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PHYTOCHEMICAL INVESTIGATION ANTIUROLITHIATIC ACTIVITY OF COMMIPHORA MUKUL

Surina Yadav*[1], Shilpi Mishra[2], Ashish Mishra[2], Ragini Singh[1], Shivraj Singh Bhaduria[1]

Research Scholar[1], Assistant Professor[2]

Advance Institute of Biotech & Paramedical Sciences, Naramau, Kanpur-209217.

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***Corresponding Author**
Surina Yadav
Research Scholar, Advance Institute of Biotech & Paramedical Sciences, Naramau, Kanpur-209217.

ABSTRACT

Commiphora mukul is a well known plant for its flavonoids and a potent source of anti oxidants. The present research was aimed to evaluate its anti-urolithiatic potential with the possible mechanism of action. methods: After collection, authentication and drying of Resins Extracted *Commiphora mukul* were extracted. Phytochemical evaluations are carried out for detection of phytoconstituents. different ratios were used to isolate the phytoconstituents through preparative TLC method. After selecting the appropriate solvent system isolation of compounds were done through column chromatography studies were performed. After assumption of the compounds the in-silico along with in-vitro enzyme inhibition studies were performed by selecting a target enzyme. For pharmacological screening two methods of in-vitro i.e. method of mineralization and Titrimetry as well as two methods of in- vivo studies i.e. Ethylene glycol and sodium oxalate induced urolithiasis was performed. results: Phytochemical analysis revealed that ethanolic extract contains the majority of phytoconstituents including flavonoids. Using appropriate solvent systems, three flavonoids were isolated by column chromatography. Various spectroscopic analyses were performed to identify the compounds. In-silico and In-vitro enzyme inhibition studies confirmed the superior potency of Eth-03 by inhibiting enzyme Glycolate Oxidase. During pharmacological screening, the ethanolic extract demonstrated its efficacy by reducing the elevated biochemical parameters of urine and serum induced by the administration of chemicals that induce urolithiasis. Histopathological and urine microscopy investigations also confirmed this result by analyzing the recovered kidney's internal

structures with reduced number of crystals in urine specimen.

KEYWORDS: flavonoids, Antioxidants, Urolithiasis, glycolate oxidase, kidney.

INTRODUCTION

Urolithiasis: Kidney stones

Urolithiasis is a disorder known from old times, yet at the same time now the causes responsible for the creation of a some of the distinctive sort of stones are obscure, and thus proficient helpful medicines have never again however been created. Urinary tract stones are not unusual, greater so in guys, and amongst Asians and Caucasians, particularly in warm climates. They are associated with more utilization of animal protein and refined sustenance's, and reduced fluid admission. There is couple of, most usually comprising of oxalates and calcium phosphates; others are made out of uric acid, magnesium ammonium phosphate (struvite) or cystine. Struvite stones are related to urinary tract diseases, often of urease-secreting microbes that boom concentration of ammonium in urine. Calcium stones may create for a some of reasons, consisting of hypercalciuria and hyperoxaluria. Hypercalciuria may emerge from changes to calcium resorption from bone, or renal and GI tract coping with of calcium; those are often related to changes to parathyroid hormone secretion. Hyperoxaluria may rise up from accelerated production or improved intestine absorption. Different elements causing hypercalciuria incorporate renal tubular acidosis or 4 conditions that lessen the urinary concentrations of specific inhibitors, for example citrate and magnesium. Uric acid and cystine stones can produce because of expanded formation or urinary concentration of the essential constituents.

Clinical Features

Pain is the most well-known sign of ureteric calculi and is brought about by the stone impeding the urinary tract. The three most normal destinations of obstruction are the pelviureteric intersect, pelvic brim and vesicoureteric junction (Disregarding the way that obstruction can happen anytime along the system). Classical renal colic is described by an intense beginning of agony in the flank that transmits to the groin and scrotum (or labia majora). Regularly, the agony is severe and the patient will move around to get relief. Not under any conditions inducing peritonism, the pain will not be vanished being in same position. Queasiness and retching are additionally basic with intense renal colic, to some degree because of a level of ileus.

Type of kidney stones

Calcium-containing stones

The mostly recognizable kind of kidney stones overall contains calcium. They ordinarily contain calcium oxalate either alone or in blend with calcium phosphate as apatite or brushite. In India, 12% of the populace is expected to have urinary stones, out of which half may wind up with loss of kidneys or renal harm.[4]

Uric acid stones

About 5-10% of stones are created from uric acid. Individuals with certain metabolic diseases, including corpulence might create uric acid crystals. They likewise may produce in relation with conditions that form hyperuricosuria presence or absence of hyperuricemia. They might in like manner produce in association with diseases of acid/base digestion where the urine is too much acidic (lower pH)

Causes kidney stones

Physicians don't generally comprehend what makes a stone to develop. While certain sustenances may advance stone formation in individuals who are defenseless, researchers don't accept that eating a particular nourishment makes stones form in individuals who are not susceptible. An individual with a family ancestry of kidney stones might be bound to create stones.

