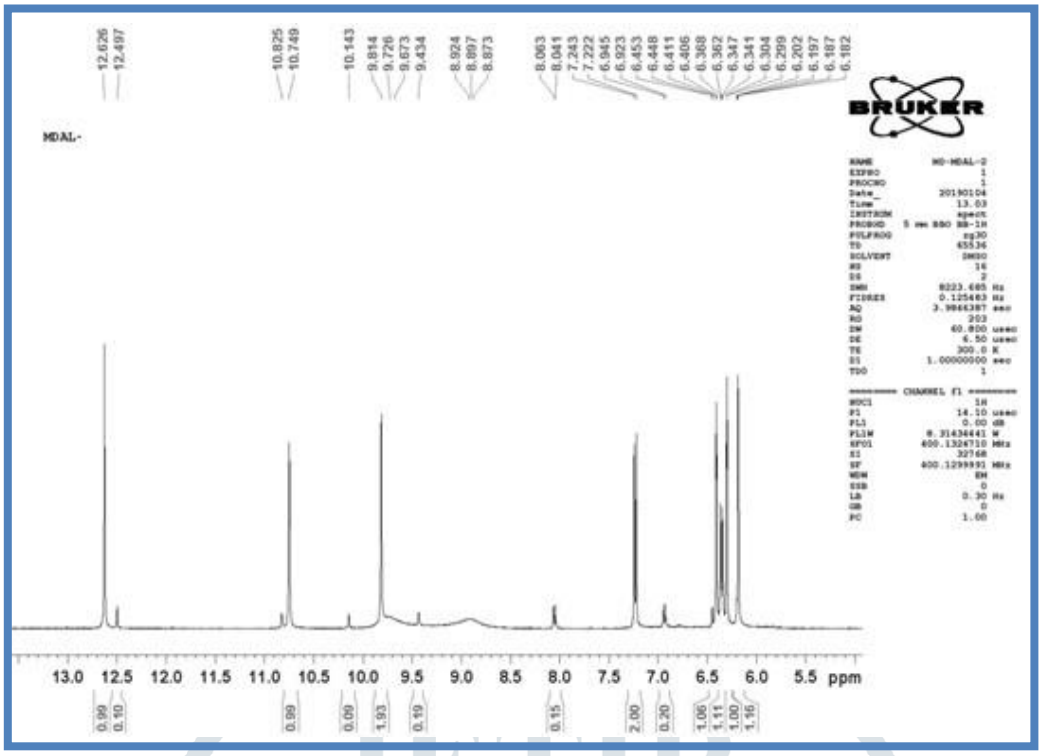


Figure 15 ^{1}H NMR spectrum of compound DP-3 isolated from ethanolicextract of *Diplocyclos palmatus fruits*

^{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.

