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Hepatoprotective and antioxidant capacity of *Melochia corchorifolia* extracts

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ABSTRACT

Objective: To evaluate hepato protective and antioxidant capacity of *Melochia corchorifolia (M. corchorifolia)* aerial part extracts. **Methods:** Antioxidant activity was evaluated by using three free radicals (Superoxide, Hydroxyl and DPPH) and hepatoprotective activity was assessed against CCl_4 induced liver intoxication in rats. **Results:** The extracts produced concentration dependent percentage protection in decrease of serum enzymes and percentage inhibition on free radicals. Among all extracts methanol extract showed better activity with percentage protection of SGOT (78.98%), SGPT (79.65%), ALP (82.48%) and total bilirubin (80.0%) levels against CCl_4 liver intoxication and also methanolic extract showed better activity with IC_{50} values on superoxide, hydroxyl and DPPH radicals were 127 μg, 240 μg and 179 μg. **Conclusions:** From the results obtained during the study it could be concluded that *M. corchorifolia* aerial part extracts have antioxidant and hepatoprotective components. Further study is necessary for isolation and characterization of bioactive molecules which are responsible for hepatoprotective and antioxidant activity.

1. Introduction

Exogenous chemical and endogenous metabolic processes in the human body or in the digestive system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. The uncontrolled production of oxygen derived free radicals is involved in triggering many diseases such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis as well as in degenerative processes associated with aging and various acute and chronic liver diseases[1, 2].

Liver diseases are one of the most serious health problems in the world today but, despite tremendous advances in modern medicine, their prevention and treatment options still remain limited. Recently, the most common *in vivo* model used in the investigation of new hepato protective agents has been a well-characterized rodent model of liver injury induced by carbon tetrachloride (CCl_4), a chemical hepato toxin that causes a free radical -mediated hepato cellular damage[3, 4]. Hepatic damage induced by CCl_4 resulted in an increase in Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase, Alkaline Phosphatase and Total bilirubin concentrations. The elevation of concentrations of serum enzymes such as SGOT and SGPT is generally regarded as one of the sensitive markers of hepatic damage[6, 7] and also it has been reported that CCl4 intoxication results in the peroxidation of lipids and lipid membranes of rats observed an increase in lipid peroxidation (LPO) as a result of CCl_4 treatment. Effectively, herbal products are widely used in the treatment of hepatic disorders all over the world[8]. Hepatoprotective studies by many researchers on medicinal plants reported that plants have active ingredients that are capable of free radical scavenging in living systems (Hepato protective)[10, 11].

The *Melochia corchorifolia* (Chocolate Weed) *(M. corchorifolia)* is an annual or perennial type of herb, typically seen in the wastelands. *M. corchorifolia* have been traditionally utilized for several remedies. For example, leaves were used to reduce ulcers, abdominal swelling, headache, snakebites and chest pains. *M. corchorifolia*

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contain several compounds Cyclopepetide alkaloids franganine, frangufoline[12], Melofoline, Melochicorine and several other constituents[13, 14], 5, 7 Hydroxy flavones, apigenin, kaempferol and quercetin[15]. Earlier there were no reports on biological activities of *M. corchorifolia*. Now, the present study was aimed to evaluate Hepato protective and antioxidant activities of *M. corchorifolia* aerial parts.

2. Materials and methods

2.1. Drug and chemicals

Silymarin, Carbon tetrachloride (CCl_4) and 1, 1- diphenyl-2-picrylhydrazyl was purchased from Sigma chemicals, USA. Nitroblue tetrozolium was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. Riboflavin was purchased from Loba Chemie Pvt Ltd., Bombay. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), Serum Total bilirubin (T.Bil) kits were purchased from Span diagnostics Ltd, Gujarat, India. All other chemicals used were of analytical grade.