Description: - he tree exudes a yellowish resin called gum guggul or guggulu and has a balsami codor.

Leaves: Leaves with 1-3 foliate leaflets, sessile or sub sessile, obovate with a serrated margin, 15cm long, 0.5 to 2.5 cm broad. Lateral leaflets are half the size of terminal leaflets.

Fruit: Fruit is ovoid, drupe, red after ripe, marked with 2 white longitudinal lines (or grooves), mucronate; mesocarp yellow, sometimes orange, four lined and fused at base; epicarp dehiscing from base upwards. When ripe, it becomes red and splits into two. In female flower, stamens are replaced by staminoids.

Seeds: Two kinds of seeds-black and white are located in mature fruits. While the black ones are viable, the whitish-black ones are not-viable because of the absence of embryo. The gum resin excreted by way of the bark of the plant is referred to as Guggal. The plant is grown through out the north India. The herb has been playing a prime role inside the traditional

medicinal drug of India. It is also known as Guggal gum, Guggal, guggulsterone, guggulu and gum Guggal. Guggal gum extraction is high all through April-*May***.**

External use of guggul

Paste: A paste of guggual may be implemented to the exterior of the body to promote healthy skin, free of movement within the joints, reduction of swelling, and detoxification of the tissues.

Gargling

Internal use of guggul

The flavor plays such an important role in the digestive technique and indicators the frame to provoke its supportive mechanisms, Ayurveda traditionally recommends tasting herbs. Guggul may be taken internally alone, however, is usually taken in combination with different herbs. It may be taken as a powder or a tablet.

Chemical composition of guggul

Guggal gum is a combination of 61% resin, 29.3% gum, 6.1% water, 0.6% volatile oil and 3.2% foreign matter. Guggal, the gum-resin exudate from the plant. *Commi phoramukul*, is a complicated combination of gum and management should be followed in this plant for future sustainability. This review could open a new window on the judicious use of this herbal plant.

Benefits of guggal

Gum resin is used as incense, as a fixative in perfumery, in medicine such as the replacement for *Commiphora mukul*. Guggal possesses strong disinfecting properties and is a weight reduction and fats burning agent. It lowers expanded serum cholesterol and triglycerides, while keeping or improving the HDL to LDL ratio. The herbwillincrease whiteblood cellsto be counted and decreases the danger of coronary artery disease.

Properties & uses 3.6.: - Commiphora Mukul Medicinal Uses Guggulu is a gum resinous exudate, which is tapped by specific traditional methods. Such resinous mass undergoes a process of purification to make it fit for human use. This is one of the important drugs used by Ayurveda in the treatment of joint disorders and heart diseases.

Chemical constituents: - Guggulu contains diterpenoids, triterpenoids, steroids, longchain aliphatic tetrols, aliphatic esters, ferulates, lignans, carbohydrates, and a variety of inorganic ions besides minor amounts of sesamin and other unidentified constituents.

MATERIALS AND METHODS

Plant material

Commiphora mukul (Burseraceae) were collected in the month of April 2024 from the locality of Kanpur district of Uttar Pradesh, India.

Identification and Authentication of plant material

Commiphora mukul was recognized and validated by Chandrashekhar Azad University Kanpur Botanical Survey of India, kanpur and voucher specimen (No. CM) was deposited at that institute.

Pharmacognostic Investigations

Organoleptic characters

Plant parts of Commiphora mukul (Gum) were analyzed for color, odor, taste and shape.

Microscopical characters

A) Preparation of specimen

Samples were fixed in FAA (Farmalin-5ml + Acetic acid -5ml + 70% Ethyl alcohol- 90ml). After twenty-four hours of fixing, the samples were got dried with t-Butyl alcohol. Filtration of the samples was performed by continuous addition of paraffin wax (M.P. 58-60ºC) until TBA solvent achieved super saturation. The samples were cast into paraffin blocks.

B) Photomicrographs

Microscopic explanations of tissues are accompanied with micrographs. Photographs were taken with different magnifications with Nikon Labphot 2 Microscopic Unit. Bright field was used for normal observations. Crystals, lignified cells and starch grains were examined using polarized light. The scale-bars indicate the figure magnification.

C) Powder microscopy

Plant materials were washed with water and dried sunlight and then place it for shade drying. Then plant materials were powdered by using wood grinder and sieved (sieve no. 60) separately and proceed for powder microscopy. Take chloral hydrate and insert powdered plant material in it and place it for boiling for 5-10 miutes. Then go for staining with phloroglucinol and toluidine and observed for microscopic features under high power (40 x).

Physicochemical characters

A) Total ash value

Plant parts are powdered and two gram of that was measured and transferred into the crucible. Support the crucible with the tripod stand and then heat it with a burner till vapours almost cease and heat strongly till the carbon is burnt off. Residue moistened with about 2 ml water, it gives you the carbon free ash. After which, it should be dried on a water bath, and ignited. Cool it and calculate its percentage.

