Original Article

Evaluation Of Antihyperlipidemic And Phytochemical Screening Of Vitex Trifolia Linn And Corchorus Trilocularis In Rat

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Abstract

Aim & Objective: Effect of Antihyperlipidemic and Phytochemical Screening of *Vitex Trifolia Linn* and *Corchorus Trilocularis* In Rat

Material & Method: Fresh leaves (immature, mature and senescence) were collected, air dried and the crude powder was prepared for phytochemical and antioxidant analysis. The powder was mixed with 70% methanol and the supernatant was separated. The antioxidant activity of this ethanolic extract was measured on the basis of the scavenging activity 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical with slight modifications. Phytochemical analysis was performed according to standard laboratory protocol. Screening of antihyperlipidemic was done by using *Corchorus trilocularis* and *Vitex trifolia linn.* Leaves part extracts used for antihyperlipidemic activity leaves (200mg/kg and 400mg/kg). Hyperlipidemia was administered in experimental animals using high fat diet. In order to collect blood for high fat diet-induced hemolysis, heart punctures were performed after 14 days hyperlipidemia. Antihyperlipidemic activity of *Corchorus trilocularis* and *Vitex trifolia linn* plant extracts were evaluated by estimation of lipid profiles and standard drug Atorvastatin for 14 days.

Results: The results indicated the presence of different phytochemicals viz. glycosides, steroids, cholesterol, alkaloids, phenols, flavonoids, riboflavin, saponins and terpenoids. The sample also showed antioxidant activity by inhibiting DPPH radical. The significant antioxidant activity of methanolic leaf extract of might be due to the presence of saponins, phenols, flavonoids and alkaloids found in the preliminary phytochemical analysis. Estimation of lipid profile shows that *Corchorus trilocularis* and *Vitex trifolia linn* leaves extract (200mg/kg and 400mg/kg) shows the less significant antihyperlipidemic activity. It decreases TC, TG, LDL, VLDL and increases HDL levels. *Corchorus trilocularis* and *Vitex trifolia linn* leaves extract 200mg/kg and 400mg/kg shows significant antihyperlipidemic activity as standard drug Atorvastatin.

Conclusion: Present study reveals that the jute leaf possesses different phytonutrients and exhibited DPPH radical scavenging activity, and therefore, may be used for therapeutic purposes According to the phytochemical screening it reveal that the hyperlipidemic effects of *Corchorus trilocularis* and *Vitex trifolia linn* (200mg/kg and 400mg/kg) are similar to the effect of standard drug Atorvastatin.

Key-Words: *Corchorus trilocularis* and *Vitex trifolia linn,* High Fat Diet x-100, Atorvastatin, Phtochemical Screening.

INTRODUCTION

Hyperlipidemia is defined as an elevation of lipids in plasma [1]. Several studies have showed that an intimate correlation exists between coronary heart diseases and hyperlipidemia [2 & 3], consequently a rational approach to the treatment and prevention of coronary heart diseases could be by decreasing any elevated levels of lipids in plasma [4]. For that purpose, many studies have been conducted to evaluate the potential hypolipidemic effects of synthetic and naturally occurring compounds. Triton WR-1339-induced hyperlipidemic rats are a globally accepted model used to evaluate potential hypolipidemic drugs [5]. Triton WR-1339 is a nonionic detergent that prevents catabolism of triacylglycerol-rich lipoproteins by lipo-protein lipase and is commonly used for *in vivo* determination of triacylglycerol production, and very low density lipoprotein (VLDL) secretion or clearance rate [6 & 7].

Phytochemicals are known to work as immunomodulators and may have anti-inflammatory, anticancer and antimicrobial activities. All these properties of the phytochemicals are attributed to its effective antioxidant mechanisms against the endogenously produced harmful free radicals. Our body has effective antioxidant defence systems, which constitute enzymes, such as superoxide dismutase (SOD), catalase and compounds, such as ascorbic acid, tocopherol, and glutathione [8]. But all these endogenous antioxidants are not sufficient in protecting the body against oxidative stress. Therefore, dietary supplementation through natural antioxidants in place of synthetic antioxidants is necessary for

strengthening the antioxidant system of the body by inhibiting free radical generation and thus preventing chronic diseases. Recently, much attention has been directed towards exploring natural antioxidants because they are natural products that are considered to be a safe source [9].

