World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.com/
Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *HEDYCHIUM CORONARIUM* J. KOENING FOR ANTIUROLITHIATIC ACTIVITY

Yogendr M. Bahuguna and Neeraj Kumar

Division of Pharmaceutical Sciences, S.G.R.R.I.T.S., Patel Nagar, Dehradun-248 001, Uttarakhand, India

Received: 17-10-2013 / Revised: 22-10-2013 / Accepted: 17-12-2013

ABSTRACT

Kidney stones formation or Urolithiasis is a complex process that results from series of several physicochemical events including super – saturation, nucleation, growth, aggregation and retention within the kidneys. Among the treatments include extracorporeal shock wave Lithotripsy (ESWL) and drug treatment. Even this ESWL treatment may cause acute renal injury, decrease in renal function and increase in stone recurrence. Data from in-vitro trails reveal that phytotherapeutic agents could be useful as either could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. In the indigenous system of medicine, roots of *Hedychium coronarium* J. Koening are reported to be useful in the treatment of urinary stones. Hence, in the present study, the roots of *Hedychium coronarium* J. Koening have been selected for antiurolithiatic activity on experimental kidney stones. (In-vitro activity) *Hedychium coronarium* J. Koening is one of the ingredients of reputed herbal formulation Cystone for the treatment of kidney stones. In this study Alcoholic extract & Aqueous extract of roots part of the plant were evaluated for their potential to dissolved experimentally prepared kidney stones calcium oxalates, by an In-vitro model. Alcoholic extracts obtained from roots part demonstrated highest dissolution of Calcium oxalate (Kidney Stones) when compared to test extracts at 10 mg concentration. Reference standard formulation Cystone was found to be equally effective (39.12%) when compared to alcoholic extract of roots part.

Key words: *Hedychium coronarium* J. Koening**,** Kidney stones, Urolithiatic, Calcium Oxalate**.**

INTRODUCTION

Urolithiasis is the presence of calculi in urinary tract. The male-to-female incidence ratio is 4:1. Eighty percent of calculi are composed of calcium (either oxalate or phosphate), with others composed of struvite, uric acid, or cystine [1]. Approximately 1 million Americans develop a kidney stone each year and an estimated 12% of the population forms a stone some time during their life [2]. A kidney stone is a hard mass developed from crystals that separate from the urine and build up on the inner surface of the kidney. Normally, urine contains chemicals that prevent the crystals from forming. This inhibitor does not seem to work for everyone, however, so some people from stones. If the crystal remains tiny enough, then they will travel through the urinary tract and pass out of the body in the urine without being noticed [3, 4]. Kidney stones may contain various combination of chemicals the most common type of stone contains calcium in combination with either oxalate or phosphate. These chemicals are part of a person's normal diet and make up important parts of the body, such as bone muscles. A less common type of stone is because by infection in the urinary tract. This type of stone is called a struvite or infection stone. A bit less common is the uric acid stone. Cystine stone are rare. Urolithiasis is the medical term used to describe stones occurring in the urinary tract. Other frequently used terms are urinary tract stones disorder and nephrolithiasis [5, 6].

MATERIALS AND METHOD

Description of Plant [7-8]: *Hedychium coronarium* J. Koening, the White Ginger Lily is a vigorous tall-growing ginger from the Himalayas and consist multiple stems per pot. It is hard, perennial, erect, branched, annual weed up to 3-6 feet height. The leaves are simple arranged in alternate manner with undulate margin. Lanceolate, oblong, pinnate, deciduous, 8-12 inch long, green

***Corresponding Author Address:** Dr. Yogendr Bahuguna, Asst. Prof., Division of Pharmaceutical Sciences, Patel Nagar, Dehradun – 248 001, India; E-mail: yogendr.bahuguna@gmail.com*

in colour. The flowers are white in colour and have pleasant fragrance; summer flowering; fall flowering. The trunk is green in colour, very thick, typically multi-trunked or clustems.

Collection of Plant material: The leaves, flower and roots of *Hedychium coronarium* J. Koening was collected from Shanti Kunj of Haridwar, Uttarakhand, India during June-2013. A voucher specimen of the plant was deposited in the Botanical Survey of India Herbarium. The assertion No. of the specimen is BSI/NRC/Tech (Ident.)/2013-14/352. The certificate of the authentification is given in annexure A-1. The roots were shade dried at room temperature and coarsely powdered in such a way that the material passed through sieve no. 20 and was retained on sieve no. 40 for desired particle size. Organoleptic evaluation of roots of *Hedychium coronarium* J. Koening is given in Table No. 01.