2.2. Collection of plant material and preparation of extracts

M. corchorifolia plant was collected from Jagityala, Andhra Pradesh. The aerial parts were separated, dried under shade and powdered. The coarse powder was extracted with 70% v/v ethanol, methanol, ethyl acetate and hexane separately in a Soxhlet apparatus. The liquid extracts were filtered and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and then four extracts were used for further investigation.

2.3. Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250 g were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of (23±2) ℃ having (50±5) % relative humidity with 12 h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. Acute toxicity studies

Acute toxicity study was conducted according OECD Guide lines No.423. After fasting overnight, mice were administered with extracts of *M. corchorifolia* in a single dose up to the highest dose of 2 000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms or mortality.

2.5. Phytochemical analysis

Phytochemical studies were carried out for hexane, ethyl acetate, hydro alcoholic and methanol extracts of *M. corchorifolia* aerial parts to detect the presence of different phytochemical constituents like steroids, terpenoides, tannins, flavanoids, saponins, glycosides, amino acids etc by using standard procedures[16, 17].

2.6. Quantification of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteau reagent[18]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765 nm. The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/g (GAE).

2.7. Quantification of total alkaloid content

Total alkaloid content was determined by the Fazel *et al*., method[19]. The plant extract (1 mg/mL) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One mL of this solution was transferred to a separating funnel and then 5 mL of BCG solution along with 5 mL of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. By using standard atropine calibration curve, measured the concentration of alkaloid content in atropine equivalents using unit's mg/g.

2.8. In vitro anti oxidant activity

For the assessment of free radicals scavenging activity, hexane, ethyl acetate, Ethanol (70%v/v) and methanol extracts were dissolved in dimethyl sulphoxide (DMSO) respectively.

2.8.1. Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich method[20], which depends on light induced superoxide generation by

riboflavin and the corresponding reduction of nitroblue tetrazolium.

2.8.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances[21]. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

2.8.3. DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*.,[22]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity[23].

2.9. Assessment of hepatoprotective activity against CCL_4 induced liver intoxication

Carbon tetrachloride intoxication in rats is an experimental model widely used to study necrosis and statues of liver[24, 25]. The animals were divided in to XV groups, each consisting of 6 animals. The vehicle, standard and test group animals were treated with 5% gum acacia, 50 mg/kg dose of silymarin and 125 mg/kg, 250 mg/kg and 500 mg/kg doses of 70% ethanol, methanol, ethyl acetate and hexane extracts of *M. corchorifolia* for 5 days. On 6th day, 1 h after treatment with standard and test doses, the animals were intoxicated with CCl_4: liquid paraffin (1:1) (1 mL/kg, *p.o.*). On 7th day the blood samples were collected and analyzed for biochemical parameters like serum enzymes, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) were estimated by Reitman and Frankel, 1957 method, Serum Alkaline Phosphatase (ALP) by King and Armstrong, 1980 method and Serum Total bilirubin (T.Bil) by Jendrassik and Grof, 1938 method by using commercial reagent kits in Autoanalyzer (RM4000, Biochemical systems International, Italy)[26-28].

2.9.1. Statistical analysis

Data was analyzed by using One-Way ANOVA followed by post hoc Dunnett's test using Graph pad Prism-5 software. The results are expressed as Mean±S.E.M. $P<0.05$ was considered as significant.

3. Results

3.1. Phytochemical analysis

Qualitative phytochemical screening of *M. corchorifolia* aerial part extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavanoids, alkaloids, glycosides, tannins and carbohydrates. The extracts gave negative results for the amino acids, oils, quinines and saponins. All extracts revealed the presence of phenols, alkaloids, carbohydrates, steroids and glycosides and gave negative results to amino acids, saponins, quinines and oils. The ethanol (70%), methanol and ethyl acetate extracts reveals the presence of terpenoids, flavanoids and tannins but the hexane extract gave negative results.