Corchorus Trilocularis Linn

The genus Corchorus (Tillaceae family) contains an estimated 40 to 100 species of flowering plants native to tropical and subtropical regions throughout the world. The crop 'jute' belongs to the genus Corchorus and is the most important natural fibre crop next to cotton [10]. Jute is a native plant of tropical Africa and Asia but also has been spread to Australia, South America and some parts of Europe. It has been grown extensively in India, Bangladesh, China, Myanmar and Nepal.[11]

Botanical Name: *Corchorus trilocularis Linn*. (Tiliaceae)

Kingdom: Plantae – Plants
Subkingdom: Tracheobionta – Vascularplants
Super division: Spermatophyta – Seed plants
Division: Magnoliophyta – Flowering plants
Class: Magnoliopsida – Dicotyledons
Subclass: Dilleniidae
Order: Malvales
Family: Tiliaceae – Linden family
Genus: Corchorus L. – corchorus
Species: Corchorus trilocularis L. – threelocule corchorus

Vitex Trifolia Linn

This plant is known to possess various active constituents such as essential oils, diterpenes, vitetrifolins with several pharmacological properties such as antipyretic, antibacterial, works against asthma and allergic diseases.Five compound are isolated from the fruits of *Vitex trifolia linn* and are identified to be rho-hydroxybenzoic acid, beta-sitosterol, beta-sitosterol-3-O-glucoside, casticin and, 3,6,7- trimethylquercetagetin. Several oils were extracted from the leaves of the plant that showed considerable mosquito repellent activity. The active compound identified was rotundinal, a cycloterpene aldehyde. The plant also showed considerable potential as a botanical pesticide.*Vitex trifolia linn* extracts exhibited anticancer activity on the proliferation of mammalian cancer cells, evaluated by sulforhodamine B, which is widely used in traditional Chinese medicine. The fruit extracts of this plant showed analgesic, antipyretic and anti-inflammatory activity. Flavonoids, isolated from V. trifolia exhibited bacteriostasis activity. Vitetrifoline E, isolated from V. trifolia leaves has been reported to exhibit tracheospasmolytic activity. V. trifolia exhibited antimalarial activity in the range of 10-100 × 10−6 g/mL against Plasmodium falciparum . Aqueous and ethanolic extracts of leaves of V. trifolia were investigated for hepatoprotective activity against carbon tetrachloride (CCl4) induced liver damage.[12,13,14]

Botanical Description of *Vitex trifolia linn*

Species: Trifolia
Genus: Vitex
Family: Verbenaceae/ Lamiaceae
Order: Lamiales
Class: Magnoliopsida
Phylum: Tracheophyta
Kingdom: Plantae

MATERIALS AND METHODS

Preliminary Phytochemical Screening

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures.[15]
Preliminary phytochemical screening of the hydro alcoholic extract of *Corchorus trilocularis and Vitex trifolia linn leaves* has shown the presence of alkaloids, tannins, saponins, steroids and flavanoids.

Test for alkaloids

a) Preliminary test: A 100gm of an alcoholic extract was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendroff's and Mayer's reagents. The treated solution was observed for any precipitation.
b) Confirmatory test: Five grams of the alcoholic extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10ml portions of chloroform. Chloroform extracts were combined and concentrated to about 5ml. chloroform extract was then spotted on thin layer plates.

Solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatograms and detected by spraying the chromatograms with freshly prepared Dragendroff's spray reagent. An orange or dark colored spots against a pale yellow background was confirmatory evidencefor presence of alkaloids.

