Phytoconstituents and Biological Activities of The Aerial Parts of *Caesalpinia gilliesii* (Hook) Family Caesalpinacae Growing in Egypt

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Abstract: In this study, phytoconstituents and biological activities of leaves, flowers and pods of *Caesalpinia gilliesii* (Hook) Family Caesalpinacae were evaluated. Cardiac glycosides, cyanogenic glycosides, saponins, flavonoids, tannins and coumarins were present in all extracts with different ratios while anthraquinones and alkaloids were absent. *In-vivo* hepatoprotective activity of the plant extracts (300 mg/kg) was studied using CCl_4 - induced hepatotoxicity. Histopathological and histochemical study of liver tissues was investigated. Leaves and flowers extracts showed the highest hepatoprotective activity comparable with standard silymarin. Different extracts of *C. gilliesii* were examined spectrophotometrically for its radical scavenging activity against 1, 1-diphenyl-2-picryl hydrazyl (DPPH). Ethyl acetate extract of flowers had the highest radical scavenging activity (IC_{50} = 7.43 μg ml $^{-1}$). This activity may be contributed to the presence of phenolic compounds. In this sense, extract from *C. gilliesii* could be a valuable alternative for obtaining compounds with great commercial potential, given its high antioxidant potential.

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1. Introduction

Free radicals from exogenous chemicals and endogenous metabolites are capable of oxidizing various biomolecules resulting in tissue damage and cell death. It has been implicated in the cause of several diseases such as liver cirrhosis, atherosclerosis, cancer and play an important role in ageing. Oxidative stress can also contribute to the development of neuro-degenerative disorders, such as Alzheimer's and Parkinson's as well as other diseases (Koheil MA. *et al*., 2011). Antioxidant supplements are used to reduce oxidative damage from free radicals and active oxygen species. Other synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and trolox are widely used as antioxidants in the pharmaceutical and food industry. However, they have been shown to have toxic and/or mutagenic effects (Halliwell *et al*., 1992). Because of their toxicity, Herbal medicine development is one of the important subjects of study focusing on the discovery of new antioxidants and hepatoprotective drugs. Phenolic compounds, represented mainly by tannins and flavonoids, stand out as the major group of natural antioxidants. They act as efficient scavengers of free radicals and interrupt oxidative chain reactions (Carlos *et al.,* 2011).

Caesalpinacae represents approximately 11% of the known legume flora, mostly tropical and subtropical in distribution. *Caesalpinia gilliesii* (Yellow Bird of Paradise) is a fast growing shrub or small tree. It is ornamental plant, semi-evergreen with bipinnate and long leaves. The showy yellow flowers have bright red protruding stamens (10-12.5 cm length). Pods are oblong and flat, being indehiscent or dehiscent. Seeds are brown in colour with 2 large cotyledons. *C. gilliesii* are native to Argentina (South America). The plant cultivated in Egypt (Borg El Arab). Caesalpinacae is rich in tannins, saponins, flavonoids, alkaloids, terpenoids and cardiac glycosides (Sivasankari *et al.,* 2010). Cassane-type diterpenoids are characteristic components of the genus *Caesalpinia* (Kalauni *et al.,* 2006). Genus *Caesalpinia* was reported to have anti-inflammatory (Manoj *et al.,*2009, Chakraborthy *et al.,*2009), antidiabetic (Kannur *al.,* 2006, Farook *et al.,*2011), anticancer (Nakamura *et al.,*2002), antioxidant (Penpun *et al.,*2005), antibiotic (Tasleem *et al.,*2009), antiviral (Jiang *et al.,*2002) activities.

However from current literature no *in-vivo* experimental data is available on the hepatoprotective or antioxidant properties of this plant. Therefore, this study was designed to study the phytoconstituents and investigate the *in- vitro* antioxidant activity and *in-vivo* hepatoprotective activity of *Caesalpinia gilliesii*.

2. Material and Methods

Plant Material and Extraction

Aerial parts (leaves, flowers and pods) of *C. gilliesii* were collected from Egypt (Borg El Arab) in May. The taxonomical features were kindly confirmed by Agriculture Research Center, Cairo, Egypt. A voucher specimen (CP#1203) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, October 6 University. Freshly collected plant material was washed 3 times with running tap water and then with distilled water followed by shade drying. Hydro-alcoholic extracts of leaves, flowers and pods were prepared by powdering 500 g of each separately in an electric mill and then defatted by *n*-hexane (1L x 3 times). The solvent was filtered and cakes were percolated in ethanol 70% separately. Ethanol was evaporated under reduced pressure. A suspension of the extract in double distilled water containing few drops of Tween 80 was prepared to be used in biological activity.

Subsequently 60 g dried *C. gilliesii* flowers extract (CGFE) was suspended in 500 ml distilled water and sonicated for 30 min. Suspension was fractionated using solvent–solvent extraction with *n*-hexane (6 x 500 ml), methylene chloride (4 x 500 ml), ethyl acetate (7 x 500 ml) and *n*-butanol (10 x 500 ml) (Trease G.E., Evans W.C., 2002). The resulting extracts were then concentrated to dryness under reduced pressure on a rotary evaporator (Sineco Technology Co. Ltd, Shanghai, China). These extracts were refrigerated until used.

Phytochemical study

The phytochemical study was carried out for the different aerial parts extracts using the standard procedures to identify the components. The phytochemical screening was carried out for the components such as volatile substance (Wagner H. and Baldt S., 1996), carbohydrates (Tiwari P., *et al.*, 2011), proteins and amino acid (Kokate C. K., 2003; Tiwari P. *et al.*, 2011), saponins, alkaloids (Tiwari P. *et al.*, 2011), flavonoids (Trease G.E. and Evans W.C., 2002), anthraquinones, cardiac glycosides (Trease G.E. and Evans W.C., 2002), sterols, terpenoids, triterpenoids (Tiwari P., *et al.*, 2011), coumarins (Kokate C. K., 2003), tannins (Himesh S., *et al.*, 2011) and cyanogens (Harborne J. B, 1972).

Thin Layer Chromatography (Wagner H. *et al.*, 1996, Harborne J. B,, 1998)

1. **Detection of sterols and/or triterpenes:** 1g of the extracts was extracted individually with 10 ml chloroform and filtered. The filtrates were used to test for sterols and/or triterpenes.
2. **Detection of cardiac glycosides:** 1g of the extracts was extracted individually with 3 ml 50% ethanol and 10 ml 10% lead acetate with reflux for 15 min. and filtered. The filtrates were extracted 3 times with 1.5 ml chloroform: isopropanol (3:2). The lower layers were separated, filtered over anhydrous sodium sulphate and evaporated till dryness. Residues were dissolved in 1 ml chloroform: isopropanol (3:2) and used for cardiac glycosides test.
3. **Detection of cyanogens:** 1g of the extracts was extracted individually with 10 ml 70% ethanol and filtered. The filtrates were used to test for cyanogens.
4. **Detection of saponins:** 1g of the extracts was extracted individually with 10 ml 70% ethanol, refluxed for 10 minutes and filtered. The filtrates were used to test for saponins.
5. **Detection of flavonoids:** 1g of the extracts was extracted individually with 10 ml methanol, heated for 5 minutes in 60°C water bath and filtered. The filtrates were used to test for flavonoids.