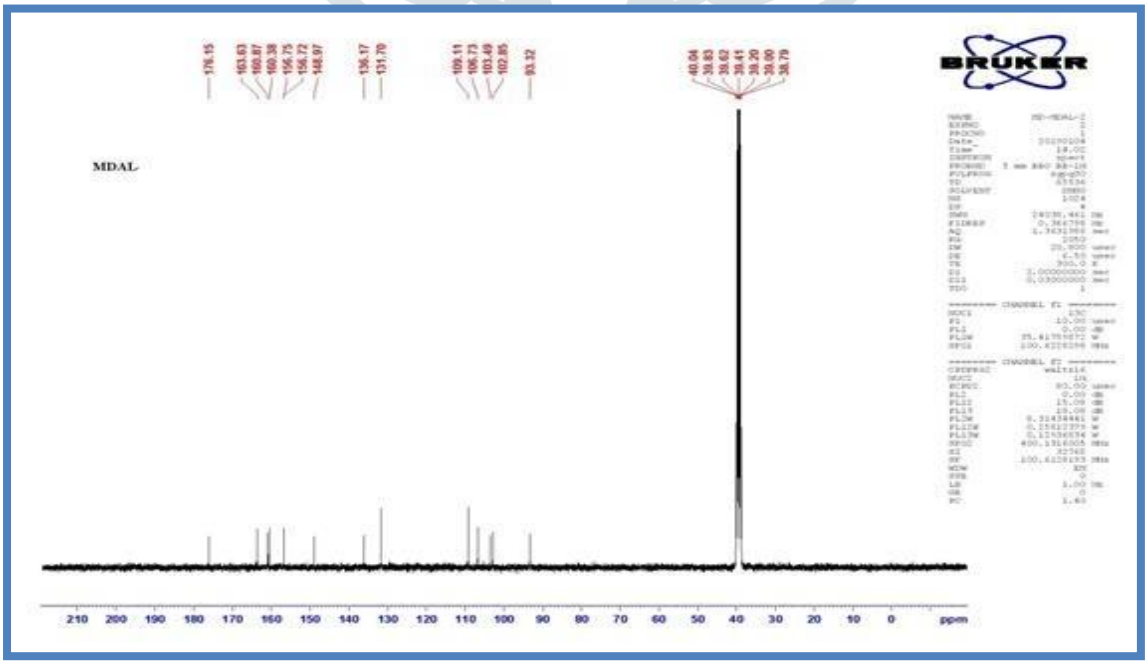


Figure 16 ^{13}C NMR spectrum of compound DP-3 isolated from ethanolicextract of *Diplocyclos palmatus fruits*

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 and 113. The molecular weight of the compound is found to be 286.

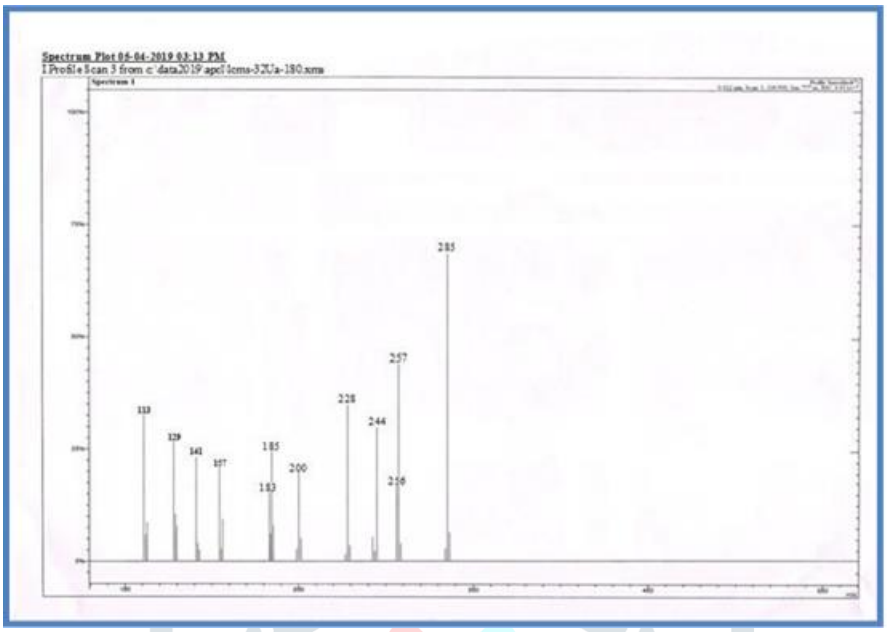


Figure 17 Mass Spectra of compound DP-3 isolated from ethanolic extract of *Diplocyclos palmatus fruits*

Table 32 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)

Molecular Docking Study

In silico molecular docking study was performed on the compounds with Molegro Virtual Docker (MVD-2013, 6.0). Figure Shows the structure (3D view) of Pig gastric H^+ K^+ ATPase in complex with BYK99 obtained from Protein Data Bank with the PDB ID: 5Y0B.

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 withamino 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. Results are tabulated in table The best dock poses of the omeprazole, Rhamnocitrin and Kaempferol is depicted in figure 5.100-5.102 respectively.