3.2. Quantification of total phenolic and alkaloid contents

The Quantified phenolic contents of *M. corchorifolia* aerial part extracts were ranging from (16.28±0.52) to (34.22±0.43) mg/g. The methanol extract have more phenolic content i.e. (34.22±0.43) mg/g than other extracts and the alkaloid content was ranging from (18.46±0.34) to (26.37±0.16) mg/g. The methanolic extract has more alkaloid content i.e. (26.37 ±0.16) mg/g than other extracts. The results were shown in Table 1.

Table 1
Total phenolic and alkaloid content (mg/gm) of M. corchorifolia aerial parts extracts.

S.no	Name of the extract	Total phenolic content (mg/g)	Total alkaloid content (mg/g)
1	Hexane	16.28±0.52	18.46±0.34
2	Ethyl acetate	24.69±0.47	20.58±0.43
3	Methanol	34.22±0.43	26.37±0.16
4	Hydro alcoholic (Ethanol 70%)	29.34±0.69	21.86±0.39

3.3. In vitro anti oxidant activity

In the present study, hydro-alcoholic, methanol, ethyl acetate and hexane extracts of *M. corchorifolia* were found to possess concentration dependent scavenging activity on tested free radicals (superoxide, hydroxyl and DPPH). The mean IC_{50} values for superoxide radical of hydro-alcoholic, methanol, ethyl acetate and hexane extracts of *M. corchorifolia* were found to be 206 μg, 127 μg, 530 μg and 901 μg respectively. The mean IC_{50} values for hydroxyl radical of hydro-alcoholic, methanol, ethyl acetate and hexane extracts of *M. corchorifolia* were found to be 384 μg, 240 μg, 490 μg and 501 μg respectively. The mean IC_{50} values for DPPH radical of Hydro-alcoholic, methanol, ethyl acetate and hexane extracts of *M.corchorifolia* were found to be 286 μg, 179 μg, 470 μg and 971μg respectively. The mean IC_{50} values of ascorbic acid were found to be 59.3 μg, 66 μg and 16 μg.

The selected plant extracts were produced concentration dependent percentage inhibition of superoxide radical and produced maximum activity at concentrations of 160 and 320 μg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. Among the four extracts of *M. corchorifolia*, the methanolic extract showed better activity than other extracts on the tested three free radicals. The order of activity in the following manner: Ascorbic acid >methanol extract >Hydro-alcoholic extract > ethyl acetate extract> hexane extract.

3.4. Hepatoprotective activity

The CCl_4-induced hepatotoxicity model is extensively used to evaluate the hepato protective effects of drugs and plant extracts. The hepato protective effect of Ethanol 70%, Methanol, Ethyl Acetate and hexane extracts of *M. corchorifolia* aerial parts at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg assessed by measuring liver related biochemical parameters (SGOT, SGPT, ALP and total serum bilirubin) following CCl_4-induced hepatotoxicity. The percentage protection produced by the standard drug and extracts were calculated based on reduction of SGOT, SGPT, ALP and total serum bilirubin levels on 7th day of experiment in each case and the results were given in Table 2. In our studies, CCl_4-damaged rats that were previously treated with extracts showed a significant decrease in serum GOT, GPT, ALP and T. bilirubin. This is evidence that both stabilization of the plasma membrane and repair of $CC1_4$-induced hepatic tissue damage. The standard drug silymarin and higher dosages of extracts showed a strong hepato protective effect against CCl_4-induced liver injury.

Group showed no significant change in the biomarkers of enzymes (SGOT, SGPT, ALP and total serum bilurubin). Group II was treated with CCl_4, there is increase in SGOT, SGPTT, ALP, total serum bilurubin levels. Group III was treated with Silymarin, at a dose of 50 mg/kg and after one hour followed by CCl_4 intoxication, produces increase in SGOT, SGPT, ALP and total serum bilirubin levels and the percentage protection offered by the sylmarin against the increase in SGOT, SGPT, ALP, and total seru bilurubin levels 93.55%, 94.32%, 89.04% and 80% respectively.