B) Acid-insoluble ash value

Plant parts are powdered and two gram of that was measured and transferred into the crucible. Support the crucible with the tripod stand and then heat it with a burner till vapours almost cease and heat till all the carbon is burnt off. Place the sample in desiccator to cool and then wash the ash with 25 ml dil. HCl. The solution was placed for boiling for five minutes. Filtered through whatmann filter paper, wash the residue twice with hat water. Place filter paper and residue for heating in crucible till all carbon was removed. Residue weighed and calculated acid insoluble ash.

C) Water soluble ash

Plant parts are powdered and two gram of that was measured and transferred into the crucible. Support the crucible with the tripod stand and then heat it with a burner till vapours almost evolved and heated till the carbon was burnt off. Powdered plant material cooled in desiccators and then ash washed using twenty five ml of H_2O. The solution boiled for 5 minutes. Filtered through filter paper, the residue washed twice with hot water. Place filter paper and residue for heating in crucible till all carbon was removed. The residue weighed and calculated water soluble ash.

D) Alcohol soluble extractives

Plant parts are coarsely grinded and about 4 gram of that was measured and transferred to conical flask. Add 100 ml methanol and cork the flask and shake it well and allow standing for one hour. Reflux condenser connected to the container and boiled for one hour. Cooled, weighed and adjust the volume using methanol. Shaken well and filter. Take crucible, transfer 25 ml of filtrate to it and evaporate it in an oven at 105˚C. Place it for cooling and weighed immediately. Calculated the percentage w/w of extractive.

E) Water soluble extractives

Plant parts are coarsely grinded and about 4 gram of that was measured and transferred to conical flask. Add 100 ml water and cork the flask and shake it well and allow standing for one hour. Reflux condenser connected to the container and boiled for one hour. Cooled, weighed and adjust the volume using methanol. Shaken well and filtered. Take crucible, transfer 25 ml of filtrate to it and evaporate it in an oven at 105˚C. Place it for cooling and weighed immediately. Calculated the percentage w/w of extractive.

F) Loss on drying

About 1.5 gram of the grinded plant parts transferred to the porcelain dish. Dried in the oven at 100˚C, until 2 continuous weighings don't vary by greater than 0.5 mg. Place it for cooling and measured. The weight loss was usually recorded as moisture.

Phytochemical studies

Successive extraction of powdered drug with different solvents

Successive extraction of Commiphora mukul were performed using Soxhlet appratus with the help of various solutions like pet. ether (60˚ – 80°C), chloroform, acetone, methanol and water according to their polar nature. The extracts were dried with the help of rotary evaporator and were placed for cooling. Extracts were measured and calculated its percentage of yield.

Preliminary phytochemical investigation of various extracts

The extracts were investigated for the presence of number of phytoconstituents /secondary metabolites which indicates the therapeutic actions of the medicine like presence of alkaloids, glycosides, carbohydrate, tannins – phenolic compounds, proteins and amino acids, gums and mucilage, flavonoids, saponins and steroids.

A) Test for carbohydrates (Molisch test)

2-3 ml of extract solution, 1-2 drops of α-naphthol was added in alcohol, shaken and conc. H2SO4 added and violet ring was created at the middle of two solutions.

B) Test for proteins (Biuret test)

2-3 ml of extract solution, 4% sodium hydroxide and some drops of 1% copper sulphate was added. Violet colour appears.

C) Test for amino acids (Ninhydrin test)

Heat 2-3 ml of extract solution, added 5% Ninhydrin in boiling water bath for ten minutes.

Purple or bluish colour appears.

D) Test for alkaloids (Dragendroff's test)

To dry extract dilute HCl was added. Shake well and filtered. To filtrate, some drops Dragendroff's solution added. Orange brown ppt. was formed.

E) Test for flavonoids (Shinoda test)

To dry extract, 5 ml ethanol added, few drops concentrated hydrochloric acid and pinch of magnesium turnings. Orange color appears.

F) Test for steroids (Salkowski reaction)

To extract solution, chloroform and conc. H_2SO_4 was added. Shake well. Red color appeared to the chloroform layer and layer of acid indicates greenish fluorescence.

G) Test for terpenoids

To extract solution, conc. H_2SO_4 was added. Formation of red/blue green color appeared.

H) Test for glycosides (Keller-Killiani test)

To extract solution, CH_3COOH, 5% $FeCl_3$ and conc. H_2SO_4 added. Reddish brown color indicated at center of the two solutions layers and upper layer shows bluish green color.

I) Test for tannins and phenols

2-3 ml of extract solution, 5% $FeCl_3$ was added. Deep blue-black colour.

J) Test for saponins

Mixture of extract and distilled water was prepared and froth produced after shaking.