Table 33 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

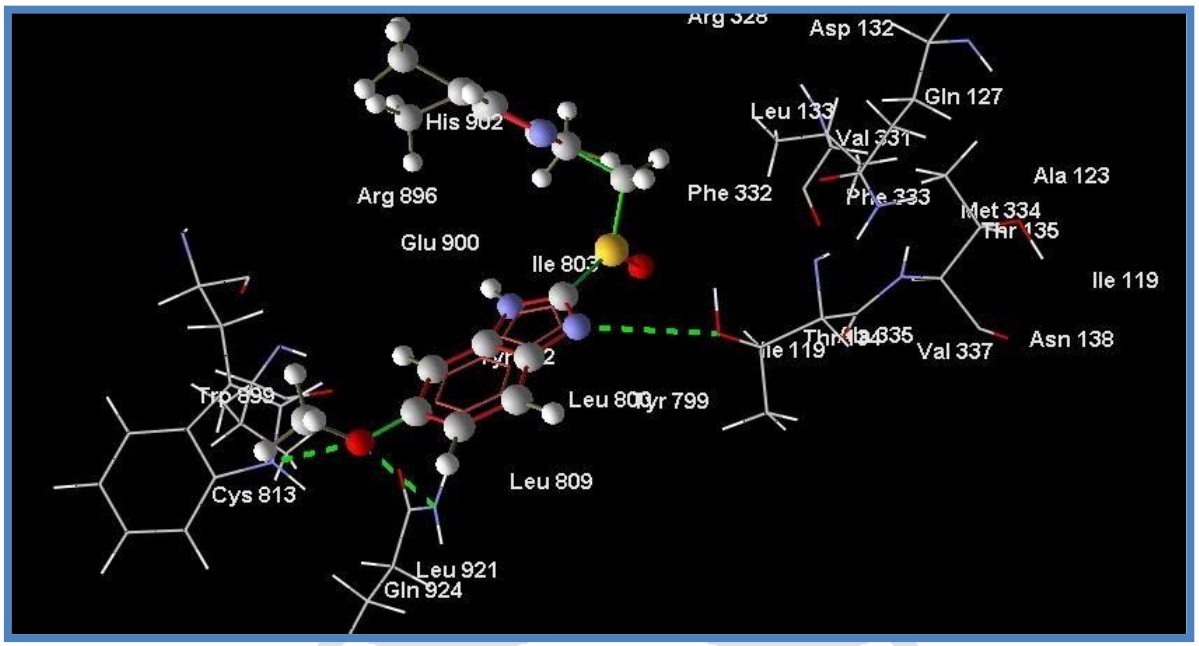


Figure 18 The standard drug Omeprazole docked in best of its conformation into the binding site of 5Y0B

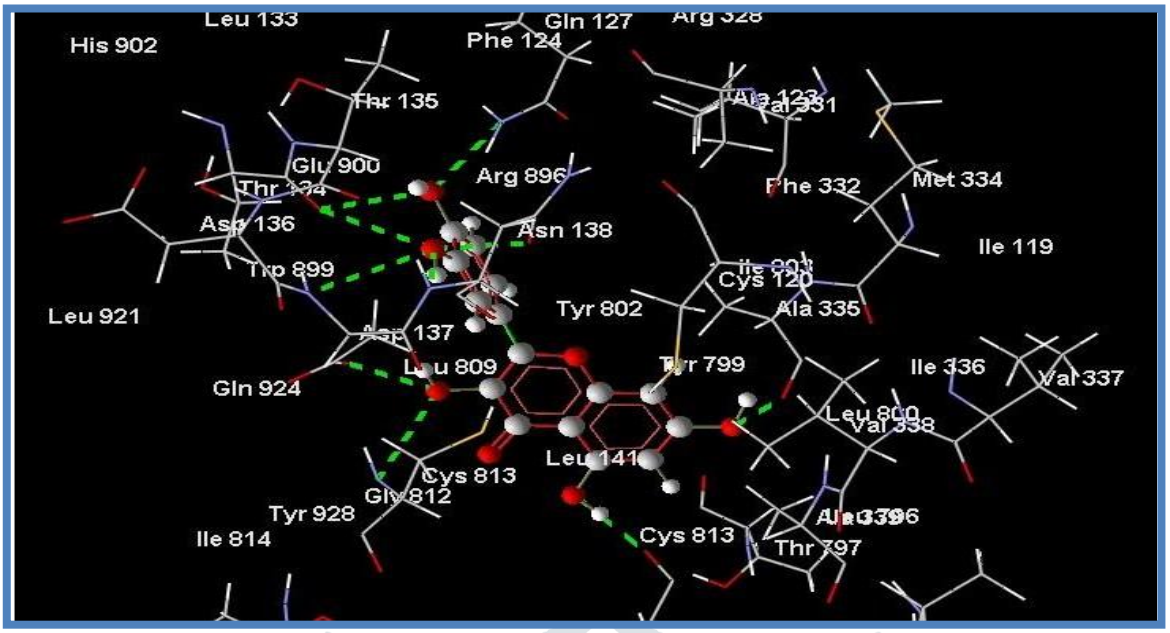


Figure 19 The compound Rhamnocitrin (CG-3) docked in best of itsconformation into the binding site of 5Y0B