In-vivo hepatoprotective study

Animals

Thirty six mature Wister strain male albino rats (animal house of NODCAR) of 3 months of age weighing about 140 ±10 g were taken for this experiment. Animals were acclimatized for a period of 7 days to our laboratory conditions prior to the experiment. Rats were housed in colony cage (6 rats per cage) at an ambient temperature of 25±2ºC with 12 h light: 12 h dark cycle. Rats had free access to standard food and water. The principles of laboratory animal care were followed throughout the duration of experiment and instruction was followed regarding injection and other treatment of experiment.

CCl_4-induced hepatotoxicity

LD_{50} of different aerial parts extracts of *C. gilliesii* were separately determined according to Karber C. 1931. Dose of 1, 2, 3, 4 and 5 g/kg body weight were chosen as dose level that would be expected to allow the identification of dose producing evident toxicity. The protocol was carried out as described by Srilakshmi V. *et al.,* 2010. Rats were divided into 6 groups and treatment schedule was as followed: Control group and CCL_4 (negative control) group, rats remain under normal conditions. Silymarin group, rats received 200 mg/ kg p.o. once daily of silymarin for 7 days, Leaves extract, flowers extract and pods extract groups, rats received 300 mg/ kg p.o. once daily of each extract for 7 days. In the 7th day all group except control group subjected to hepatotoxicity by single intraperitoneal injection of 30% CCL_4 in olive oil (1mg/kg, i.p). After 24 h of hepatotoxicity (on 8th day), blood was collected from all groups of rats by puncturing the retro orbital plexus in centrifuge tube and allowed to clot for 45 min at room temperature (Galighor A.E. and Kozloff E.N. 1976). Serum was separated and various biochemical parameters i.e., aspartate amino transferase (AST), alanine amino

transferase (ALT), alkaline phosphatase (ALP) (Szasz G., 1971) were estimated using reported methods assay and kits according to the methods described by the manufacturers.

Histological and Histochemical investigation:

After blood sampling for the biochemical analysis, the animals were sacrificed; quickly dissected and a small portions of liver were washed in saline and quickly fixed in 10% formalin. Then, the specimens were processed by standard histopathological technique. Sections of 6 µm in thickness were prepared and stained with Haematoxylin and Eosin (H&E), examined under microscope and photographed (Drury R.A.D., Wallington E.A., 1980). In the histochemical study, sections were stained with Periodic Acid Schiff (PAS) to demonstrate the carbohydrate content (Phifer R F., *et al.*, 1973) and with Bromophenol blue to demonstrate total proteins (Maize D., *et al.*, 1953). These were examined microscopically and photographed.

Radical Scavenging Activity

Plant extracts were screened in-vitro using DPPH radical photometric assay in a process guided by its discoloration in triplicate method described by Carlos H. *et al* 2011. The reaction mixture for different plant extracts consisted of 2 ml of 0.125 mM DPPH-ethanol solution, 1.8 ml of 0.05 M Tris-HCl buffer (pH 7.4) and 0.2 ml of each tested extract concentration. The absorbance was measured 516 nm at room temperature immediately after adding the various extract concentration. Analyses were performed using ethanol to reset the spectrophotometer. Absorbance was compared with a negative control consisting of DPPH ethanolic solution for each extract or standard. The antioxidant activity was calculated from Equation: $\%AA = 1 - (Abs_{sample} - Abs_{blank} / Abs_{negative\ control}) \times 100$. Then %AA plotted versus the concentrations of plant extract or standard. IC_{50} values calculated from the curve for each concentrations of extracts or standard.

Chemicals

Silymarin (Silymarin, CID Co., Giza, Egypt) was purchased from local pharmacies in Cairo, Egypt. Carbon tetrachloride, (E. Merck (I) Ltd., Bombay), DPPH**,** Rutin **(**Sigma- Aldrich Co., USA.) and olive oil was purchased from local market in Cairo, Egypt. All other chemicals and solvents used were purchased from local firms in Egypt and were of the highest purity grade available.

Statistical analysis:

The data for body weight and fasting blood glucose (FBG) level were expressed as mean ± SEM and compared using t-test. Values were considered statistically significant when $p< 0.05$ (Winer BJ., 1971).

3. Results and Discussion

Phytochemical screening

Extraction and phytochemical screening of different aerial parts of *C. gilliesii* showed that 75 g (15%), 60 g (12%) and 50 g (10%) residues were yielded from leaves, flowers and pods respectively. Carbohydrates, fats and terpenoids have been seen in all extracts while volatile substance, amino acids and proteins were absent (Table 1). Cardiac glycosides, cyanogenic glycosides, saponins, flavonoids, tannins and coumarins were present in all extracts with different ratios while anthraquinones and alkaloids were absent (Table1).

Preliminary phytochemical screening revealed that flowers extract contain large amount of tannins, flavonoids and sterol compounds, which were reported to possess antioxidant and hepatoprotective activity (Miyako T. and Shibamato T., 1997). Fractionation of flower extract yielded 12%, 2%, 9%, 29% and 48% for *n*-hexane fraction (HF- CGFE), methylene chloride fraction (MF- CGFE), ethyl acetate fraction (EF- CGFE), *n*-butanol fraction (BF-CGFE) and aqueous fraction (AF- CGFE) respectively.

Hepatoprotective activity

The LD_{50} of different extracts of *C. gilliesii* aerial parts were found more than 5 g/kg b.wt, p.o. in mice. Therefore, 300 mg/kg b.wt is considered to be safe and convenient dose. Rats receiving these extracts also showed normal values for the serum biochemical parameters determined at the selected dose (300 mg/kg, p.o.) regimen. Liver injuries induced by carbon tetrachloride was found to be the best characterized system of xenobiotic-induced hepatotoxicity besides that the changes associated with CCl_4-induced liver damage were similar to those of the acute viral hepatitis. Silymarin was used as standard hepatoprotective compound since it was reported to have a protective effect on the plasma membrane of the hepatocytes (Srilakshmi V.S., *et al.*, 2010). The hepatotoxicity of CCl_4 is due to its active metabolite, trichloromethyl radical (Srivastava S. P. *et al.*, 1990, Johnston D.E., Kroening C. 1998). This radical binds to the macromolecules inducing lipid peroxidative and degradation of biomembranes of the endoplasmic reticulum which is one of the principal causes of hepatotoxicity of CCl_4 (Cotran R. S. *et al.*, 1994).

