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INVESTIGATION OF PHYTOCHEMICALS AND ANTIUROLITHIATIC ACTIVITY OF THE LEAVES EXTRACTS OF *COMMIPHORA BERRYI*

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ABSTRACT

This study investigates the *in vitro* antioxidant and antimicrobial activities of extract obtained from the dried leaves of *Commiphora berryi*. The urine and Serum Creatinine, uric acid, urea, and calcium (mg/dl) level of normal, control & treated was found to be significant. The plant extract showed significant when compared to Control group. Calcium, urea, uric acid, and Creatinine values of serum of rats are decreased in serum may be drug effects metabolism of calcium, urea, uric acid and Creatinine. May it effect oxalate formation as it is main constituent in inducing urolithiasis. Intense pain may lead to decrease in the food consumption which may further result into decrease in the body weight. Thus by consideration of urine and serum parameters Hydroalcoholic extract of *Commiphora berryi* (Leaves) has anti urolithiasis effect.

Keywords: Hydroalcoholic extract, *Commiphora berryi*, Anti urolithiasis effect.

INTRODUCTION

The man has been blessed by the Mother Nature with rich resources of remedies to cure all ailments of mankind. The knowledge of traditional medicines has accumulated over thousands of years as a result of man's hunt for acquiring the knowledge from nature so that today he possesses many effective means of treatments for various diseases ensuring good health. The history of herbal medicines dates back to human civilization on earth. The available documents, which are of great knowledge since ages and reveals that plants were used as medicines in China, India, Egypt and Greece long before the beginning of the Christian era[1].

In India nature is the prominent source for Ayurvedic medicines in the crude form which includes mainly dried herbal products or their extracts or mixtures of products. Other alternative traditional systems of treatment are being practiced for many centuries in most part of the world and apart from these alternative

systems. There has been a rich heritage of ethno botanical usage of natural sources by various tribal communities in India[2].

Uroliths or stones of the urinary tract are a compact mass of crystalline and non- crystalline substances held together by numerous layers of organic matrix .Urinary stones mark a complex disease termed 'urolithiasis' which encompasses the generation and deposition of Uroliths at any segment within the urinary tract. Its complexity derives from its understated and multifaceted aetiology In simple terms, it is put forward as a disorder that results from disruption of equilibrium between promoters and inhibitors of crystallization in urine[3]. Roots of existence of Urolithiasis are immensely deep that run deep down to 4800 BCE, as the presence of oldest bladder stones were reported in approximately 7000 years old Egyptian mummies at El Amrah of Egypt[4-5].

Evidences for its prevalence in various civilizations are also drawn from pieces of work like treatment strategies for urinary stones documented in Egyptian Ebers papyrus (1500 BCE), Mesopotamian stone tablets, and later in works of Hippocrates (fifth century BCE). Similar findings including elaborate descriptions of the disease, the associated complications and treatment of stones by surgical intervention are also evident in Sushruta Samhita (sixth century BCE) and writings of Charaka. While Sushruta provided approximately three hundred surgical procedures for stone removal ,crushing of stones to facilitate removal was first proposed and developed in third century BCE by Ammonius of Alexandria who also coined the term "lithotomus" which means "cutting the stones" [6].

Most crucial contributions that mark the dawn of urology are those of Cornelius Celsus who provided vivid details on perineal lithotomy which was practiced from the advent ofthe Christian era till the 18th century. Eventually, various instruments and surgical procedures were developed, but the most remarkable progress was depicted in 1824 when Jean Civiale developed a lithotripter equipped with stone grasping and fragmenting tools. Since then, a revolution in more sophisticated instruments for endourological, stone fragmentation and removal procedures have been witnessed[7].

Medicinal plants are always remained important source of drugs. Some medicinal plants and proprietary composite herbal preparations are reported to be effective in the treatment as well as prevention of recurrence of renal calculi with minimal side effects. *Commiphora berryi* (Arn) Engl, (Tamil name: Mudgiluvai, Family: Burseraceae) is a small fragrant, thorny tree occurring in the dry forests of North Coimbatore hills and is also commonly grown as hedge plant throughout South India. It yields a fragrant gum resin obtained by incision of the bark. The resin is used in folklore medicine as an ingredient in multi-component indigenous formulations used as astringent, antiseptic, carminative, diuretic, appetite

stimulator, uterine stimulant and emmenagogue. The study carried out was to evaluate in vitro anti-urolithiatic activity from extract of *Commiphora berryi.*

MATERIAL AND METHODS

Collection and authentification of plant material:

The leaves of selected plant namely *Commiphora berryi* was identified and collected from various areas of Madhya Pradesh on the basis of geographical availability. The entire plant drug was authenticated by expert botanist of Department of Botany Barkatullah University, Bhopal. The collected plant drug was cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction of Plant Material

Leaves powder of the plant *Commiphora berryi* were cut into little pieces using sterile scissor, washed under running tap water to remove the dust impurities. Then the plant Leaves was dried at room temperature (under shade). After complete drying, it was powdered using the motor and pestle.