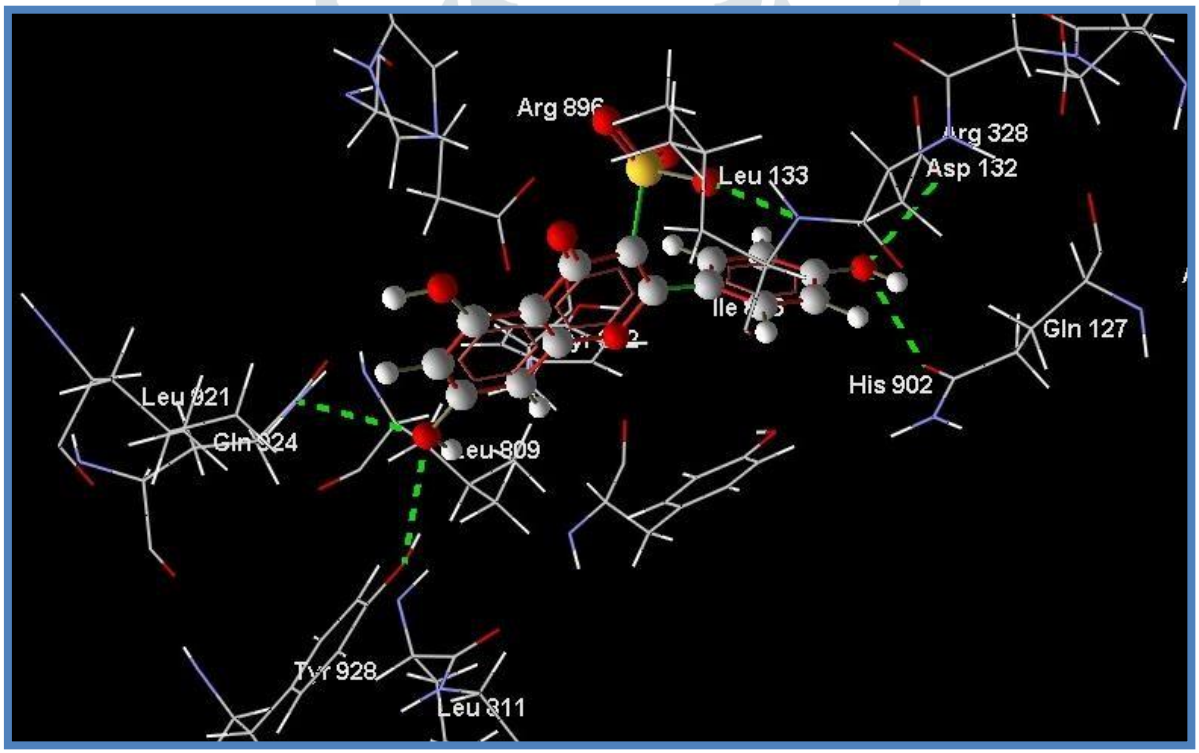


Figure 20 The Compound Kaempferol (DP-3) docked in best of itsconformation into the binding site of 5Y0B

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 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

Table 34 H^+K^+ ATPase inhibitory action of CG-3 and DP-3

Concentration (µg/ml)	Treatment		
	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

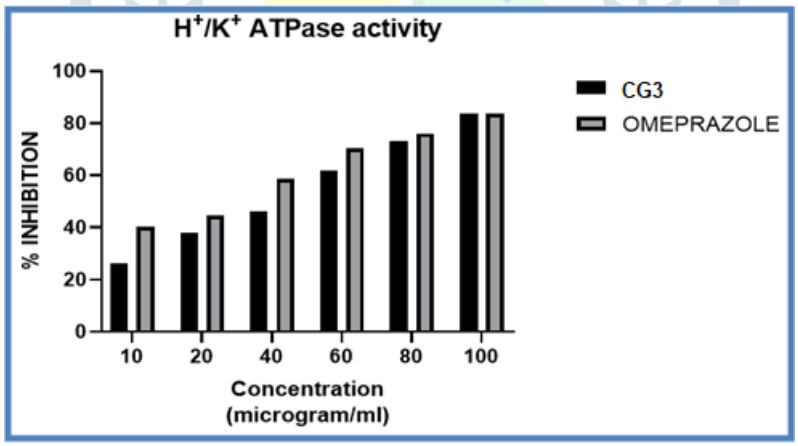


Figure 21 Percentage H^+ K^+ ATPase activity of isolated compound CG-3 andStandard Omeprazole