After CCL_4 injection (8^{th} day), There was extremely significant ($P< 0.001$) elevation in serum biochemical parameters AST, ALT and ALP of CCL_4 (negative control) group compared to control group. Rats that received 200 mg/kg body weight of the standard silymarin (positive control) were protected considerably against the elevation in the levels of the biochemical parameters when compared with the CCl_4 group ($P< 0.001$). These parameters came towards the control level in rats pretreated with leaves extract and

flowers extract comparable to CCl_4 group ($P< 0.001$) and insignificantly when compared with standard silymarin group ($P> 0.05$). The results were summarized in (Table 3). Leaves and flowers extract showed significant protection against the elevation in the levels of these serum markers, indicating the protection of the hepatic cells. The potent and significant hepatoprotective activity of that extracts of the aerial parts of *C. gilliesii* may be attributed to its high content of phenolic compounds (Miyako T. and Shibamato T. 1997, Jain A., *et al.*, 2008).

Histological and Histochemical investigation:

Although biochemical method is generally used for investigate the hepatoprotective effect of different treatment, the microscopic observation provided good information about organ morphology and function. There was no histopathological or histochemical alteration observed and the normal histological structure of liver cells was recorded in control group, which shows normal liver with normal hepatocytes with well preserved cytoplasm, nucleus, nucleolus and central vein. Normal concentration and distribution of carbohydrate and protein contents were also detected (Figure 1). In CCL_4 group (negative control) the liver sections showing total loss of cellular architecture with enlarged nuclease, marked degenerative changes and fatty changes with dilated liver sinusoids, as well as areas of inflammatory cell infiltration. Marked carbohydrate depletion with abnormal distribution and mild depletion of protein with irregular distribution were also shown (Figure 2).

The protection against depletion of carbohydrate and protein contents in the liver tissues indicated maintaining of liver function, storage and synthesis, as carbohydrate is one of the most important components stored in the liver and proteins are typically synthesized in its tissues (Anthea M. *et al.*, 1993). Pretreatment of rats with silymarin (positive control) resulted in mild degree of cellular degeneration with more or less normal hepatocytes with mild fatty changes, mild infiltration of inflammatory cells and moderate dilation of liver sinusoids. Marked protection against depletion of carbohydrate and protein caused by CCl_4 with abnormal distribution of glycogen with marked fatty changes around portal vein (Figure 3).

Rats pretreated of with leaves or flowers extract of *C. gilliesii* showed normal hepatocytes with minimum fatty changes, portal vain congestion and mild inflammatory cell infiltration around portal vain. It showed also a significant protection against depletion of carbohydrate and protein with irregular distribution of glycogen and protein (Figures 4, 5). Pretreatment with pods extract showed mild protection against the toxic effects of CCl_4 on the liver cells (Figure 6).

Table (1): Phytochemical Screening of The Aerial Parts of *C. gilliesii*

No.	Phytoconstituents	Phytochemical test	Leaves	Flowers	Pods
1	**Steam volatile substance**	Hydrodistillation test	-	-	-
2	**Carbohydrates and/or glycosides**	Molisch's test	++	++	++
		Fehling test	+	++	++
3	**Protein and amino acids**	Biuret test	-	-	-
		Xanthoprotic test	-	-	-
4	**Fixed oil and fats**	Spot test	+	++	+++
		Saponification test	+	++	+++
5	**Phytosterols**	Salkowski test	+	++	+
		Liebermann test	+	++	+
		Cu acetate test	+	++	+++
6	**Cardiac glycosides**	Baljet test	+	+++	++
		Keller killiani test	+	++	++
7	**Anthraquinon glycosides**	Bornetrager's test	-	-	-
		Modified bornetrager's test	-	-	-
8	**Flavonoids**	Amyl alcohol test	+++	+	++
		Na OH test	+++	+	++
		Shinodas test	+++	+	++
9	**Alkaloids**	Mayer's test	-	-	-
		Dragendorff's test	-	-	-
10	**Cyanogens**	Picric acid test	+	+	+++
11	**Tannins**	Fe CL_3 test	+++	+++	+++
		Gelatin test	++	++	++
12	**Coumarins**	Na CL test	+	+	+
13	**Saponins**	Froth test	+++	+	+

(+++): large amount, (++): moderate amount, (+): traces, (-): absent.

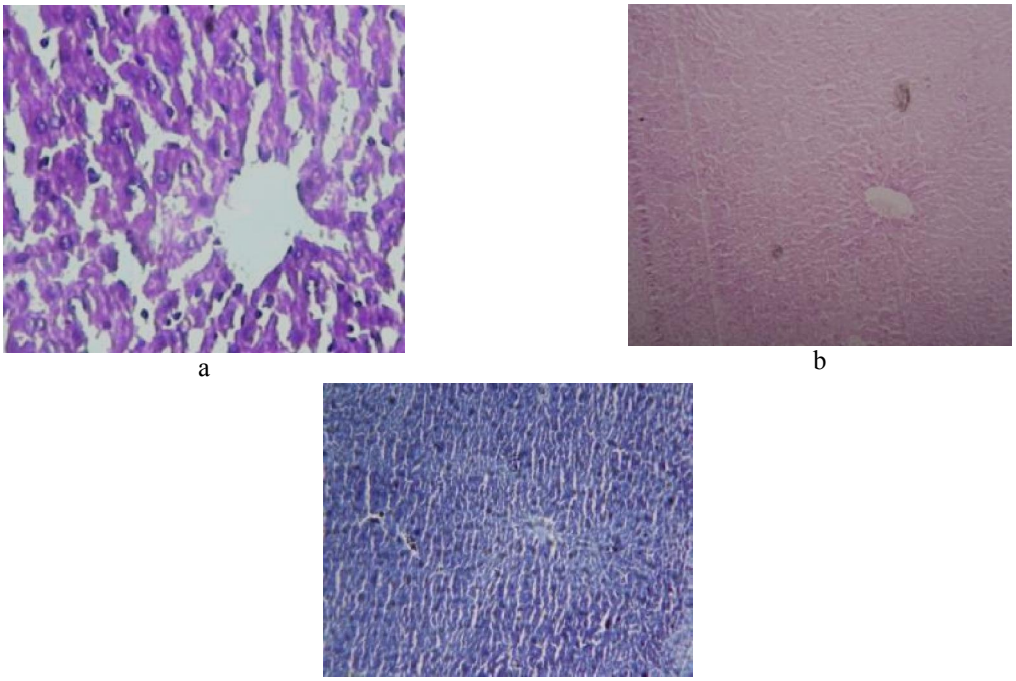
Table 2: Secondary Metabolites Screening using Thin Layer Chromatography