Groups IV, V and VI was treated with hydroalcoholic extract of *M. corchorifolia* orally at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication produced increase in SGOT, SGPT, ALP and total serum bilirubin levels. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 31.87%, 37.65%, 57.48% and 40.0%, 52.30%, 48.24%, 69.29%, and 60.00%, 74.91%, 70.24%, 78.46% and 80.00% respectively.

Groups VII, VIII and IX was treated with *M. corchorifolia* methanolic extract orally at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication produced increase in SGOT, SGPT, ALP and total serum bilirubin levels. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 44.65%, 40.06%, 57.95% and 50.0%, 57.86%, 56.47%, 64.65% and 70.00%, 78.98%, 79.65%, 82.48% and 80.00% respectively.

Groups X, XI and XII received *M. corchorifolia* ethyl acetate extract orally at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg and after one hour followed by CCl4 intoxication produced mild increase in SGOT, SGPT, ALP and total serum bilirubin levels. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 27.13%, 27.41%, 54.41% and 50.0%, 44.14%, 50.35%, 62.52% and 60.00%, 60.75%, 66.82%,

Table 2
Enzymes levels due to the effect of vehicle, CCl_4, silymarin and *M. corchorifolia* extracts at different doses in 7th day of experiment.

Name of the extract	Amount of the extract											
	125 mg				250 mg				500 mg			
	SGOT (U/L)	SGPT(U/L)	ALP(U/L)	Total bilirubin (mg/dL)	SGOT (U/L)	SGPT(U/L)	ALP(U/L)	Total bilirubin (mg/dL)	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Total bilirubin (mg/dL)
Vehicle (5% gum acacia)	96.17±2.85	56.00±1.46	217.50±1.06	0.17±0.01	96.17±2.85	56.00±1.46	217.50±1.06	0.17±0.01	96.17±2.85	56.00±1.46	217.50±1.06	0.17±0.01
CCL_4	194.30±2.73	141.00±1.88	471.50±12.60	0.27±0.00	194.30±2.73	141.00±1.88	471.50±12.16	0.27±0.00	194.33±2.73	141.00±1.88	471.50±12.16	0.27±0.00
Silymarin (50 mg/kg)	102.50±1.61	60.83±1.08	245.33±2.70	0.17±0.01	102.50±1.61	60.83±1.08	245.33±2.70	0.17±0.01	102.50±1.61	60.83±1.08	245.33±2.70	0.17±0.01
Ethanol (70%)	163.00±0.93	109.00±0.36	325.50±1.28	0.23±0.01	142.70±0.76	100.00±0.45	295.50±1.06	0.21±0.01	120.80±0.60	81.30±0.49	272.20±0.75	0.19±0.00
Methanol	150.50±0.76	102.70±0.49	324.30±0.61	0.22±0.00	137.50±0.67	93.00±0.68	307.30±0.71	0.21±0.00	116.80±1.08	73.30±1.02	262.00±0.68	0.19±0.01
Ethyl Acetate	167.70±0.51	117.70±0.61	333.30±0.90	0.22±0.00	151.00±0.33	98.20±0.50	312.70±1.05	0.21±0.00	134.70±0.56	84.20±0.44	281.30±0.90	0.20±0.00
Hexane	173.30±0.99	22.20±0.98	343.20±1.01	0.23±0.00	153.00±0.68	111.00±0.76	323.70±1.00	0.21±0.00	139.50±0.56	98.30±0.71	293.20±1.08	0.20±0.01

All groups were compared with CCl_4 group. Values are mean±S.E.M., n = 6 animals per group. Values in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection. The percentage of the protection is calculated as 100 × (values of CCl_4 −values of sample)/ (values of CCl_4 control − values of vehicle).

74.88% and 70.00% respectively.