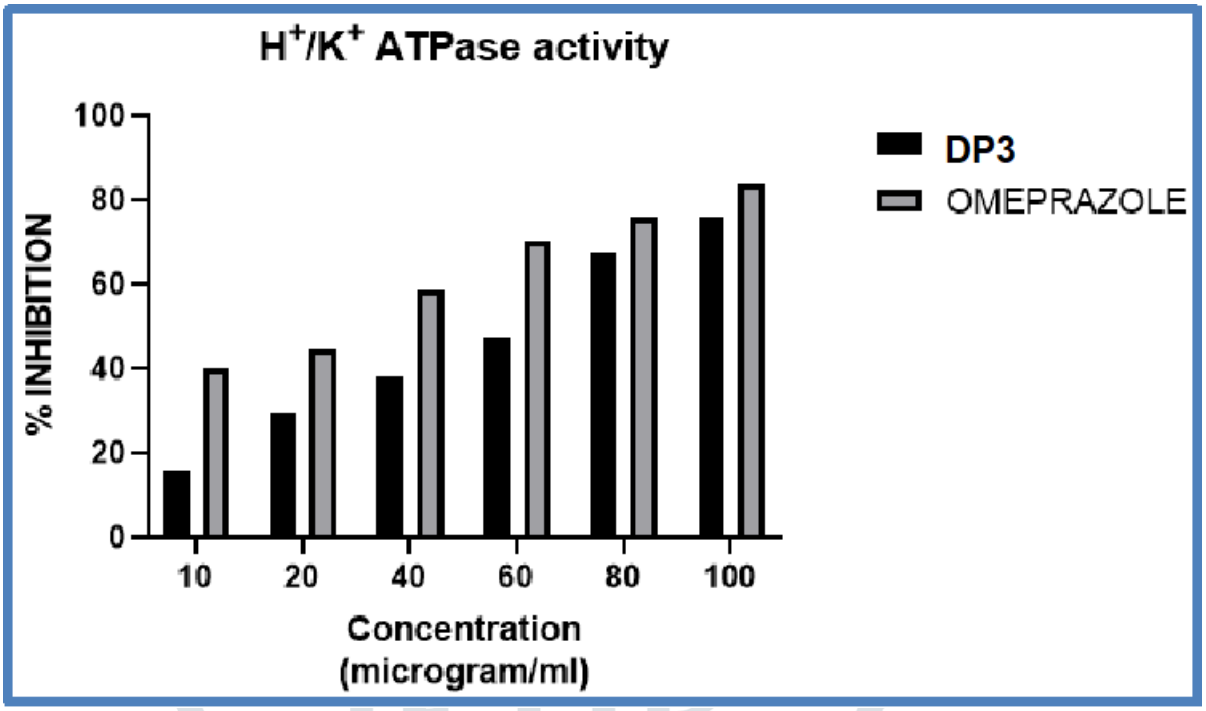


Figure 22 Percentage H^+ K^+ ATPase activity of isolated compound DP-3 andStandard Omeprazole

Discussion :

Cocinnia grandis fruits and *Diplocyclos palmatus* fruits plants were chosen for the current study to assess their efficacy in preventing and treating ulcers, respectively. According to a review of the literature, both plants are mentioned in the Ayurvedic work "Bhavprakash Nighantu" under the chapter "Guduchyadi Varga" as having the vranaropan virtue (wound healing) as pittashamak, dahashamak, and sheetal In many ethnic groups, the chosen herbs were historically used to cure ulcers. However, no studies were conducted to determine the plants' capacity for treating ulcers. Therefore, the goal of the current study was to identify, characterise, and assess the active phytoconstituents that are responsible for the pharmacological effects of *Cocinna grandis* and *Diplocyclos palmatus* fruits on gastric ulcer.

In comparison to water soluble extractive value (9.6%), chloroform soluble extractive value (7.28%), and petroleum ether soluble extractive value (3.76%), the alcohol soluble extractive value of *Cocinnia grandis* fruits (14.24% w/w) was greater. Fruits from *Diplocyclos palmatus* had a greater alcohol soluble extractive value (16.08%) than their water, chloroform, or petroleum ether counterparts (8.4%, 8.96%, and 4%, respectively).

Fruits from *Cocinnia grandis* and *Diplocyclos palmatu*s were reported to have loss on drying values of 6.71 % and 8.3 %, respectively .

The purity and quality of a powdered medicine must be determined by determining the Ash value. An ash of . *Cocinnia grandis fruits* were found to have a total ash value of 9.43% w/w, a water

soluble ash value of 3.69% w/w, and an acid insoluble ash value of 1.57% w/w, respectively, whereas *Diplocyclos palmatus* fruits were found to have a total ash value of 8.67% w/w. The amount of ash that is soluble in water was found to be 4.29% w/w, and the amount that is soluble in acid was determined to be 1.31% w/w. Results show that a powdered medication had inorganic particles, but not to the required levels.

Fruit powder from *Cocinnia grandis* and *Diplocyclos palmatus* was repeatedly extracted using various solvents such petroleum ether, chloroform, and ethanol. . The percentage of each extract yield is shown. After recovering the solvent, extracts were dried, and a phytochemical analysis was done to evaluate the presence of various chemical components.

A crucial first step in the study of medicinal plants is to check them for the presence of various phytoconstituents. In order to identify the many secondary metabolites that the plant produces, preliminary phytochemical screening is helpful.