Chemical class	Solvent system	Plant parts	bands	Spray reagent
Sterols and triterpenes	Ethyl acetate : methanol: water (77:15:8)	Leaves	9	Vanillin / H_2SO_4 110 C^o
		flowers	7	
		pods	7	
	Benzene: Ethyl acetate (2:1)	Leaves	8	
		flowers	2	
		pods	1	
Cardiac glycosides	Ethyl acetate : methanol: Ethanol: water (81:11:4:8)	Leaves	5	Chloramine trichloroacetic acid (TCA) and UV light 365 nm
		flowers	3	
		pods	4	
Cyanogenic glycosides	Butanol: Ethanol :water (40:11:14)	Leaves	1	Amm. Ag NO_3
		flowers	2	
		pods	3	
	Chloroform : methanol (8:2)	Leaves	1	2% alc. α –naphthol then conc. H_2SO_4 110 C^o
		flowers	1	
		pods	1	
Saponins	Chloroform :glacial acetic acid: methanol: water (64:32:12:8)	Leaves	7	Vanillin / H_2SO_4 110 C^o
		flowers	8	
		pods	9	
Flavonoids	Ethyl acetate :glacial acetic acid: formic acid: water (100:11:11:26)	Leaves	12	Al Cl_3 and UV light 365 nm
		flowers	11	
		pods	14	
Flavonoids	Ethyl acetate : methanol: water (100:13:10)	Leaves	9	Al Cl_3 and UV light 365 nm
		flowers	7	
		pods	11	
Flavonoids	Chloroform : methanol (9:1)	Leaves	12	Al Cl_3 and UV light 365 nm
		flowers	6	
		pods	14	
Flavonoids	Chloroform : methanol (6:4)	Leaves	8	Al Cl_3 and UV light 365 nm
		flowers	6	
		pods	11	
Coumarins	Toluene: ether (1:1) saturated in 10% acetic acid	Leaves	13	Al Cl_3 and UV light 365 nm
		flowers	8	
		pods	8	

Table 3: Effect of Different Extracts of *C. gilliesii* Aerial Parts on Serum Biochemical Parameters in CCl_4-Induced Hepatotoxicity rats (values are mean ± SEM of 6 rats)

Groups	Serum biochemical parameters					
	AST	ALT	ALP	Protein	Cholesterol	Triglycerides
Control	206±25	84±2	434±50	6.9±0.1	91.5±9	43.3±3
CCL_4 (negative control)	2018±85^a	1193±32^a	1207±21^a	4.9±0.4	145±19	85.8±9
Silymarin (positive control)	469±38*	216±9*	375±38*	6.7±0.2	95.8±6	80.6±8
Leaves extract	573±53*	485±35*	459±45*	6.7±0.2	93.5±8	75.8±9
Flowers extract	800±59*	466±52*	452±61*	5.4±0.4	105±11	43.1±4
Pods extract	1403±95$^{a\#}$	895±68^a	761± 60$^{b\#}$	5.7±0.4	135±15	70.6±5

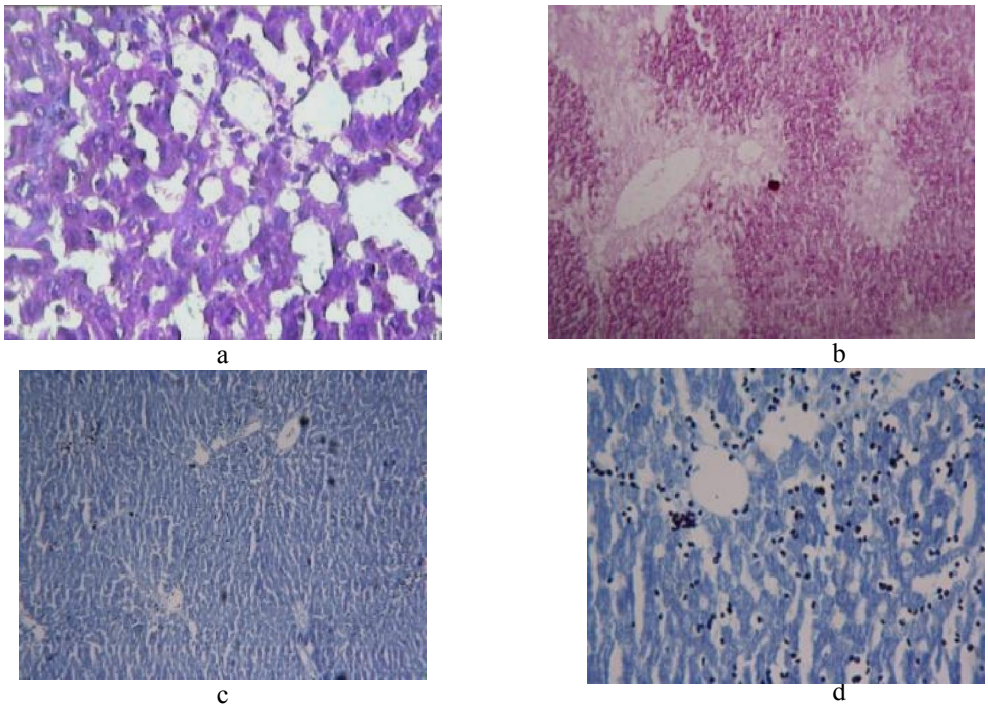
Superscripts a, b donate statistical significance in comparison to normal group $P< 0.001$, <0.05 respectively.
Superscripts *, # donate statistical significance in comparison to CCl_4 group $P < 0.001$, <0.01 respectively.



a b c

Figure 1: Photomicrograph of liver section of control group rats

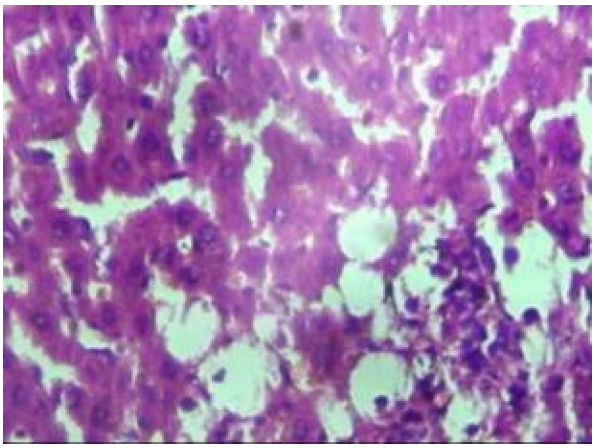
a) Showing histological normal liver lobules and hepatocyte threads with normal portal vein (H&E, X=400).
b) Showing normal concentration and distribution of carbohydrate (PAS, X= 100).
c) Showing normal content and distribution of protein (Bromophenol blue, X=100).



a b c d

Figure 2: Photomicrograph of liver section of CCL_4 group rats

a) Showing marked degenerative changes and fatty changes with dilated liver sinusoids (H&E, X= 400).
b) showing marked carbohydrate depletion with abnormal distribution (PAS, X= 100)
c) Showing mild decrease of protein content with irregular distribution (Bromophenol blue, X= 100).
d) Showing irregular protein distribution and moderate dilation of liver sinusoids (Bromophenol blue, X= 400).