Test steroidal compounds

a) Salkowski's test: 0.5g of the alcoholic extract was dissolved in 2ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown color at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).

b) Lieberman's test: 0.5g of the alcoholic extract was dissolved in 2ml of acetic anhydride and cooled well in an ice-bath. Concentrated sulfuric acid was then carefully added. A color change from purple to blue to green indicated the presence of a steroid nucleus i.e. aglycone portion of the cardiac glycosides.

Test for phenolic compounds

a) To 2ml of filtered solution of the ethanolic extract of *Corchorus trilocularis and Vitex trifolia linn* of the plant material, 3drops of a freshly prepared mixture of 1ml of 1% ferric chloride and 1ml of potassium ferrocyanide was added to detect phenolic compounds. Formation of bluish-green color was taken as positive.

b) The dried alcoholic extract (100mg) was dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of Phenolic compounds.

Flavonoids

a) Test for free flavonoids: Five milliliters of ethyl acetate was added to a solution of 0.5g of the extract in water. The mixture was shaken, allowed to settle and inspected for the production of yellow color in the organic layer which is taken as positive for free flavonoids.

b) Lead acetate test: To a solution of 0.5g of the extract in water about 1ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

c) Reaction with sodium hydroxide: Dilute sodium hydroxide solution was added to a solution of 0.5g of the extract in water. The mixture was inspected for the production of yellow color which considered as positive test for flavonoids.

• Quantitative estimation of flavonoids and polyphenols

Since the extracts showed strong positive reaction for the flavonoids and polyphenols both were estimated quantitatively.

• Estimation of total phenols

Overall phenolics present in various fractions were assessed by the Folin-Ciocalteu method (81). Two hundred µl of the diluted fraction solutions were blended with 1mlof Folin-Ciocalteu reagent (Merck, Mumbai, India) and 800 µl of saturated sodium carbonate (Himedia, Mumbai, India). The resultant mixture was kept in an incubator(Technico, Chennai, India) for 40 min at a temperature of 45°C. After incubation, theoptical densities of the resultant mixture were read at 765 nm Shimadzu UV-Vis spectrophotometer UV-1700 pharmaspec, Kyoto, Japan at room temperature (82). The same process was followed for construction of calibration curve using gallic acid at aconcentration of 0-50 µg/ml. The analyses were carried at least thrice and the resultswere denoted as gallic acid equivalent, (mg GAE/g) to dry weight of fractions and expressed as mean value ± SD.

• Estimation of total flavonoids

The quantity of flavonoids existing in various fractions was estimated by aluminiumchloride technique (83). 1 ml of solution containing 100 µg/ml of fraction was addedwith 1 ml of methanolic 2% aluminium chloride (Merck, Mumbai, India) solution. The O.D. of the solution was measured at 430 nm using Shimadzu UV-Visspectrophotometer UV-1700 pharmaspec, Kyoto, Japan at room temperature. The same process was followed for construction of calibration curve using rutin at a concentrationof 0-50 µg/ml. The analyses were carried at least thrice and the outcomes were denoted as rutin equivalent, (mg RE/g) to dry weight of fractions and expressed as mean value ± SD.

- Spectral analysis
- Identification of phytoconstituents by GC-MS

1. Test for saponins

Froth test: 0.5g of the alcoholic extract was dissolved in 10ml of distilled water in test tube. The test the test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30minute period of time. If a "honey comb" froth above the surface of liquid persists after 30min. the sample is suspected to contain saponins.

2. Test for tannins

a) Ferric chloride test: A portion of the alcoholic extract was dissolved in water. The solution was clarified by filtration. 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in color to bluish black.

b) Formaldehyde test: To a solution of about 0.5g of the extract in 5ml water, 3 drops of formaldehyde and 6 drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for l min and cooled. The precipitate formed (it any) was washed with hot water, warm alcohol and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a colored residue after washing, indicated the presence of phlobatannins.

c) Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as an evidence for the presence of phlobatannins.

d) Modified iron complex test: To a solution of 0.5g of the plant extract in five milliliter of water a drop of 33% acetic acid and 1g sodium potassium tartarate was added. The mixture was warmed and filtered to remove any precipitate. A 0.25% solution of ferric ammonium citrate was added to the filtrate until no further intensification of color is obtained and then boiled. Purple or blackish precipitates which is insoluble in hot water; alcohol or dilute ammonia denotes pyrogallol tannin present.