The ethanolic extract of *Cocinnia grandis* fruits (EECG) demonstrated the presence of many phytoconstituents, including carbohydrates, steroids, glycosides, flavonoids, alkaloids, and tannins, according to the results of the phytochemical screening of the plants. Steroids and triterpenes were visible in the petroleum ether extract of *Cocinnia grandis* fruits (PECG), whereas steroids, glycosides, flavonoids, and alkaloids were visible in the chloroform extract of *Cocinnia grandis* fruits (CECG). In comparison to petroleum ether and chloroform, it was shown that ethanol was able to extract more secondary metabolites from *Cocinnia grandis* fruits. Our data support the earlier conclusions.

Diplocyclos palmatus fruits' preliminary phytochemical screening revealed the presence of sugars, steroids, glycosides, flavonoids, tannins, and triterpenes in its ethanolic extract (EEDP). When compared to ethanolic and chloroform extract (CEDP), the petroleum ether extract of *Diplocyclos palmatus* fruits (PEDP) revealed the presence of less phytoconstituents. Glycosides, tannins, and steroids were all detected in PEDP. Carbohydrates, steroids, glycosides, flavonoids, tannins, and triterpenes were all detected by CEDP.

Phenolic chemicals play a significant part in the plant's antioxidant action since they contain a hydroxyl group. The plant's phenolic content is primarily responsible for its scavenging capacity . By using the Folin-Ciocalteu reagent technique, the phenolic content of the extracts of both plants was estimated. Based on the absorbance readings of sample solutions that react with Folin-Ciocalteu's reagent and comparison to the standard solution of gallic acid equivalents, the total phenolic content was calculated. In comparison to PECG and CECG, EECG was shown to have a greater total phenolic content. EEDP was discovered to have a greater phenolic content in the *Diplocyclos palmatus* fruit extract 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 when the dosage was increased up to 5000 mg/kg, the LD50 could not be identified in this investigation. Both behavioural and neurological and autonomic responses remained unchanged. However, higher than 5g/kg of *Cocinnia grandis* fruit LD50 has been reported . The fruits of *Diplocyclos palmatus* have also been shown to be safe up to doses of 15g/kg body weight .

Using pylorus ligation-induced ulcers, the antiulcer effectiveness of PECG, CECG, EECG, PEDP,CEDP, and EEDP was evaluated.

The most established and reliable model for creating ulcers and determining how different drugs affect acid secretion is the pylorus ligation induced ulcer model.

By tying up the stomach at the pyloric end using surgical sutures, it is possible to cause an acid buildup in the stomach. With the use of this model, other parameters such as stomach volume, pH, total acidity, and free acidity may be determined.In this paradigm, an essential role in the formation of ulcers is played by an imbalance between aggressive forces like the release of the stomachic acid pepsin and defensive factors like cytoprotection.

When compared to the control group, the ulcer index significantly decreased in the EECG and EEDP groups. The ulcer index did not significantly decrease in PECG, CECG, PEDP, or CEDP. 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.When compared to the group receiving Omeprazole as a conventional treatment, EECG and EEDP shown a decrease in ulcer index. This may be because fruits from *Cocinnia grandis and Diplocyclos* palmatus contain phytoconstituents like flavonoids. Preliminary pharmacological research involved doing the a forementioned in vivo anti-ulcer experiments. The outcomes of PECG, CECG, PEDP, and CEDP were not encouraging. Due to their significant anti-ulcer efficacy, EECG and EEDP were chosen for more research. Antioxidants are helpful compounds that lessen the pathological cell damage brought on by free radicals and unstable molecules that are created in response to external and internal stresses. By snatching up free radicals or preventing lipid peroxidation, antioxidants help create a protective barrier against oxidative stress.

DPPH radical scavenging activity, reducing power assay, hydroxyl radical scavenging activity were used in in vitro antioxidant research. The DPPH scavenging In the process, a proton-donor antioxidant agent lowers the amount of DPPH in the methanol to create stable DPPH-H. In methanol, the unpaired electron of DPPH provides a persistent violet hue that is used to measure the

amount of free radicals that antioxidants scavenge. A concentration-dependent DPPH scavenging activity was shown by EECG and EEDP. The reference standard utilised was quercetin.

The purpose of the reducing power test is to ascertain whether a chemical has the antioxidant capacity to break the chain of free radicals. The Fe3+ combination is further reduced to ferrous form by a hydrogen atom, which results in the formation of pearl's Prussian blue hue, which is detected at 700 nm . The reducing activity of EECG and EEDP increased as extract concentration increased, with IC50 values of 107.23 5.88 and 120.60 8.07, respectively..

The primary goal of the current research was to identify the active phytoconstituents that provide *Cocinnia grandis* and *Diplocyclos palmatus* fruits their anti-ulcer properties. For the purpose of isolating active chemicals, column chromatography was also used to EECG and EEDP.