- **Fluorescence analysis**

It was performed on powdered drug under UV-light. Powdered drugs were analysed for fluorescence characteristics by using chemicals. The observations relating to their colour in day light and under UV light (short wave length and long wave length) were.

- **Isolation of classes of phytoconstituents by various chromatographic techniques**
- **Sorbent used:** Silica gel 60 GF_{254} / Pre-coated TLC plates on aluminium sheet
- **Support material:** Glass plates (for handmade TLC plates)
- **Plate size:** 10 x 10 cm / 20 x 20 cm
- **Solvents:** Initially plates were developed using single solvent (100%) as per table.

Based on the separation pattern, the combinations of more than two solvents were used for effective separation of phytoconstituents.

From the preliminary phytochemical investigation, the *Commiphora mukul* indicated that

flavonoids, saponins, triterpenoids, tannins and phenols are present as characteristic secondary plant metabolites. Furthermore, qualitative TLC/high performance TLC was performed using different solvent systems and specific visualizing reagents for the separation and identification of these phytoconstituents.

The qualitative TLC analysis was performed using Linomat V sample applicator, TLC 3 densitometric scanner and WinCATs software (Camag, Switzerland; Version 1.2.3) on precoated TLC plates [Merck Ltd.; Catalogue No. 1.5554.0007].

Table List of solvents based on their physico-chemical characteristics.

Solvent	Polarity index #	Dielectric constant (20bzw.25°C)	Dipole moment	Boiling point (°C)
Cyclohexane	0.0	2.0	0	80.7
n-Hexane	0.0	2.0	0	69
Petroleum ether	0.0	-	0	-
Toluene	2.3	2.4	0	110.6
Benzene	2.7	2.28	0	80
Diethyl ether	2.8	4.34	1.15	35
Dichloromethane	3.4	9.1	1.60	40
n-Butanol	3.9	17.8	1.66	118
Ethyl acetate	4.3	6.0	1.78	78
Chloroform	4.4	4.1	-	61.7
Methanol	5.1	33	1.70	68
Ethanol	5.2	24.3	1.69	78
Acetone	5.4	20.7	2.88	56
Acetonitrile	6.2	36.6	2.91	81
Glacial acetic acid	6.2	6.15	1.75	118
Formic acid	8.6	58	1.41	100
Water	9.2	80.2	1.85	100

Based on Rohrschneider Data

Table Different solvents utilized for TLC study.

Solvent System No.	Solvents	Composition	Phytoconstituents to be separated
SS-1	Benzene + Ethyl acetate + CH_3COOH	60 + 40 + 0.5	Terpenoids, Phenylpropanoids, Saponins, Bitter principles
SS-2	Ethyl acetate + Methanol + Water	100 + 13.5 + 10	Anthraquinones, Flavonoids, Mono- and Diterpenoids
SS-3a	Toluene + Ethyl acetate	93 + 7	Terpenoids, Phenylpropanoids,

			Saponins, Bitter principles
SS-3b	Toluene + Ethyl acetate	70 + 30	Terpenoids, Phenylpropanoids, Saponins, Bitter principles
SS-4	Chloroform + Methanol + Water	64 + 50 + 10	Saponins, Sapogenins
SS-5	n-Butanol + Chloroform + Ethyl acetate + Formic acid	2 + 1 + 1 + 2	Triterpnoids, Saponins, Flavonoids
SS-6	n-Butanol- Glacial acetic acid-Water	5 + 1 + 4, Upper layer	Flavonoids, Triterpnoids, Saponins, Amino acids

Table 5: Different visualizing / derivatizing reagents used for TLC stud.

Reagent No.	Visualizing reagent	Detection	Phytoconstituents to be visualized
VR-1	Anisaldehyde-sulphuric acid reagent	Different visible colours	Terpenoids, Phenylpropanoids, Steroids, Saponins, Bitter principles
VR-2	Komarowski reagent	Different visible colours	Terpenoids, Sapogenins, 3-keto steroids
VR-3	Ferric (III) chloride reagent	Blue - blue green visible spot; bright fluorescence in long wave UV light (366 nm)	Tannins and Polyphenolic compounds
VR-4	Aniline phthalate reagent	Different visible colours	Sugars, Sugar derivatives, Sugar alcohols
VR-5	Potassium hydroxide reagent	Visible colours; fluorescence in long wave UV light (366 nm)	Anthraquinones, Anthrone, Coumarins
VR-6	Aluminium chloride reagent	Yellow fluorescence in long wave UV light (366 nm)	Flavonoids
VR-7	Vanillin-sulphuric acid reagent	Different visible colours	Triterpnoids, Saponins, Steroids, Phenylpropanoids

For Commiphora mukul extract and its fraction

Pet. ether fraction (TI_1), Chlororform fraction (TI_2), methanol fraction (TI_3) and remaining aqueous fraction (TI_4) of *Commiphora mukul* were screened for number of phytochemicals.