Around 100 gm of air-dried powdered plant material was Placed in Soxhlet apparatus, starting form Petroleum ether then Hydroalcohol (ethanol: water; 75:25) for Leaves powder of the plant *Commiphora berryi*. Every time before removing with next dissolvable, powdered material was air dried beneath 100^0C. The extracted solvent was evaporated using the water bath at 100^0C. After the evaporation the extracted samples were stored in cold for further analysis[8]. **Phytochemical screening**

Phytochemical screening activity of Leaves powder of the plant *Commiphora berryi* was carried out to analyze the presence of the following compounds present in the plants such as[9-10]:

Alkaloids:

Alkaloids exist as the salts which are organic acids forms. They are easily soluble in water and alcohols. Hence for the study the solvent chemicals which were used were Hydroalcohol (ethanol: water; 75:25). Various tests are there to determine the alkaloid content in the plant extract.

Meyer's Test

Few drops of the Meyer is reagent, 5mg of Hydroalcoholic extract was added. White and pale-yellow precipitate formation indicates the presence of Alkaloids.

Dragendorff'sTest

To 5 mg of the Hydroalcoholic extract 5ml of distilled water was added, few drop of hydrochloric acid was added until the acid reaction occurs. To this few drops of Dragendorff's is reagent was added, formation of orange or orange red precipitate shows the presence of alkaloid.

Wagner's test

5mg of Hydroalcoholic extract with 1.5% of hydrochloric acid. To this few drops of Wagner's reagent was added. Brown ppt indicates the presence of alkaloid.

Flavonoids:

Flavonoids are the group of secondary compounds which are present in plants and plays important role in various plant metabolic activities. Qualitative detection of alkaloids can be done by different methods.

Alkaline reagent test

To 10mg of Hydroalcoholic extract 2ml of sodium hydroxide solution is added. Formation of intense yellow colour which on addition of 0.1% HCL gets colorless which indicated the presence of Flavonoid.

Shinoda test

10mg of the Hydroalcoholic extract was dissolved in the irrespective diluents. To this10 drops of dilute HCL was added and small pieces of magnesium was added. Formation of pink, brown or reddish color ppt indicates the presence of Flavonoid.

Carbohydrates:

Carbohydrates are the group of the plant secondary metabolites which are present in the form of sugars. They may be present in the form of monosaccharide, disaccharide and oligosaccharide. There are various tests to determine the carbohydrates in plant extract.

Anthrone test

5mg of Hydroalcoholic extract was shaken with 10ml of water, the solution was filtered and the filtrate was concentrated. 2ml of anthrone reagent solution was added to the solution. Formation of green blue color indicates the presence of carbohydrates.

Benedict's test

5mg of Hydroalcoholic extract was mixed with 10ml of water, filtered and the filtrate was concentrated and to this solution 5ml of Benedict reagent in solution was added and heated for 5mins. Formation of brick red colored ppt confirms the presence of carbohydrates.

Triterpenoids

Triterpenoid are a gathering of mixes which are available in all plants parts. They assume an essential job in the different restorative fields in improvement of anti-toxins. In plants they can be separated utilizing different solvents, for example, ethanol, methanol and watery concentrates. Different test are there to decide the terpenoids nearness and substance of it in plant extricates.

Salkowski's test

5mg of plant extract was dissolved in 2ml of the chloroform and 2 ml of concentrated sulphuric acid was added from the sides of the test tube. Upper layer turns red and lower layer turns yellow with light green color fluorescent which indicating presence of the tri-terpenoids.

Liebermann-Burchard's test

2mg of plant dry extract was dissolved in acetic anhydride, boiling and the cool down fast and to it 1ml of concentrated sulphuric (H_2SO_4) was added along the sides of the test tube slowly. Formation of pink color indicates presence of triterpenoids.

Saponins

Saponins are also a group of secondary metabolites which are present in the plants are grouped under amphipathic which produces foam formation when the aqueous solution is shaken vigorously for 5 to10 mins. Different sources are there from which the saponins have been isolated they may be either plant source or marine. They possess anti fedants and they also have the activity of plants against microbes and fungi. Various tests are there to identify the presence of saponins.

Honeycomb test

5ml extract was taken in the test tube and to it few drops of 5% sodium bicarbonate solution was added. The test tube was then shaken vigorously and kept for 3 minutes. Honeycomb like froth formation shows the presence of saponins.

Foam test

1 mg of plant extract was taken in a test tube and 20ml of distilled water was added and the tube was shaken vigorously for 15mins. Formation of foam to the length of 1cm indicates the presence of saponins.