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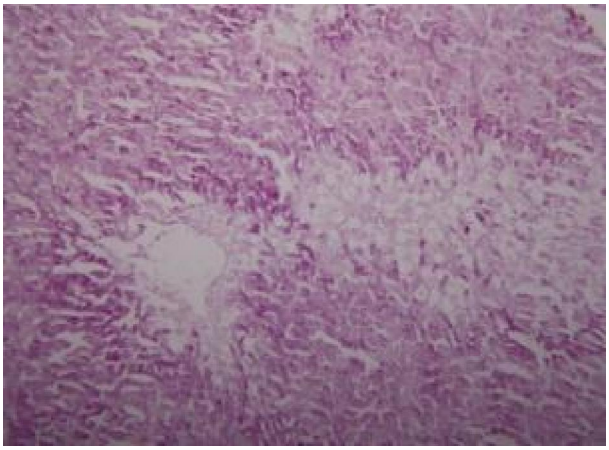

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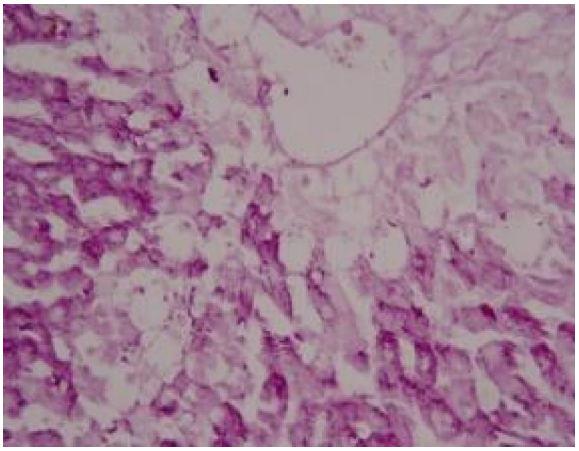

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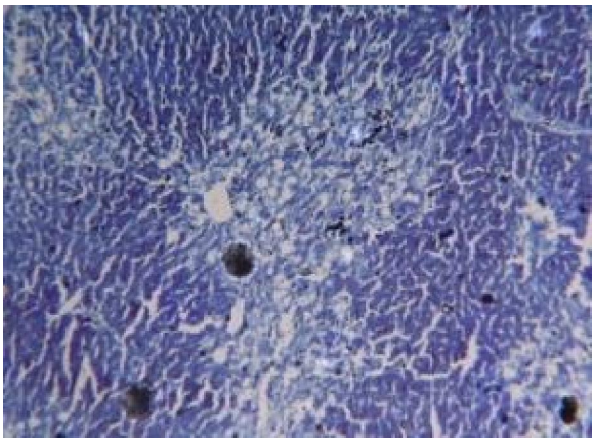

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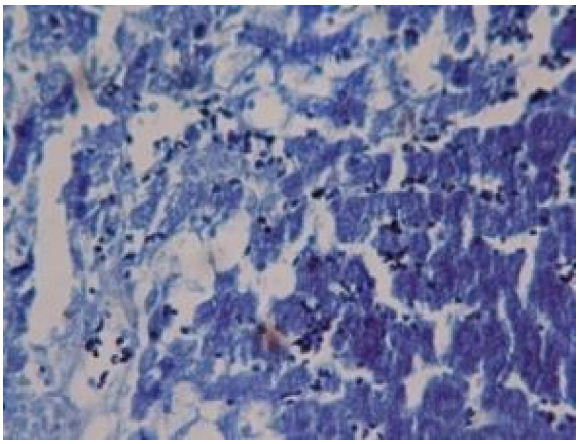
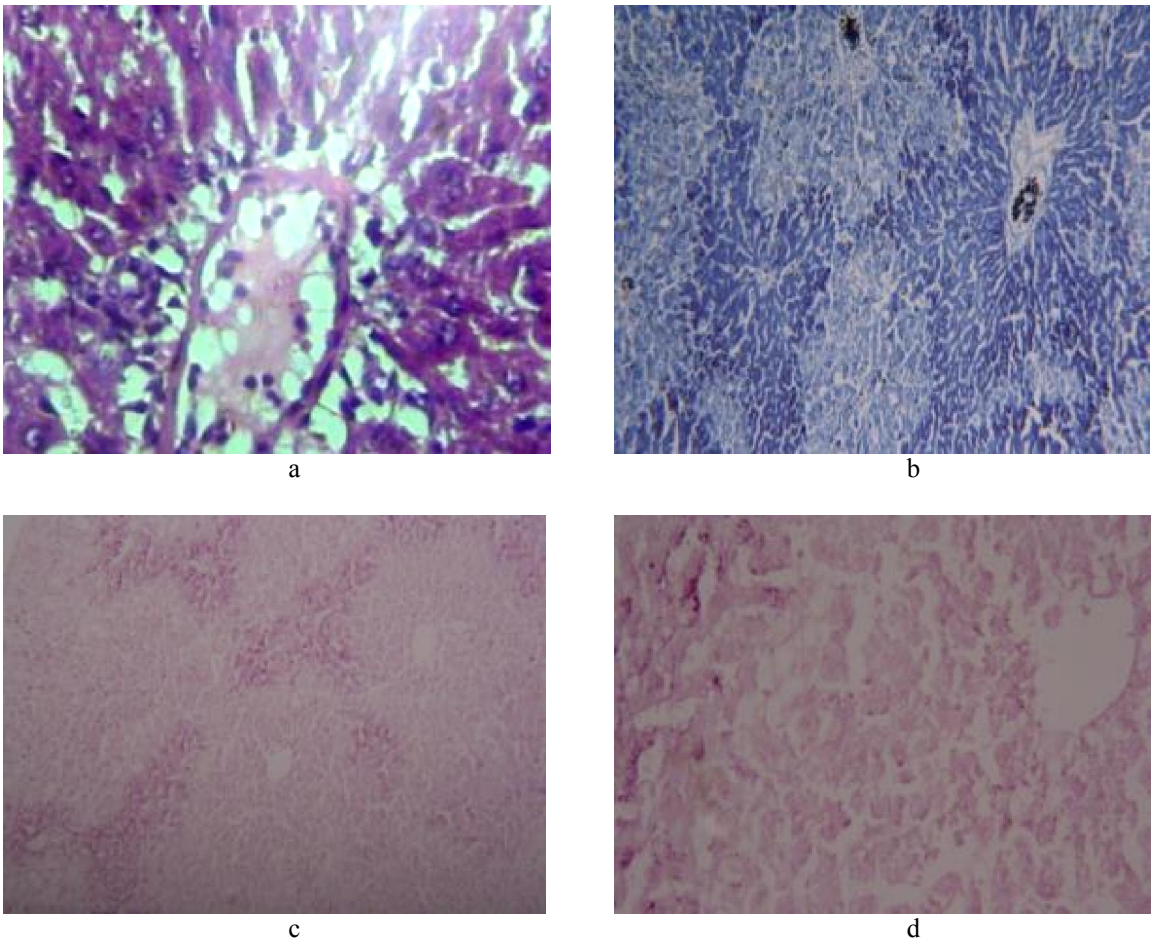