Table Qualitative TLC analysis for Commiphora mukul

Plate No.	Solvent system	Visualizing reagent	Target phytoconstituents
1	SS-1	VR-1	Terpenoids, Phenylpropanoids, Steroids, Saponins, Bitter principles
2	SS-1	VR-2	Terpenoids, Sapogenins, 3-keto steroids
3	SS-1	VR-3	Tannins and Polyphenolic compounds
4	SS-3b	VR-7	Terpnoids, Saponins, Phenylpropanoids
5	SS-4a	VR-7	Triterpenoids, Saponins, Steroids, Phenylpropanoids, Bitter principles
6	SS-5	VR-7	Triterpenoids, Saponins, Sapogenins

Isolation of compounds using preparative TLC

From the qualitative TLC study of selected bioactive extracts and their fractions of all three plants, five major compounds were selected for isolation by preparative chromatographic techniques. The common parameters for preparative TLC were as follows.

- **Sorbent used:** Silica gel 60 GF254 (Pre-coated TLC plates on aluminium sheet)
- **Plate thickness:** 0.5 mm
- **Particle size:** 100 µ
- **Size of plate:** 10 x 10 cm
- **Band width:** 86 mm
- **Sample application quantity:** 160 µL per plate

Safety and Pharmacological Screening

Animal selection

Wistar rats of either sex weighing somewhere in the range of 150 and 200 g were chosen for toxicity study and for the antiurolithiatic activity. The animals were acclimatized to standard research facility states of temperature (22±3°C) and kept up on 12:12 h light:dark cycle. They were nourished with regular rat chow (Lipton India Ltd., Mumbai) and D.W. ad libitum. The animal care and experimental protocols were as per control and supervision of experiments on animals (CPCSEA) and institutional animal ethical committee (IAEC).

Chemicals and equipments

Ethylene glycol was procured from from Merck Laboratories, Mumbai, India. Allopurinol tablets (Zyloric® tablets, GlaxoSmithKline Pharmaceuticals Limited, Dr. Annie Besant Road, Mumbai, Bangalore, India; Batch No. L416; Mfg. Date: Jan 2007; Exp. Date: Dec-2009) were used as standard antiurolithiatic drug.

Metabolic cages for rats (B.I.K. Industries Ltd. Mumbai, India) were utilized for urine collection. For biochemical investigation of urine Flame photometer (Elico Ltd., India) was used. For urine, serum and kidney homogenate investigation, Biochemical kits (Erba Mannheim Gmbh, Germany) and autoanlyzer were used. Urocolor strips (Standard Diagnostic Inc. South Korea) and Uro-Dipcheck 300 urinaluzer (Erba Mannheim Gmbh, Germany) were used for routine analysis

Test principle

It is dependent on a stepwise technique with the utilization of a basic number of rats per step; adequate data is gotten on the intense toxicityof the test substance to empower its classification. The substance is directed orally to a group of rats at one of the characterized portions. The substance is tried utilizing a stepwise system, each progression utilizing three rats of a one sex (ordinarily females). Nonappearance or nearness of compound related mortality of the creatures dosed at one stage will decide the subsequent stage.

- No further testing is required.
- Dosing of three extra rats with a similar portion.
- Dosing of three extra rats at the following higher or the following lower portion level.

The technique empowers a judgment as for ordering the test substances to one of the arrangement of toxicity classes (for example GHS grouping) characterized by fixed LD50 cut-off value.

Housing and feeding

The temperature in the animal room was kept 22±3°C. Lighting was kept up for 12:12 h light:dark cycle. The creatures were furnished with rodent chow and distill water ad libitium.

Animals

Screening of antiurolithiatic activity of ETGE on ethylene glycon and ammonium chloride induced rat model

In an animal model of calcium oxalate urolithiasis, the antiurolithiatic action of ETGE and Cystone was identified.[7] For this investigation, the dosage that had significantly increased urine production during the diuresis trial was used. sixteen- male swiss rats (weighing between 150 and 200 g) were separated into 4 groups of 16 animals each, with matched body weights, and then randomly assigned to undergo the following treatments. Group-1 As a vehicle-treated control group, rats were given intraperitoneal (i.p.) injections of normal saline

(2.5 mL) once every 24 hours and free access to water every day for 28 days. Group-II Rats were subjected to a 28-day stone-inducing regimen consisting of 0.75% (w/v) EG in water with 1% (w/v) ammonium chloride for five days, followed by 0.75% EG alone in water with saline treatment (positive control urolithiatic group). GroupIII Rats were given the conventional medication Cystone (100 mg/kg) by gastric gavage while also receiving daily stone-inducing therapy akin to the positive control (treatment group, standard medication). Group-IV Rats were stomach gavaged with 50 mg/kg of ETGE, and at the same time, the treatment group (low dosage) underwent daily stoneinducing therapy for 28 days. After this Rats were stomach gavaged with ETGE (100 mg/kg) and given daily stone-inducing therapy comparable to the positive control for 28 days (treatment group, high dosage).