EECG was submitted to column chromatography utilising the gradient elusion technique with the mobile phase being a mixture of n-hexane, ethyl acetate, and ethanol. Four compounds with Rf values of 0.78, 0.69, 0.59, and 0.38 were recovered from EECG and given the names CG-1, CG-2, CG-3, and CG-4. Because of its extremely low yield, CG-1 was scrapped.

Through column chromatography and the gradient elusion method with 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 equivalent 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 ligationAspirin's corrosive activity on mucosa by inhibiting prostaglandin production may have contributed to the ulcers that were created in the aspirin plus pylorus ligated induced ulcer model . Damage to the mucosa alters gastric permeability, increases the amount of pepsin and protein, and produces free radicals that promote the production of reactive oxygen species

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 CG-3 and DP-3..

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 224–227 ^{0} 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.

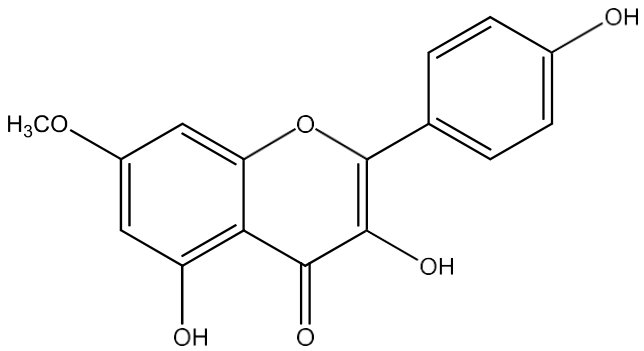
The sample CG-3's 1H NMR spectrum included singlets at 3.897 for three aliphatic protons, 6.051 for one aromatic proton, 6.453 for one aromatic proton, 6.850 to 6.893 for two aromatic protons, 8.151 to 8.201 for two aromatic protons, 9.951 for one proton, 10.732 for one proton, and 12.430 for one proton.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

One aliphatic carbon was identified in the sample CG-3's 13C NMR spectra at a singlet at 56.00; the fourteen aromatic carbons were identified at 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), and 160.00 (s);

There is evidence of carbonyl carbon at 162.70 (s), 166.00 (s), and a singlet at or about 175.50. One aliphatic carbon, fourteen aromatic carbons, and a carbonyl carbon were all detected by 13C NMR.

Fragments at m/z 299, 284, 271, 255, 243, 227, 185, 183, 157, 141, 129, and 113 were seen in the mass spectra of sample CG-3. The chemical has a 300 molecular weight, according to research.

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.

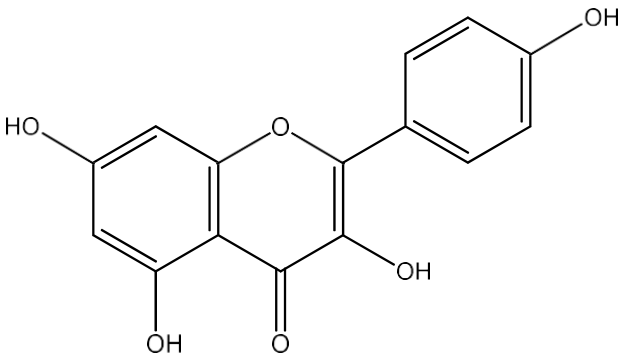
Due to the presence of a hydroxyl group, the IR spectra of compound DP-3 revealed the presence of a band at 3460 cm-1. The existence of aromatic C-H and aliphatic C-H stretching are shown by the absorption bands at 3363 and 3230 cm-1, respectively. A band at 1290 cm-1 is caused by the stretching of the 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.

15 aromatic carbons were detected in the molecule DP-3's 13C NMR spectra at positions 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), and 163.63 (s), and a singlet at 176. A carbonyl carbon and fifteen aromatic carbons were detected using 13C NMR.

Sample MD-MDDP-2's mass spectra revealed fragments at m/z 285; 257; 256; 244; 228, 183; 157; 141; 129; and 113. The chemical has a 286 molecular weight, according to analysis.

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



Kaempferol

Rhamnocitrin and Kaempferol were accessed by computer assisted molecular docking simulation study utilising Molegro Virtual Docker (MVD-2013, 6.0) for H+ K+ ATPase inhibitory activity based on the pharmacological activity and structure revealed. Rhamnocitrin and Kaempferol had MolDock values of -86.456 and -89.377, respectively, whereas the normal omeprazole's MolDock score was -112.113. The contact between omeprazole and rhamnocitrin (compound CG-3) was found to be with the same amino acid Cys813, but the interaction between omeperazole and kaemepferol (compound DP-3) was with two amino acids, Leu921 and His902. Through non-covalent interaction, CG-3 (rhamnocitrin) and DP-3 (kaempferol) demonstrated excellent interaction with little binding energy in the enzyme active region. The results of the molecular docking suggest that the antiulcer activity of both separated drugs may be influenced by their potential H+K+ ATPase inhibitory action.