Groups XIII, XIV and XV received *M. corchorifolia* Hexane extract orally at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication produced mild increase in SGOT, SGPT, ALP and total serum bilirubin levels. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 21.42%, 22.11%, 50.5% and 40.00%, 39.02%, 36.04%, 55.98% and 50.00%, 53.42%, 50.23%, 70.19% and 70.00% respectively. All the results were given in Table 2, Figures 1, 2 and 3.

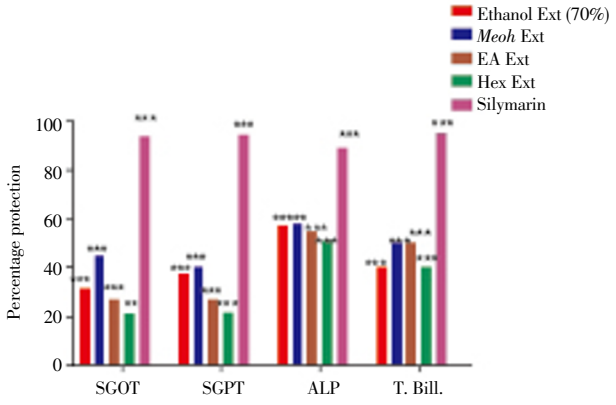

Figure 1. Percentage protection produced by different extracts of *M. corchorifolia* at a dose of 125mg/kg.

***P<0.001. Results were analysed by using two-way ANOVA followed by Bonferroni post-hoc test. All gropus were copmared with Silymarin group. Ethanol (70%): Hydro-alc.extract, MeOh Ext: Methanolic extract; EA Ext: Ethyl acetate extract; Hex Ext: Hexane extract.

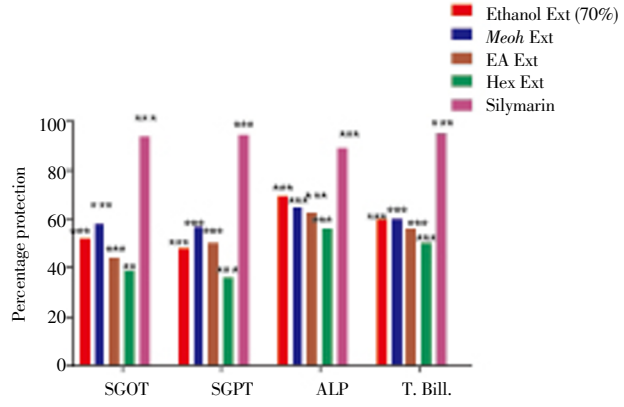

Figure 2. Percentage protection produced by different extracts of M. corchorifolia at a dose of 250 mg/kg.

***P<0.001. Results were analysed by using Two-way ANOVA followed by Bonferroni post-hoc test. All gropus were copmared with Silymarin group. Ethanol (70%): Hydro-alc.extract, MeOh Ext: Methanolic extract; EA Ext: Ethyl acetate extract; Hex Ext: Hexane extract.

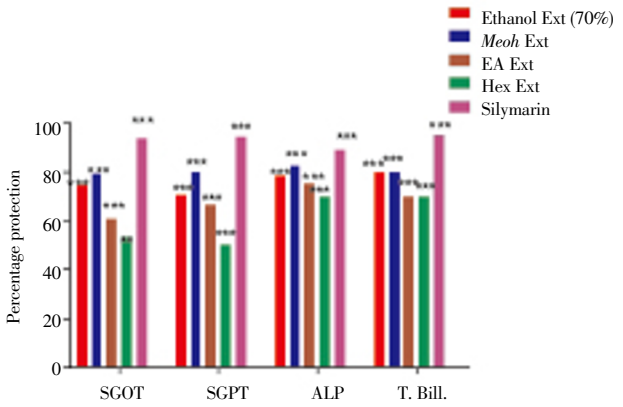

Figure 3. Percentage protection produced by different extracts of *M. corchorifolia* at a dose of 500 mg/kg.