Evaluation of antiurolithiatic activity

A) In-Vivo study

Ethylene glycol induced urolithiasis

Rats were isolated in 16 groups containing 5 in each and placed in metabolic enclosures. All rats had free access to rodent chow and drinking water ad libitium for 28 days. Renal calculi were initiated in II to IX group by enhancing with 0.75%v/v ethylene glycol in drinking water. III to IX Group were treated with plant concentrates beginning from fifteenth day to 28th day (Curative routine). VI to VII group were treated with plant concentrates beginning from first day to 28th day (Preventive routine).The particulars are given in

Protocol for antiurilithiatic activity

I ==Normal Control

II == Standard

III = commiphora mukul 100 mg/km

IV =commiphora mukul 200 mg/km

V ==commiphora mukul 200 mg/km

Table Protocol for antiurilithiatic activity

Group	Treatment		Dose
I	Normal Control		-
II	0.75% ethylene glycol in drinking water for 14 days		-
III		Standard (glycinen and ammonium chloride)	5mg/kg
IV		Optimized commiphora mukul drug (Test)	50 mg/kg
			200mg/kg
			400mg/kg

Collection and assessment of urine

Urine samples (24 h) were gathered For 28th day. Store urine at 4°C after adding few amount of conc. HCl. Urinary calcium, phosphate and oxalate content were determined. Urine was centrifuged to pool the crystals and observed under light electron microscope. Size and shape of crystals were observe.

Microscopic evaluation

Fresh urine samples collected (day 14 and day 28), were examined at ×50 magnification using compound microscope to ascertain the presence of characteristic crystals of CaOx and CaPh. Their photomicrographs were taken using a digital Microtel CCD camera supported with Aver cap software [Aver media Technologies, version 1.0.0.10]

Urine biochemistry

Before being used at 4°C Conc. HCl was added to the urine. Using biochemical estimation kits, calcium and phosphate content was analyzed in urine; while, urinary oxalate content was estimated using modified method of Hodgkinson and Williams.

Histopathological examination of kidney

Each selected kidney was embedded in paraffin using conventional methods and cut into 5 µm thick sections and colored with help of hematoxylin-eosin dye and placed in diphenyl xylene. Then each section was examined using microscope for histopathological characters in kidney and photomicrographs were taken at ×50 magnification. By visualizing different fields, a general method of scoring was adopted to observe the extent of nephritic damage and the recovery. About 10 fields for each kidney slide were observed and assigned for severity of renal damage and progression of recovery utilizing scores on a scale of none (ND), mild (+), moderate (++) and severe (+++) damage.

B) In vitro study

Ions analyzed by TLC

This test was performed by the method proposed by Atmani et al., 24 h urine test sample was stored in propylene bottle. Before storing thr test urine sample in propylene bottle, it was filled with 1 ml of 20% sodium azide, it act as an antibacterial agent. 2 ml of urine aliquotes was distributed in to the tubes and maintained at 37 °C. Then, add extract solution of 50 µl to the tubes. Some tubes used as control without adding extract solution. After that 50 µl 0.1M sodium oxalate added to tubes and then allows it for incubation at 37 °C for 30 min. lastly,

solutions are placed to examine optical density at 620 nm by TLC And finally those samples placed under light microscope for observation at x45.

RESULTS AND DISCUSSIONS

PHARMACOGNOSTIC INVESTIGATION

Organoleptic characters

The *Commiphora mukul* gum were evaluated for organoleptic characteristics and macromorphological characteristics. The observed characteristics were compared with the literature to authenticate the crude drug under examination. Observations have been summarized in table gum.

Table Organoleptic and macromorphological characteristics

Characteristic	Commiphora mukul
Colour	Brown
Odour	Not characteristic
Taste	Tasteless
Shape	Irregular shaped
Size (Average)	1.6 cm long
Surface texture	Testa hard, shiny and smooth

Microscopical characters

The commiphora mukul, were evaluated for microscopical characteristics. The observed characteristics were compared with the literature to authenticate the crude drug under examination.

Physiochemical characters

The dried powders of commiphora mukul, were devoid of any visible foreign matter. The loss on drying for commiphora mukul, were observed less than 10.0% w/w, it indicates that the plant parts dried properly. The acid insoluble ash values were observed to be greater than 1.0% w/w indicating that commiphora mukul, contain any silicious material like sand, clay etc. The extractive values were observed to be gretaer than 5.0% w/w for plants with polar solvents (such as methanol and water) and below 3.0% w/w for plant parts extracted using non- polar solvents. So, it is observed that the polar phyto constituents are present in plants.

Table Physico-chemical characterization Gum.