Test for Anthraquinones

a) Test for free anthraquinones (Borntrager's test)
The hydro-alcoholic extract of the plant material (equivalent to 100mg) was shaken vigorously with 10ml of benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.

b) Test for O-anthraquinone glycosides (Modified Borntrager's test)
For combined anthraquinones, 5g of the plant extract was boiled with 10ml of 5% sulphuric acid for 1 hour and filtrated while hot. The filtrate was shaken with 5ml benzene; the benzene layer separated and half of its own volume of 10% ammonia solution added. The formation of a pink, red or violet color in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract.

Test for carbohydrates

The extracts were treated with 3ml of alpha naphthol in alcoholic and conc. Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of two liquids indicates presence of carbohydrates.
Fehling's Test: To the sample Fehling's solution Aand B was added and heated for twominutes. Appearance of reddish-brown color indicates presence of reducing sugars.
Benedict's test: To the sample Benedict's solution was added and heated, appearance ofreddish orange precipitate indicates presence of reducing sugars.
Barfoed's Test: The sample were treated with Barfoed's reagent and heated, appearance ofreddish orange precipitate indicates presence of reducing sugars.

Test for Proteins

- Biuret's Test: To the extracts copper sulphate solution followed by sodiumhydroxide solution, a violet color precipitates indicates presence of proteins.
- Million's Test: To the extracts million's reagent was added, appearance of pink colorindicates presence of proteins.

Test for Gums and Mucilage

The extracts were treated with 25ml absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties.

Test for Glycosides

500 mg of chloroform or ethyl acetate or alcoholic fractions of both the plants was combined with 20 ml of 20 % dilute hydrochloric acid (Merck, Mumbai, India) and the resultant mixture was heated well in boiling water bath for 2 h to induce hydrolysis.
After 2 h the resultant mixture was exposed to the succeeding tests for detecting different types of glycosides A pinch of the extract were dissolved in glacial acetic acid and few drops of ferric chloride solution was added followed by the addition of Conc. Sulphuric acid, formation of red ring at the junction of the two liquids indicates presence of glycosides.

Legal's test

1 ml of hydrolysate obtained from the aforementioned treatment was blended with 1 ml of pyridine ((Merck, Mumbai, India) followed by three drops of sodium nitroprusside(Merck, Mumbai, India) solution. The solution was then appended with sodium hydroxide (Merck, Mumbai, India) to make the mixture alkaline. The resultant mixture was checked for the appearance of pink colour (Cardenolide nitroprusside complex)within 2 min, which is an indication for the presence of cardiac glycosides.

Borntrager's test

Two ml of hydrolysate was mixed with 2 ml of chloroform (Merck, Mumbai, India)and shaken manually in a separating flask. The chloroform layer was separated fromthe separating flask and was appended with dilute ammonia (10% w/w) (Merck, Mumbai, India). The resultant mixture was investigated for the formation of pink colour (anthraquinone derivatives) in the ammonia layer immediately is an indication for theexistence of anthraquinone glycosides.

Test for cyanogenic glycosides

100 mg of plant extracts were added seperately with 2 ml of chloroform or ethanol and kept in a closed tube using a stopper. A small piece of filter paper impregnated withsodium picrate (Whatmann filter paper grade 1 (5.0 X 1.5 cm) were soaked in an aqueous solution of 0.05 M picric acid, previously neutralized with sodium bicarbonate,and filtered. The impregnated paper was left to dry at ambient temperature) was hungwith the help of a cork and incubate

at ambient temperature for 2 h. A colour change of the sodium picrate paper from yellow to brownish red (release of hydrogen cyanide)indicates the presence of cyanogenic glycosides.