***P<0.001. Results were analysed by using two-way ANOVA followed by Bonferroni post-hoc test. All gropus were copmared with Silymarin group. Ethanol (70%): Hydro-alc.extract, MeOh Ext: Methanolic extract; EA Ext: Ethyl acetate extract; Hex Ext: Hexane extract.

The order of hepatoprotective activity of *M. corchorifolia* based on SGPT (ALT) levels is as follows:

Silymarin (50 mg/kg)(93.55%) > Methanolic extract (500 mg/kg) (79.65%) > Hydro-alcoholic extract (500 mg/kg)(70.24%) > Ethyl acetate extract (500 mg/kg)(66.82%) > Hexane extract (500 mg/kg)(50.23%).

4. Discussion

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent biological (pharmacological) activities, low toxicity and economic viability.

Oxidation-one of the body's natural chemical processes-can produce "free radicals," which are highly unstable molecules that can damage cells. For example, free radicals are produced when the body breaks down foods for use or storage. They are also produced when the body is exposed to tobacco smoke, radiation, and environmental contaminants. Free radicals can cause damage, known as "oxidative stress," which is thought to play a role in the development of many diseases, including Alzheimer's disease, cancer, eye disease, heart disease, Parkinson's disease, and rheumatoid arthritis. Reducing power, reflecting the electron donation capacity, is one of the most important indicators of antioxidant activity of bioactive compounds[29]. By donating electrons, antioxidant substances are able to block radical chain reaction by converting reactive oxygen species to more stable products. The health-promoting effect of antioxidants from plants is thought to arise from their protective effects

by counteracting reactive oxygen species (ROS)[30].

Numerous natural products are effective antioxidants, and many medicinal plants with a long history of use in folk medicine in different countries against a variety of diseases have turned out to be rich sources of antioxidants[31-33]. Recently, many researchers have taken a great interest in medicinal plants for their phytochemical constituents and related total potential biological activities including antioxidant activity[34, 35]. It is reported that some medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity.

Various reactive oxygen species, they can damage a wide range of essential biomolecules such as amino acid, protein, and DNA in human body. Therefore, it would be of great significance to discover some compounds with good free radical scavenging activity for the oxidative stress induced diseases. The present studies have shown that the extracts of M. corchorifolia have free radicals scavenging ability. Among all the extract of plants the methanol extract showed the better percentage inhibition on free radicals.

Carbon tetrachloride (CCl_4) a known hepatotoxicant produced rise in biomarker enzymes and serum bilirubin by three folds clearly indicated that the liver damage produced by the intoxicant. Administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of CCl_4. In the present study the extracts (70% ethanol, methanol, ethyl acetate and hexane) of *M. corchorifolia* showed significant hepato protective activity against CCl_4 intoxication, which was comparable with standard drug silymarin. The decrease in the SGOT, SGPT, ALP and TB levels produced by the higher dose (500 mg/kg) of the extracts was comparably similar with the silymarin. The protection offered by the plant extracts may be due to the stabilization of membrane of the hepatocytes and by scavenging the free radicals or by both mechanisms. Among all extracts methanol extract produced significant activity compared to other extracts.

The plant extracts give the positive results for different phytochemical compounds such as phenols, alkaloids, steroids, glycosides, flavanoids, tannins etc., in the qualitative phytochemical screening. In the quantification of total phenolic and alkaloid contents the methanolic extract contain more amount. The results of the present study indicated that different extracts of *M. corchorifolia* possess antioxidant and hepatoprotective properties and this may be by the presence of important antioxidative factors like phenolic, alkaloid and flavanoids compounds[36-42].

Therefore, further studies along these lines would be worthwhile for isolation and characterization of the common constituents of all extracts of the *M. corchorifolia* and screening of the pharmacological action for the isolated compounds to identify an efficient antioxidant and hepato protective drugs.

Conflict of interest statement

The authors state no conflict interests.

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