The gastric parietal cell's ATP-dependent H+K+ exchanger pump is crucial for gastric acid secretion. The final stage in the secretion of acid is the release of H+ ions by H+K+ ATPase . Gastric ulceration is primarily caused by hyperactivity of the proton pump-like H+K+ ATPase enzyme . Therefore, a pump inhibitor would be highly successful in reducing the oversecretion of stomach acid

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. Positive in vitro H+K+ ATPase inhibition activity results supported CG-3 (Rhamnocitrin) and DP-3 (Kaempferol)'s in vivo antiulcer efficacy as well as computer assisted molecular docking studies.

The human body is continuously subjected to oxidative stress, including ROS and free radicals, which may damage biomolecules including lipids, proteins, and nucleic acids in both reversible and irreversible way. These elements influence the development of oxidative stress-related illnesses such cancer, heart failure, ulcers, and aging. In order to address pathologic oxidative stress, antioxidant medications and naturally occuring compounds with antioxidant capabilities have been the focus of study. Fruits from *Cocinna grandis* and *Diplocyclos palmatus* have

demonstrated outstanding in vitro DPPH radical, nitric oxide,superoxide anion, reducing power, and hydroxyl radical scavenging action in this study. Along with reactive oxygen species, the overproduction of stomach acid is the primary factor in the pathophysiology of peptic ulcers . Rhamnocitrin, an isolated chemical from *Cocinnia grandis* 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. DP-3 (kaempferol), which was evaluated using in vitro and molecular docking experiments.

We come to the conclusion that the fruits of *Cocinna grandis and Diplocyclos palmatus* have promising potential as antiulcer medications. This study provides scientific support for the traditional use of these plants for the treatment of ulcers and provides a framework for the creation of polyherbal formulations that include the fruits of *Cocinnia grandis and Diplocyclos palmatus.* More thorough investigation into the molecular mechanisms behind rhamnocitrin and kaempferol's inhibition of the H+K+ ATPase is possible.

SUMMARY AND CONCLUSION

The goal of the current investigation was to verify the traditional use of *Cocinnia grandis* and *Diplocyclos palmatus* fruits as ulcer-healing supplements. It was intended for research to separate the active components from these plants and conduct in vitro and in vivo activities to assess their anti-ulcer effectiveness.

Utilising the DPPH radical, , Reducing power, and Hydroxyl radical scavenging assays, in vitro antioxidant activity of EECG and EEDP was examined assay. The antioxidant activity of EECG and EEDP increased with concentration.

Using column chromatography, an effort was undertaken to separate the main phytoconstituents from EECG and EEDP. Fruits of *Cocinnia grandis* were used to isolate four chemicals, designated as CG-1, CG-2, CG-3, and CG-4. Since the yield of CG-1 was insufficient, it was not taken into consideration for future research. *Diplocyclos palmatus* fruits contained three chemicals that were identified as DP-1, DP-2, and DP-3.

Using several ulcer generating models, such as the Aspirin plus pylorus ligation caused ulcer model,. Maximum antiulcer activity was seen for CG-3 and DP-3 at a dosage of 20 mg/kg.

CG-3 and DP-3 were characterised and structurally clarified using a variety of spectrum techniques, including UV, FTIR, NMR, and mass spectroscopy. The acquired spectrum data was analysed and contrasted with the information from previously published literature. Flavonoids Rhamnocitrin and Kaempferol were identified as CG-3 and DP-3, respectively.

Using Molegro Virtual Docker (MVD-2013,6.0), 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.

Additionally, the in vitro antiulcer efficacy of CG-3 and DP-3 was assessed in order to correlate with its in vivo action. It was discovered that CG-3 and DP-3 have strong H+ K+ ATPase activity.We draw the conclusion that the antiulcer activity of the fruits of *Cocinnia grandis* and *Diplocyclos palmatus* is caused by the presence of the flavonoids rhamnocitrin and kaempferol, respectively, based on the overall findings of the current study effort. The antioxidant and H+K+ ATPase inhibitory capabilities of rhamnocitrin and kaempferol may be responsible for their antiulcer efficacy.*Cocinnia grandis and Diplocyclos palmatus* fruit extracts in petroleum ether, chloroform, and ethanol were initially tested for antiulcer efficacy utilising ulcer-inducing models such pylorus ligation,

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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.

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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 CG-3 and DP-3.

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