Phytosterols

Two hundred mg of various fractions was added with 10 ml of suitable solvent and was subjected to Liebermann's test, Liebermann-Burchard test and Salkowski test in seperate test tubes.

Liebermann's test

The solution was added with 0.5 ml of concentrated sulphuric acid (Merck, Mumbai,India) and 4 drops of saturated solution of sodium nitrate (Merck, Mumbai, India). The formation of red colour on dilution (condensation of nitrosophenol and phenol to indophenol) and when added with 10% sodium hydroxide (Merck, Mumbai, India) solution turning to blue indicates the presence of phytosterols.

Liebermann-Burchard's test

To 1 ml solution containing the fractions of plants was added with 2 ml of Liebermann- Burchard reagent (Acetic anhydride (Merck, Mumbai, India): 95-97% Sulphuric acid,(Merck, Mumbai, India) (1:1). The formation of purple colour progressing to green (conjugation of unsaturation in the fused ring) indicates the formation of phytosterols.

Salkowski's test

To 5 ml of the solution containing the fractions of plants was added with 3 ml of chloroform (Merck, Mumbai, India) and 3 ml of 95-97% Sulphuric acid in a test tube.The test tube was noticed for the formation of brownish red colour (sulfination of bicholestadiene) immediately in the lower layer which is an indication for the existence of phytosterols.

Test for Terpenes

The extracts were treated with tin and thionyl chloride, appearance of pink color indicates presence of terpenes.

Animal study

Healthy wister rats of either sex (both male and female) weighing about 150-200g used for the study. The animals were caged individually and kept in air conditions room at temperature of 22±2^{0}C with 50%±10% relative humidity with 12hrs light and dark cycle. Throughout the study, Animals were maintained at normal laboratory conditions. Animals were maintained at standard rat pellet diet and pure drinking water *ad libitum.* Animals were acclimated to laboratory conditions 7 days prior to initiation of experiments. The Experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) and all the experiments were carried out according to guidelines of committee for the purposeof control and supervision of experiments on animals, India.

Acute toxicity studies

Wister Albino female rats weighing 150-200g were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines (acute toxic class method). The rats were fasted overnight and the weight of each rat was recorded just before the experiment. Animals were divided randomly into four groups each group consisting of two rats. Each group received orally the 70% hydroalcoholic extract of all three medicinal plant leaves in a dose of 50, 300, 1000 and 2000 mg/kg according to OECD-423 guidelines. Animals were kept under close observation for 4 hours after administering the extract, and then they were observed daily for seven days for any change in general behavior and/or other physical activities.

Atherogenic Diet

Diet Components	5 kg.	10 kg.
Dairy Butter	922.5 g.	1845 g.
Corn Oil	100 ml.	200 ml.
Sucrose	2500 g.	5000 g.
Cellulose (Alphacel)	203.5 g.	407 g.
Casein-Vit. Free	1000 g.	2000 g.
Cholesterol	48 g.	96 g.
Sodium Cholate (Cholic Acid)	25 g.	50 g.
Ain 76 Mineral Mix	250 g.	500 g.
Ain 76 Vitamin Mix	50 g.	100 g.
DL-Methionine	15 g.	30 g.
DL-a-Tocopheol	6.5 g.	13 g.
Choline Chloride (0.5 gr./ml.)	100 ml.	200 ml.
Distilled Water	+/- 175 ml.	+/- 350 ml.

EXPERIMENTAL ANIMALS

Wistar rats weighing 130-165g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee, Patel College of Pharmacy, Madhyanchal University, Bhopal, Madhya Pradesh, India.

Blood Collection

Blood samples were withdrawn from the retro orbital venous plexus of rats for the separation of serum at the end of treatment schedule. After collection the blood in the micro- centrifuge tubes, kept aside for 20min at room temperature and then serum was isolated by centrifugation at 2500 rpm for 15 min and stored until analyzed for biochemical estimation oflipid parameters includes Serum Total Cholesterol, Triglycerides, and HDL-cholesterol.