e

Figure 3: Photomicrograph of liver section of silymarin group rats

a) Showed more or less normal hepatocytes with mild fatty changes, mild infiltration of inflammatory cells and dilated liver sinusoids (H&E, X= 400).
b) Showing abnormal distribution of glycogen (PAS, X= 100).
c) Showing abnormal distribution of carbohydrates with fatty changes around portal vein (PAS, X= 400).
d) Showing mild changes in protein content (Bromophenol blue, X= 100).
e) Showing mild changes in protein distribution with moderate dilation of liver sinusoids (Bromophenol blue, X= 400).

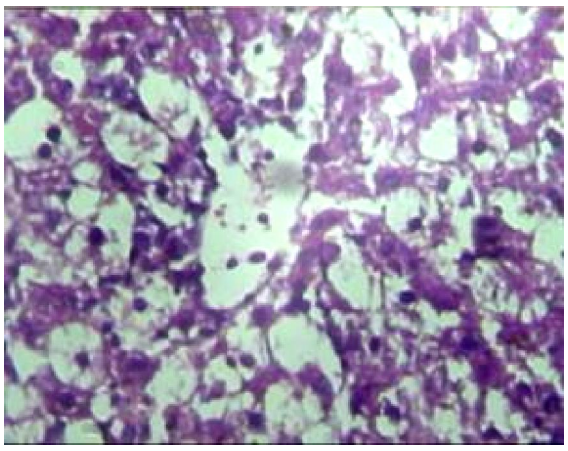


a b

c d

Figure 4: Photomicrograph of liver section of leaves group rats

a) Showing normal hepatocytes with minimum fatty changes, portal vain congestion and mild inflammatory cell infiltration around portal vain (H&E, X= 400).
b) Showing mild changes in protein content (Bromophenol blue, X= 100)
c) Showing regular distribution of glycogen (PAS, X= 100).
d) Showing varying degree of carbohydrates distribution all over the tissue with mild dilated of liver sinusoids (PAS, X= 400).



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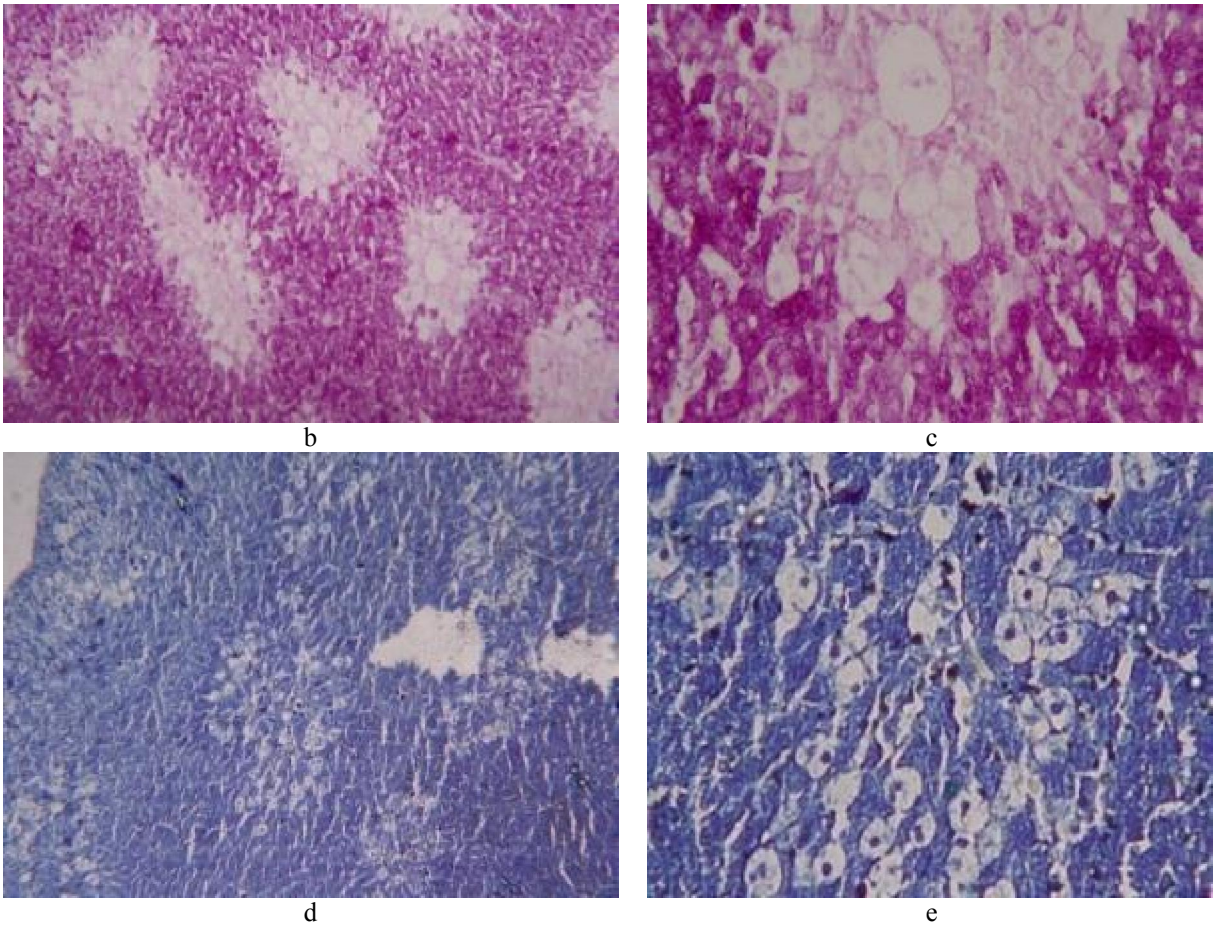
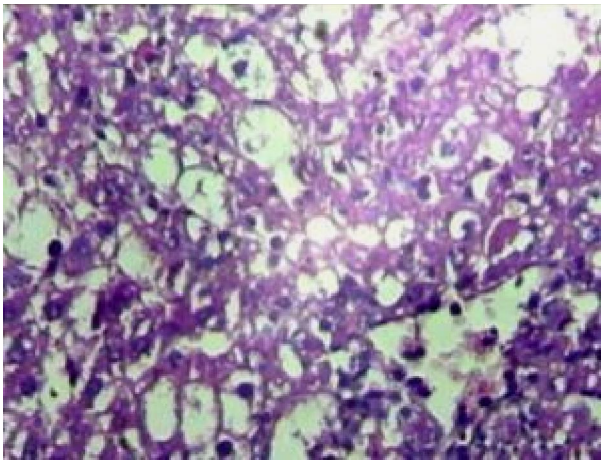