Sr. No.	Parameter	Commiphora mukul (Gum)
1	Loss on drying (at 105^0C)	8.1 %w/w
2		
	• Total ash	9.8 %w/w
	• Acid insoluble ash	3.6 %w/w
	• Water soluble ash	5.5 %w/w
	• Pet. Ether (40- 60^0C) soluble extractives	11.6 %w/w
	• Chloroform soluble extractives	3.8 %w/w
	• Alcohol soluble extractives	22.2 %w/w
	• Aqueous soluble extractives	15.8 %w/w

PHYTOCHEMICAL STUDIES

Successive extraction of powdered drug with different solvents

The extraction of commiphora mukul, showed maximum percent yield with methanol; while in case of higher percentage yield was observed with water. The summary of colour, percent yield and consistency of each successive solvent is given in table.

Table Yield of successive solvent extracts.

Extracts	Colour	Consistency	Yield (%w/w)
i) commiphora mukul, Gum			
Petroleum Ether (40- 60^0C)	Yellow	Greasy	11.6
• Chloroform	Green	Sticky	3.8
• MtE	Brown	Sticky	22.2
• AqE	Brown	Sticky	15.8

The preliminary qualitative phytochemical investigation of various extracts of commiphora mukul, indicated steroids, carbohydrates (primary metabolite), tri-terpenoids, volatile oils, alkaloids, tannins and phenolic compounds (secondary metabolites). Summary of results given in table.

Table Summary of preliminary phytochemical screening of plant commiphora mukul

Sr. No	Phytoconstituents	Pet ether (60 ^{o} - 80^o C)	Chloroform	Methanol	Aqueous
1.	Alkaloids	-	-	-	-
2.	Carbohydrates	-	-	+	+
3.	Glycosides	-	-	-	-
4.	Flavonoids	-	-	+	+
5.	Phenol & tannins	-	+	+	+

6.	Steriods	-	-	-	-
7.	Triterpenoids	-	-	+	-
8.	Saponins	-	+	-	-
9.	Proteins	-	-	-	+
10.	Amino acids	-	-	-	-
-= Absent ; + = Present					

Fluorescence analysis

The extracts plant materials in various solvents showed characteristic fluorescence along and short wavelength of UV light. Summary of results given in table.

Sr. No	Type of extract		Commiphora mukul (Gum)
1.	Pet ether (60 ^{o} -80^{o} C)	Day light	Yellow Brown Greenish
		V short light	
		V long light	
2.	Chloroform	Day light	Green Dark green Black
		V short light	
		V long light	
3.	Methanol	Day light	Brown Deep brown Black
		V short light	
		V long light	
4.	Aqueous	Day light	Brown Deep brown Black
		V short light	
		V long light	

Safety and pharmacological screening

Acute oral toxicity study

As per GHS classification, AqE and MtE extracts of commiphora mukul, were found to be of class 4 (>300 to 2000 mg/kg, b.w.). However, on the basis of mortality in either steps of acute oral toxicity test guidelines 423, different LD50 cut-off dose (in mg/kg, b.w.) were determined. Accordingly, the individual effective dose was determined (in mg/kg, b.w.) as 1/10th of the respective LD50 cut-off dose. Results have been summarized in table.

Table Summary of acute oral toxicity

Sr. No.	Extract	GHS category	Dose calculations (in mg/kg, b.w.)	
			LD50 cut-off value	Effective dose
1.	Ethanol of commiphora mukul,	Class 1	>300 to 2000	400
	AqE of commiphora mukul,	Class 1	>300 to 2000	400
2.	ChE of commiphora	Class 3	>300 to 2000	400

	mukul,			
	Ethanol of commiphora mukul,	Class 4	>300 to 2000	400

With 400 mg/kg dose of Ethanol and AqE of commiphora mukul, and 400 mg/kg dose of ChE and Ethanol, no adverse effects were observed on the respiratory, circulatory, autonomic and central nervous systems and somatomotor activity. Also, the behavioural pattern was found to be normal throughout the study period. Apart from the moribund rats, none showed any sign of abnormal change in skin and fur, eyes and mucous membranes. Tremors and convulsions were also not observed in any of the survivor.

Evaluation of antiurolithiatic activity

Ethylene glycol induced urolithiasis

A method of Atmani F *et al.* (2003) was used to evaluate antiurolithiatic activity in albino rats. Continuous oral administration of 0.75%v/v ethylene glycol in drinking water for 14 days rendered a condition of experimentally induced hyperoxaluria in rats. The microscopic examination of urine collected on 14th day from randomly selected groups showed characteristic crystals of calcium oxalate (CaOx) and calcium phosphate (CaPh) under 50x and 100x magnifications (Fig. 5.1). Further, curative treatment with crude extracts showed characteristic alterations in serum and renal biochemical, and renal histological parameters.

Microscopic examination of urine

The microscopic examination under 50x magnification showed that the urine of normal group animals was devoid of any crystal or similar structure. In calculi induced rats, the urine mple showed abundant, large crystals of CaOx with characteristic rectangular shape. The cystone treated animals showed very less or almost dissolved small crystals. The AqE and Ethanol of commiphora mukul,. showed less abundant crystals and on visual comparison, the size of crystals were found to be reduced with respect to that of calculi induced rats. AqE of commiphora mukul, was found to be equally effective in dissolving the preformed crystals; however, smaller and discrete fragments of crystals were seen among these groups.