Screening Models

1: High Fat Diet-induced hyperlipidemic model

The animals were selected, weighed then marked for individual identification. Rats were made hyperlipidemic by the oral administration of atherogenic diet for 20 days. The rats were then given plant extracts suspended in 2% acasia at the dose of 200mg/kg b.w. once daily in the morning through gastric intubation for 14 consecutive days.

During these days, all the groups also received atherogenic diet in the same dose as given earlier. The control animals received the hyperlipidemic diet and the vehicle. At the end of treatment period, the animals were used for various biochemical parameters. Blood was collected by heart puncturing of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum.[16]

EXPERIMENTAL DESIGN

High Fat Diet Induced Hyperlipidemic model

Animal Grouping:

Rats were divided into Seven groups (n = 6 for each group).

Control: DMSO

Standard: Atorvastatin (10 mg/kg)

1. Group I (Negative Control) receive vehicle
2. GroupII (Positive Control) receive High Fat Diet and vehicle
3. Group III receive standard drug (Atorvastatin 10 mg/kg)
4. Group IV Test group (CTAE, dose 200 mg/kg)
5. Group V Test group (CTAE, dose 400 mg/kg)
6. Group VI Test group (CTET, dose 200 mg/kg)
7. Group VII Test group (CTET, dose 400 mg/kg)
8. Group VIII Test group (VTAE, dose 200 mg/kg)
9. Group IX Test group (VTAE, dose 400 mg/kg)
10. Group X Test group (VTET, dose 200 mg/kg)
11. Group XI Test group (VTET, dose 400 mg/kg)

Table 1. Groups & Doses schedule for High Fat Diet-induced hyperlipidemic model

SL.NO	GROUP	TREATMENT	ANIMAL USED
1	I	Receive vehicle	6
2	II	2% acacia + High Fat Diet diet for 20 days	6
3	III	positive control received standard drug atorvastatin (10mg/kg/ day p.o.) for 14 days	6
4	IV	Aq. Extract of CT (200mg/kg/day) fine suspension of 2% acasia + High Fat Diet Diet for 14 days	6
5	V	Aq. Extract of CT (400mg/kg/day) fine suspension of 2% acasia + High Fat Diet diet for 14 days	6
6	VI	Ethanolic Extract of CT (200mg/kg/day) fine suspension of 2% acasia + High Fat Diet Diet for 14 days	6
7	VII	Ethanolic Extract of CT (400mg/kg/day) fine suspension of 2% acasia + High Fat Diet Diet for 14 days	6
8	VIII	Aq. Extract of VT (200mg/kg/day) fine suspension of 2% acasia + High Fat Diet Diet for 14 days	6
9	IX	Aq. Extract of VT (400mg/kg/day) fine suspension of 2% acasia + High Fat Diet Diet for 14 days	6
10	X	Ethanol Extract of VT (200mg/kg/day) fine suspension of 2% acasia + High Fat Diet diet for 14 days	6
11	XI	Ethanol Extract of VT (200mg/kg/day) fine suspension of 2% acasia + High Fat Diet diet for 14 days	6

Statistical Analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out using One-way ANOVA followed by Tukey test with the help of Graph pad instant software. Values of $P<0.05$ were considered statistically significant.

RESULT & DISCUSSION

Table 2. Preliminary phytochemical qualitative screening of differentextracts of CT, VT

Plants/Extracts		Flav	Alka	Carb	Sap	Tan	Gly	Ster	Ttr
CT	CT-PE	+	-	+	+	+	-	+	-
	CT-CH	+	-	+	+	+	+	+	-
	CT-EA	+	-	+	+	+	+	+	-
	CT-AE	+	-	+	+	+	+	+	-
VT	VT-PE	+	+	-	+	-	-	+	+
	VT-CH	+	+	-	+	-	-	+	+
	VT-AE	+	+	-	+	-	+	+	+
	VT-ET	+	+	+	+	+	+	+	+