Tannins

The tannins are a plant compounds which are distributed in many species of plants, where they play a role in protection from the predators and also plays an important role in plant growth. They are finding in both gymnosperms and angiosperms. The test to determine the plant extract was.

Lead acetate test

5mg of plants extract was mixed with 0.5ml of 1% lead acetate solution. Precipitate formations indicate the presence of the tannin.

Ferric chloride test

5mg of plant extract was dissolved with 0.5ml of 5% ferric chloride solution in the test tube. Development of dark black color indicates the presence of tannins.

Phenols

Phenols are group of plant compounds which are distributed in species of plants, and they also play a very important role in protection from the predators, and also help in plant growth. They are finding in both gymnosperms and angiosperms. The test to determine the phenols in plant extract are.

Sodium hydroxide test

5mg of Hydroalcoholic extract was dissolved with 0.5 ml of 20% sulphuric acid solution. Followed by the addition of 5 drops of aqueous sodium hydroxide solution. Solutions turn blue on addition of aqueous sodium hydroxide which indicates the presence of phenols.

Steroids

Steroids are the gathering of mixes which are available in all plant's parts. They assume an imperative job in the different therapeutic fields being developed of medications and related mixes. In plants they can be removed utilizing different solvents, for example, ethanol, methanol and fluid concentrates. Test to decide the steroids nearness and substance of it in plant extracts.

Salkowski's test

5mg of plant extract was dissolved in 2ml of the chloroform and 2 ml of concentrated sulphuric acid was added from the sides of the test tube without disturbing the ring development at the junction. Upper layer turns red and lower layer turns yellow with light green color which indicating presence of the steroid.

Glycosides

Glycosides are the group of the plant secondary metabolites which are present in the form of sugars. They are the molecules which bound to another functional group with glycosidic bonds. They play important roles in living organisms. Many of the plants stores this chemicals compounds in inactive glycoside which can be made active by enzyme hydrolysis, Test to determine the glycosides is Molisch test.

Anti-Urolithiatic activity

Animals

Albino wistar rats (200–250 g) were purchased and maintained under standard environmental laboratory conditions and fed with laboratory diet and water ad libitum and the protocol was approved by the institutional animal ethical committee[11].

Experimental design

Thirty healthy adult wistar albino strain rats of either sex weighing 200- 250 g were randomly selected and then divided into six groups with 6 animals in each group. The treatment period was considered for 10 days. Group-I served as normal received drinking water, Group-II served as Urolithiatic control

received Drinking water containing 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride, Group-III served as standard received Cystone 5ml/kg body weight per oral &drinking water containing 0.75 %(v/v) ethylene glycol and 2% (w/v) ammonium chloride, Group-IV received Hydroalcoholic extract of *Commiphora berryi* 50 mg/kg body weight per oral &drinking water containing 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride, Group-V received Hydroalcoholic extract of *Commiphora berryi* 100 mg/kg body weight per oral &drinking water containing 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride[12].

Grouping of animals

Group-I served as normal received drinking water

Group-II served as Urolithiatic control received Drinking water containing 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride

Group-III served as standard received Cystone 5ml/kg body weight per oral &drinking water containing 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride

Group-IV received Hydroalcoholic extract of *Commiphora berryi* 50 mg/kg body weight per oral &drinking water containing 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride

Group-V received Hydroalcoholic extract of *Commiphora berryi* 100 mg/kg body weight per oral &drinking water containing 0.75 % (v/v) ethylene glycol and 2% (w/v) ammonium chloride

Assessment of Anti-Urolithiatic activity

Formation of crystalluria and stone formation was verified by different biochemical marker analysis of urine and serum. At the end of the experiment, all animals kept in individual metabolic cages and 24-hour urine samples were collected and measured on the 10th day. Animals had free access to drinking water during the urine collection period (Rang & Dale., 2010). The urine was analyzed for calcium, magnesium, phosphate, urea, uric acid, oxalate and citrate[13].

Collection of Blood sample

On the 10th day, the animals were anesthetized and blood was collected from the retro-orbital sinus under mild anesthesia. Serum was separated by centrifugation at 15,000 rpm for 20 minutes and analyzed for calcium, oxalate, magnesium, phosphate, urea, uric acid and Creatinine[14].

Statistical analysis

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analyzed separately and one-way analysis of variance was carried out.