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Routine urinalysis

In CaOx urolithiasis, the pH of urine remarkably increases beyond 7.2, which initiates the nucleation of phosphate and oxalate with calcium. In calculi induced animals the mean pH of 7.58 was observed. However, the AqE of commiphora mukul, significantly decreased the elevated urinary pH to nearly normal values (6.67 to 6.92).

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Table Effect of commiphora mukul, on urine calcium, phosphate and oxalate levels in ethylene glycol induced urolithiasis in.

Sr.no	Groups	Calcium(mg/dl)	Phosphates (mg/dl)	Oxalate (mg/dl)
1.	Normal control	5.83± 0.65	5.31 ± 0.24	6.03±0.14
2.	Control	8.86 ± 0.36	17.4 ± 0.44	9.00 ±0.09
3.	Standard (Cystone)	6.5 ±0.23***	13.6 ± 0.42***	6.02 ±0.12***
4.	CMME 100 mg/kg	8.00±0.14	16.9 ± 0.42	8.38 ±0.16
5.	CMME 200 mg/kg	7.48 ±0.37*	15.1 ± 0.20**	7.97 ±0.50*
6.	CMAE 400 mg/kg	5.00±0.16***	15.2 ± 0.185**	6.17±0.20***
CMME- commiphora mukul, methanolic extract, CMAE- commiphora mukul, aqueous extract Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferonni post hoc test when compared with vehical control *P<0.05, **P<0.01, ***P<0.001.				

Table Effect of commiphora mukul,extract on crystallyzation assay.

Paramet ers mg/gener ated crystals in test tube	Group - I Contro l Gener ated Calciu m oxalate	Group- II 5mg/m l Fruse mide	Grou p- III 5mg/ ml Cyst one	Group- IV 5mg/ml pet ether extract of commipho ra mukul,	Group- V 5mg/ml chlorof orm extract of commiphor a mukul,	Group- VI 5mg/ml methan ol extract of commipho ra mukul,	Group- VII 5mg/ml aqueous extract of commiphora mukul,
Oxalate	15.02 ±0.45	14.78 [d] ±0.66	6.77 [b] ±0.50	15.44[d] ±0.44	14.87 [d] ±0.79	3. 97[a] ±0.32	6.66[b] ±0.57
Calcium	6.20 ±0. 29	5.24[d] ±0. 20	2.66 [b] ±0.21	5.84 [d]± 0. 31	6.15[d] ±0.10	2.30[a] ±0.19	2.48[b] ±0.16
		5.24[d] ±0. 20	2.66 [b] ±0.21	5.84 [d]± 0. 31	6.15[d] ±0.10	2.30[a] ±0.19	2.48[b] ±0.16

Anti-bacterial activity

The antibacterial activity of the extracts at different concentrations was screened by disc diffusion technique and by measuring the zone of inhibition. The results are given in the Table. The extract of commiphora mukul, was more effective *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Staphylococcus aureus* with zone of inhibition 27 mm, 26 mm, 25 mm and 27 mm (at concentration 1000 µg) respectively and was least effective against pseudomonas aurigenosa and proteus vulgaris. With the other tested bacterial strains, petroleum ether extract was not found to be effective.

Table Antibacterial activity of different extracts of commiphora mukul.

Extract	Conc (mg)	Zone of inhibition (mm)					
Microorganisms		**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Pet ether	2.5	15	-	-	-	-	-
	5	22	-	-	6	5	6
	10	27	6	8	11	10	-
Chloroform	2.5	11	10	6	5	-	9
	5	21	15	11	11	5	12
	10	29	18	15	17	14	16
Methanol	2.5	12	13	-	15	15	-
	5	22	21	10	18	24	18
	10	27	26	25	27	30	27
Aqueous	2.5	5	8	10	9	11	7
	5	12	11	15	11	18	13
	10	20	15	18	13	21	20
Ciprofloxacin	30 mcg/disc	22	22	20	21	21	22

SUMMARY AND CONCLUSION

In the present study, successive extracts of *commiphora mukul* were prepared and evaluated

for pharmacognostic and pharmacological activities. The preliminary phytochemical investigation of commiphora mukul extracts showed presence of glycosides, saponins and triterpenoids. Three extracts Ethanol of commiphora mukul, Ethanol possessing optimum bioactivity were subjected to fractionation using non-polar to polar solvents and all fractions were further subjected to TLC/ fingerprinting. The qualitative and preparative TLC/ study established the method of isolation and purification for five different compounds. Purity of all five isolated compounds was found >80% when assessed using method. Although, the exact phytochemistry of isolated compound (lupeol) remains unidentified; further systematic phytochemical studies would elucidate the probable structural entities in these plants and their structure activity.

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