(-)-absence of phytoconstituents, (+)-presence of phytoconstituents; **Flav**- flavonoids; **Alka**- Alkaloids; **Carb**-Carbohydrates; **Sap**-Saponins; **Tan**-Tannins; **Gly**- glycosides; **Ster**-Steroids; **Ttr**- Triterpenoids; **CT**- Chorchorus Tricularis; **VT**- Vitex Trifolia

High Pressure Liquid Chromatography (HPLC) analysis of CT, VT extracts

Table 3. High Pressure Liquid Chromatography (HPLC) analysis of *Corchorus trilocularis* leaves extracts

Phytoconstituents	Retention Time (min)				
	Std.	CT-PE	CT-CH	CT-ET	CT-AE
Caffeic acid	3.87	ND	ND	ND	ND
Gallic acid	3.83	3.88	3.89	3.89	3.89
Quercetin	6.76	6.78	6.73	6.62	6.78
Rutin	3.82	3.82	3.82	3.82	3.90
p-hydroxy cinnamic acid	6.89	ND	ND	ND	ND

CT- *Corchorus Trilocularis* leaves extract**, PE**- Petroleum Ether (60-80°C), **CH**-Chloroform, **ET**- Ethanol, **AE**-Aquous Extract, **Std.**-Standard, **ND**-Not Detected

From the above results, HPLC fingerprinting showed same retention time as that of standard gallic acid (3.83), quercetin (6.76) and rutin (3.82), CT-PE shown (3.88, 6.78 and 3.82), CT-CH (3.89, 6.73 and 3.82), CT-ET(3.89, 6.62 and 3.82) and CT-AE (3.89, 6.72, 3.90) respectively while caffeic acid and *P*- hydroxy cinnamic acid be found absent in CT-PE, CT-CH, CT-ET and CT-AE.

Table 4. High Pressure Liquid Chromatography (HPLC) analysis of *Vitex trifolia linn* leaves extracts

Phytoconstituents	Retention Time (min)				
	Std.	VT-PE	VT-CH	VT-AE	VT-ET
Caffeic acid	3.87	3.82	3.82	3.82	3.86
Gallic acid	3.83	3.94	3.96	3.82	3.86
Quercetin	6.76	ND	6.71	6.70	6.81
Rutin	3.82	3.82	3.82	3.82	3.91
p-hydroxy cinnamic acid	6.89	ND	ND	ND	ND

VT- *Vitex trifolia linn* leaves extract, **PE**- Petroleum ether (60-80°C), **CH**-Chloroform, **EA**- Aqueous Extract, **ET**-Ethanol, **Std.**-Standard, **ND**-Not Detected

From the above results, HPLC fingerprinting which showed same retention time as that of standard caffeic acid (3.87), gallic acid (3.83), quercetin (6.76) and rutin (3.82), VT-PE shown (3.82, 3.94 and 3.82), VT-CH (3.82, 3.96, 6.71 and 3.82), VT-AE (3.82, 3.82, 6.70 and 3.82), VT-ET (3.86, 3.86, 6.91 and 3.91) respectively while *P*-hydroxy cinnamic was found absent.

Phytochemical standardization of *Corchorus trilocularis* CT, *Vitex trifolia* VT

Table 5. Determination of total phenolic and flavonoid content of CT,VT extracts

Plants	Extracts	Absorbance	TPC (mg/g ofGAE)	Absorbance	TFC (mg/g ofRE)
CT	CT-PE	0.075	07.70±0.91	0.015	14.40±0.18
	CT-CH	0.084	12.31±0.86	0.016	25.16±0.12
	CT-AE	0.099	28.60±0.95	0.024	41.17±0.17
	CT-ET	0.131	71.66±0.76	0.029	66.61±0.15
VT	VT-PE	0.080	08.16±0.52	0.014	08.30±0.36
	VT-CH	0.088	15.70±0.32	0.017	20.66±0.38
	VT-AE	0.136	70.55±0.14	0.022	31.15±0.40
	VT-ET	0.185	125.02±0.15	0.025	44.66±0.60

All the determinations were **c**arried out in triplicates and expressed in µg/mg of crude extracts. Values are representatives of Mean ± SEM. **TPC**- Total Phenolic Content, **TFC**- Total flavonoidsContents, **GAE**- Gallic Acid Equivalents, **RE**-

Rutin Equivalents, **CT-** *Corchorus Trilocularis* ***leaves***extract**, VT-** *Vitex Trifolia leaves* extract, **PE-**Petroleum Ether (60-80°C), **CH**-Chloroform, **ET**- Ethanol, **AE-** Aqueous Extract.