b c

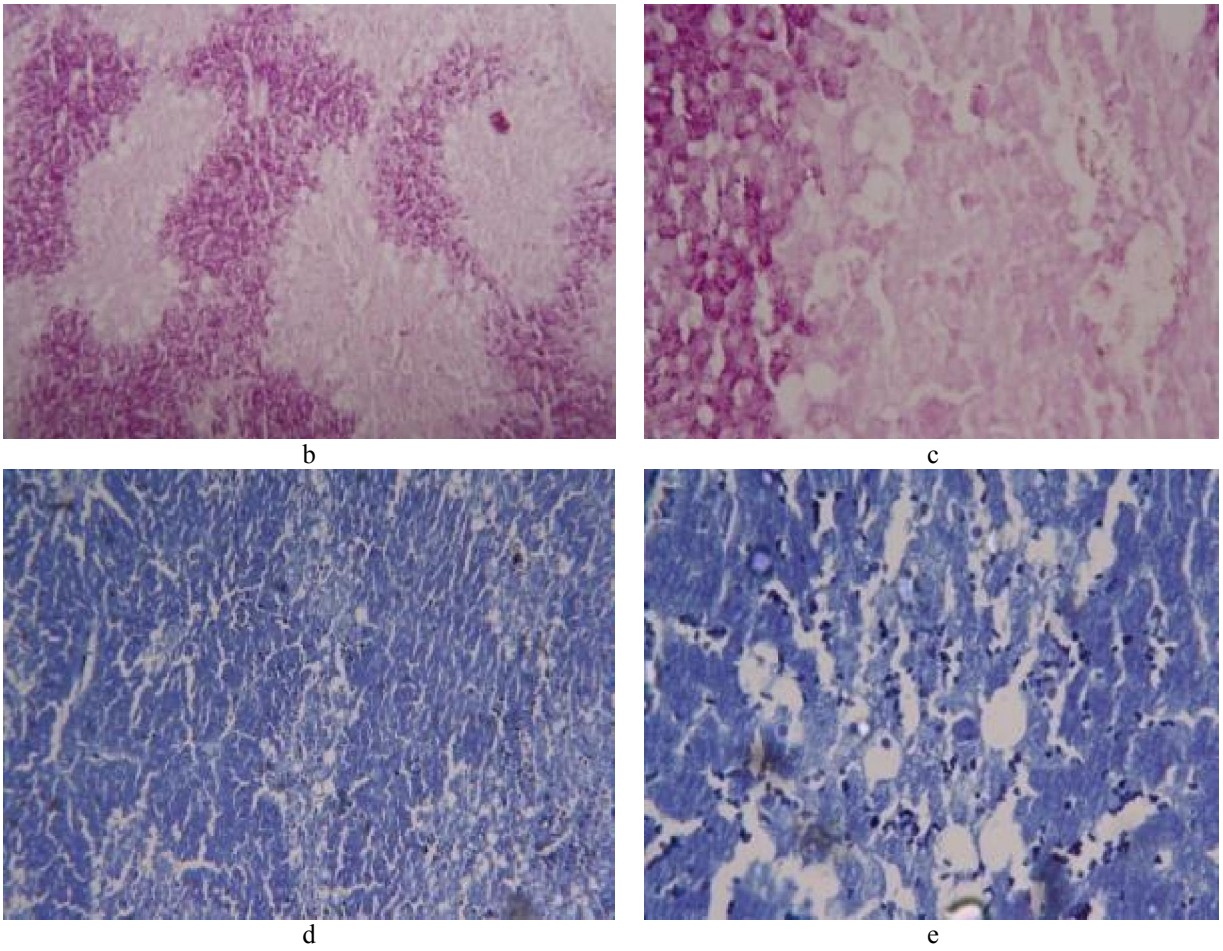
d e

Figure 5: Photomicrograph of liver section of flower group rats

a) Showed fatty and degenerative changes with inflammatory cells infiltration (H&E, X= 400).
b) Showing abnormal distribution of glycogen (PAS, X= 100).
c) Showing marked glycogen depletion or negative PAS around portal vein while hepatocytes away from portal vein loaded with glycogen (PAS, X= 400).
d) Showing abnormal distribution of protein content (Bromophenol blue, X= 100).
e) Showing some cells completely negative for bromophenol blue except nucleus which are moderately enlarged (Bromophenol blue, X=400).



a



b c

d e

Figure 6: Photomicrograph of liver section of pod group rats

a) Showed marked fatty and degenerative changes, dilated liver sinusoids, loss of cellular architecture and inflammatory cells with enlarged neucleus (H&E, X= 400).
b) Showing marked abnormal distribution of glycogen, the majority of cells are negative PAS reaction while others loaded with glycogen (PAS, X= 100).
c) Showing marked abnormal distribution of glycogen (PAS, X= 400).
d) Showing abnormal distribution of protein content (Bromophenol blue, X= 100).
e) Showing some cells completely free from protein except enlarged nucleus (Bromophenol blue, X= 400).

Antioxidant activity

Different extracts of *c. gilliesii* showed scavenging activity; where 95.3, 38.7 and 44.7 μg mL^{-1} were IC_{50} of leaves, flowers and pods respectively. From this results, flowers extract had the lowest concentration of extract needed to reduce free radicals by 50% (IC_{50} = 38.66 μg mL^{-1}) (Figure 7). Ethyl acetate fraction of flowers presented the lowest IC_{50} = 7.43μgmL^{-1} and this value was statistically lower than rutin. According to Melo J. G *et al.,* 2010, antioxidant activity can be classified based on the performance of the extract into good activity (IC_{50}<72 μgmL^{-1}, up to three times the inhibitory concentration of the standard); moderate activity (72 μgmL^{-1}< IC_{50}< 168 μgmL^{-1}, between three and seven times the inhibitory concentration of the standard) and low activity (IC_{50}> 168 μgmL^{-1}, exceeding seven times the inhibitory concentration standard). Using this classification, all flower extracts showed good activity except hexane fraction showed moderate activity (Figures 8, 9).