RESULTS AND DISCUSSION

The qualitative analysis carried out revealed the presence of the phytonutrients namely terpenoids, alkaloids, Phenols, flavonoids, quinones carbohydrates, tannins etc .The identification of the above compounds supports the use of these in traditional medicine as these been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart diseases and stroke by scavenging free radicals which are implicated in the pathogenesis of many diseases. The present study indicated that the Hydroalcoholic extract of *Commiphora berryi* (Leaves) have Excellent amount of total phenolic and Flavonoid. The urine and Serum Creatinine, uric acid, urea, and calcium (mg/dl) level of normal, control & treated was found to be significant. The plant extract showed significant when compared to Control group. Calcium, urea, uric acid, and Creatinine values of serum of rats are decreased in serum may be drug effects metabolism of calcium, urea, uric acid and Creatinine. May it effect oxalate formation as it is main constituent in inducing urolithiasis. Intense pain may lead to decrease in the food consumption which may further result into decrease in the body weight. As in urolithiasis induced control group kidney weight is increased because of accumulation of urine in kidneys as obstruction in excretion of urine.

Table 1: Yield of crude extracts

Extracts	Colour	Consistency	Yield(% w/w)
***Commiphora berryi* (Leaves)**			
Pet ether	Dark brown	Semisolid	10.45%
ethanol: water; 70:30	Brown	Semisolid	12.22%

Table 2: Results of Preliminary qualitative phytochemical tests

Sr No.	Phytoconstituents	Test	Observations
1	Alkaloid test	Mayers test	Positive
2	Flavonoid test	Alkaline reagent test	Positive
3	Phenolic content	Sodium hydroxide test	Positive
4	Tannin content	Ferric chloride test	**Negative**
5	Steroid test	Salkowski's test	Positive
6	Carbohydrate	Benedict's Test	Positive
7	Triterpenoids	Salkowski's test	Positive
8	Saponins test	Saponins test	Positive
9	Glycosides	Glycosides test	Positive

Table 3: Total Phenolic content of Hydroalcoholic extract of *Commiphora berryi* (Leaves)

Sample	Total phenolic content GAE mcg/ml
Hydroalcoholic extract 100µg/ml	14.54± 0.41

n=3, values are given in SEM

Table 4: Total Flavonoid content of Hydroalcoholic extract of *Commiphora berryi* (Leaves)

S. No.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	Hydroalcoholic extract (100µg/ml)	17.32 ± 0.52

n=3, values are given in SEM

Table 5: Effect of Hydroalcoholic extracts of *Commiphora berryi* (Leaves) on urine parameters

S. No.	Groups	Urea (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)	Creatinine (mg/dl)
1.	Normal control	76.9±0.2	4.6±0.1	5.2±0.2	0.4±0.3
2.	Negative Control	83.2±0.1	8.2±0.2	8.7±0.3	9.6±0.1
3.	Cystone 5ml/kg	91.1±0.2	4.7±0.1	6.4±0.1	3.8±0.2
4.	HACSJ 50 mg/kg	46.2±0.1	7.8±0.2	8.1±0.2	5.3±0.1
5.	HACSJ 100mg/kg	72.2±0.2	6.3±0.1	7.2±0.1	5.8±0.1

The values are expressed as mean ±SEM, n=6 in each group.

Table 6: Effect of Hydroalcoholic extracts of *Commiphora berryi* (Leaves) on serum parameters

S. No.	Groups	Urea (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)	Creatinine (mg/dl)
1.	Normal control	66.2±0.2	7.2±0.1	0.9±0.3	3.19±0.15
2.	Negative Control	131.4±0.1	5.2±0.2	7.6±0.2	0.64±0.12
3.	Cystone 5ml/kg	54.3±0.2	9.6±0.2	1.4±0.1	0.62±0.23
4.	HACSJ 50 mg/kg	111.6±0.4	4.6±0.2	4.5±0.3	0.73±0.28
5.	HACSJ 100mg/kg	71.2±0.2	3.9±0.2	3.6±0.1	0.63±0.41

The values are expressed as mean ±SEM, n=6 in each group.

Table 7: Effect of Hydroalcoholic extracts of *Commiphora berryi* (Leaves) on total body weight and kidney weight

S. No.	Groups	Body weight		Kidney weight(gm)
		Initial (gm)	Final (gm)	
1.	Normal control	206±4.5	216±3.5	0.59±0.01
2.	Negative Control	221±6.0	199±3.5	0.82±0.03
3.	Cystone 5ml/kg	202±3.5	206±3.0	0.50±0.02
4.	HACSJ 50 mg/kg	226±2.5	216±4.5	0.64±0.02
5.	HACSJ 100mg/kg	231±1.5	241±2.5	0.51±0.03

The values are expressed as mean ±SEM, n=6 in each group.

Conclusion

Change in body weight the change in body weight (%) of normal, control & treated was found to be significant. Anti Urolithiatic activity may be due to diuretic effect and antioxidant effects of flavonoids are reported and thus it may protect urolithiasis is by protecting from per oxidation of kidney apoptosis. Thus by consideration of urine and serum parameters Hydroalcoholic extract of *Commiphora berryi* (Leaves) has anti urolithiasis effect.

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