Among CT extracts CT-AE exhibit highest amount of total polyphenols (71.66±0.76), total flavonoids (66.61±0.15), in VT extracts VT- ET exhibit highest amount of total polyphenols (125.02±0.15), total flavonoids (44.66±0.62)

Table 6. Effect of *Corchorus trilocularis and Vitex trifolia* on serum biochemical parameter in diet induced hyperlipidemic model on rat.

S.No	Groups	Serum Tri Glyceride (mg/dl)	Serum Total Cholesterol (mg/dl)	Serum LDL Cholesterol (mg/dl)	Serum HDL Cholesterol (mg/dl)	Serum VLDL Cholesterol (mg/dl)
I	Control	62.61±0.49	79.93±0.62	07.61±0.17	69.93±3.66	12.6±0.98
II	Positive Control	186.83±0.32	184.84±3.73	113.14±3.43	34.63±3.48	37.16±0.88
III	Atorvastatin	121.31±0.43**	126.23±3.33**	36.44±3.29**	66.64±3.68**	24.26±0.61**
IV	CT-AE 200	184.44±0.39	180.41±3.67	103.66±3.73	40.02±3.42	36.88±0.63
V	CT-AE 400	171.72±0.63*	178.22±3.43	92.26±3.34*	61.63±3.68*	34.34±0.88*
VI	CT-ET 200	167.81±0.34**	164.41±3.67*	73.64±3.79**	67.34±3.83*	33.66±0.67**
VII	CT-ET 400	136.61±0.63**	140.71±3.44**#	60.43±3.73**#	62.81±3.63**	27.61±0.22**
VIII	VT-AE 200	183.66±0.37	180.71±3.33	106.64±3.88	38.86±3.44	36.26±0.63
IX	VT-AE 400	172.31±0.63*	170.43±3.62	93.64±3.76*	42.34±3.63	34.46±0.68*
X	VT-ET 200	166.66±0.66**	167.8±3.82	84.23±3.86**	60.66±3.61	33.13±0.41**
XI	VT-ET 400	137.63±0.38**	140.61±3.38**#	62.74±3.63**#	60.47±3.43**#	27.63±0.31**

The data obtained were analyzed by one way ANOVA followed by Tukey Multiple Comparisons Test. Each values represent the mean ± SEM; n=6. **p< 0.01*p< 0.05, p< 0.001***

a- Significant difference as compare to negative control group
b- Significant difference as compare to control group
c- Significant difference as compare to standard group
d- Significant difference as compare to aquous group

Values are expressed as mean±SEM. (n=6), ANOVA followed by Tukey test. *p<0.06 significantdifference, **p<0.00 highly significant difference when compared with Positive-control. #p>0.06 non-significant difference when compared with standard; **CT-** *Corchorus trilocularis leaves* extract**, VT-** *Vitex trifolia linn leaves* extract, **ET**- ethanol, **AE-** Aqueous Extract.

CONCLUSION

The present study of phytochemical screening and antioxidant activity of methanolic extract of leaves of Corchorus olitorius indicated the presence of different secondary metabolites and antioxidants. The presence of these bioactive compounds in jute leaf establishes it as a potential source of a natural therapeutic agent that can be used against different infectious and other diseases. C. olitorious is easily available and economically cheap and therefore can be used for the medicinal purposes. Finally, investigations on the isolation and identification of antioxidant components in this particular plant will lead us to know the chemical entities with potential for clinical use.

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