Our results showed that almost different flowers extracts of *C. gilliesii* showed radical scavenging capacity and can be used to inhibit the oxidation of vital substances by reactive oxygen species. Some studies have suggested that extracts or compounds that exhibit activity against the DPPH free radical can be considered as primary antioxidants (Fukumoto R. and Mazza, G. 2000, Almey A. *et al.,* 2010). That activity may contributed to presence of phenolic compounds, such as tannins and flavonoids, that may be linked to the antioxidant activity (Cai, Y. *et al.,* 2004).

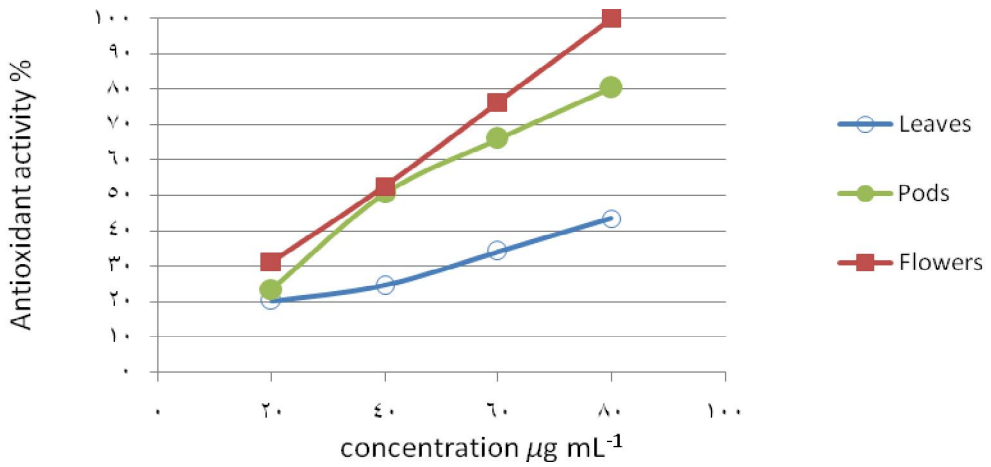


Figure 7: The chelating capacity of different aerial parts extracts of *C. gilliesii* screened *in-vitro* using DPPH radical spectrophotometric assay

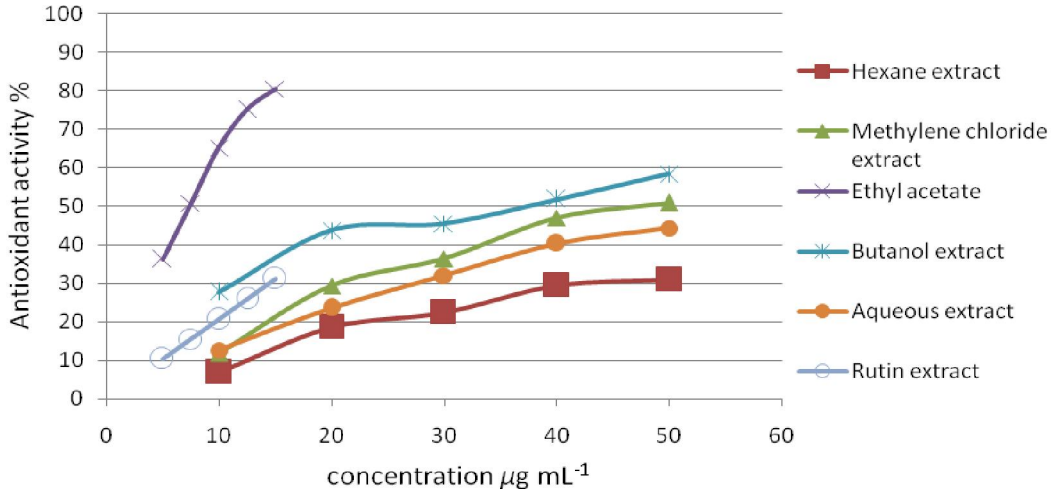


Figure 8: The chelating capacity of different flower extracts of *C. gilliesii* screened *in-vitro* using DPPH radical spectrophotometric assay

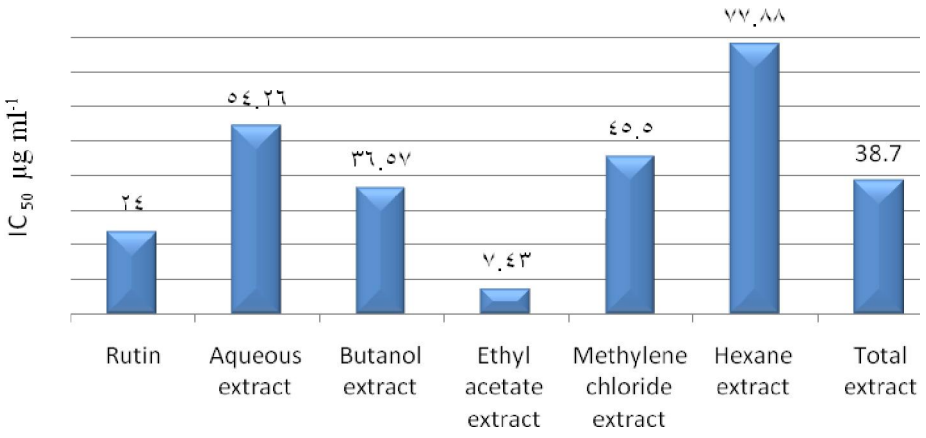


Figure 8: IC_{50} (μg ml^{-1}) values of plant extracts for free radical scavenging activity by DPPH radical. (Lower IC_{50} value indicates higher antioxidant activity)

Conclusion

The medicinal value of plants lies in some phytoconstituents that have a definite physiological action on the human body. Aligning traditional knowledge with scientific assessment through laboratory tests, *C. gilliesii* represent good candidates for the crossover from folk medicine to modern therapeutics based on their phenolic content and antioxidant activity. The aerial parts of *C. gilliesii* are rich in carbohydrates, fats, terpenoids, cardiac glycosides, cyanogenic glycosides, saponins, flavonoids, tannins and coumarins. Leaves and flowers extracts are active fractions possessing hepatoprotective activity. All flowers fractions showed good antioxidant activity. *C. gilliesii* could be a valuable alternative for obtaining compounds with great commercial potential, given its high antioxidant potential. Further studies are being conducted on this plant in order to isolate and elucidate the structure of the bioactive compounds.

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