Standardization of roots of *Hedychium coronarium* J. Koening [9]

The evaluation of crude drug involves the determination of identity, purity and quality. Purity depends upon the absence of foreign matter whether organic or inorganic, while quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine. The following standardization parameters were evaluated to obtain the qualitative information about the purity and quality of roots of *Hedychium coronarium* J. Koening. The results are shown in Table No. 3.

Determination of foreign matter: Foreign matter in herbal drugs consists of either parts of the medicinal plant or it may be any organism, part or product of an organism. It may also include mineral admixture not adhering to the medicinal plant material e.g. soil, stone, dust etc. The specified quantity of plant material is spread on a thin layer of paper. By visual inspection or by using a magnifying lens (5X or 10X), the foreign matters are picked out and the percentage is recorded.

Determination of physical constants

Loss on drying at 105^{0}C: Loss on drying is the loss of mass expressed as per cent w/w. the test for loss on drying determines both water and volatile matter in the crude. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible. An accurately weighed quantity of about 1 to 2 g of powdered drug was taken in a tared glass petridish. The powder was distributed evenly. The petridish kept open in vacuum oven and the sample was dried at a temperature between 100 to 105^{0}C for 2 h until a constant weight was recorded. Then it was cooled in a desiccator to room temperature, weighed and recorded.

Ash values: Ash values are helpful in the determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its prepration. A higher limit of acid – insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

Total ash value: Weighed accurately about 2 to 3 g of the powdered drug in a tared silica crucible. Incinerated at a temperature not exceeding 450^{0}C for 4 h, until free from carbon , cooled and weighed.

Water soluble ash value: Boiled the ash with 25 ml of water. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ingnited in a tared crucible at a temperature not exceeding 450^{0}C for 4 h. Cooled in a dessicator and weighed. Substract the weight of insoluble matter from the total weight of ash. The difference in weight represented weight of water soluble ash.

Acid insoluble ash value: Boiled the ash for 5 min with 25ml of 2 M HCl. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited and in a tared crucible at a temperature not exceeding 450^{0}C for 4 h. Cooled in desiccators and weighed.

Extractive Values

Alcohol soluble extractive value: Macerated 5 gm accurately weighed coarse powdered drug with 100ml of alcohol (90% v/v) in a stoppered flask for 24 h, shaking frequently during first 6 h. filtered rapidly through filter paper taking precaution against excessive loss of alcohol. Evaporated 25ml of alcoholic extract to dryness in a tared dish and weight it.

Water soluble extractive value: Macerated 5 gm accurately weighed coarse powdered drug with 100 ml of chloroform water I.P. in a stopper flask for 24 h, shaking frequently during first 6 h. Filtered rapidly through filter paper taking precaution against excessive loss of chloroform water I.P.

Evaporated 25 ml of chloroform water I.P. extract to dryness in a tared dish and weighed it.

Extraction [10-12]

The powdered roots of *Hedychium coronarium* J. Koening were extracted with 70% v/v alcohol by hot percolation method, separately. Aqueous extracts were also prepared by using chloroform water I.P. by maceration Process.

Preparation of alcoholic extract: About 300 g of dried powder of roots of *Hedychium coronarium* J. Koening was extracted with 70% v/v alcohol in a soxhlet extractor. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and solvent was distilled off in a Distillation assembly at 50^0C. The extract was concentrated to dry residue, in a desiccators over anhydrous sodium sulphate. The percentage yield of the extract was calculated with reference to air dried powder. Some part of the total extract was used for phytochemical investigation and rest of the extract was used for pharmacological screening.

Preparation of aqueous extracts: About 150 g of dried powder of roots of *Hedychium coronarium* J. Koening was subjected to cold maceration with chloroform water I.P. in a conical flask, for about 7 days at room temperature. The flask was securely plugged with absorbent cotton and was shaken periodically. Then the material was filtered through a muslin cloth and marc was pressed. The filtrate was re-filtered through whatman filter paper to get the clear filtrate (free from suspended material). The filtrate was concentrated to dry residue, in a desiccators over anhydrous sodium sulphate.

Successive solvent extraction: About 300 g of dried powder of roots of *Hedychium coronarium* J. Koening was extracted with solvent of different polarity in succession, starting with a highly non – polar solvent [Petroleum Ether ($60\text{-}80^0$C)], followed by comparatively less non-polar solvents (Chloroform) and finally with a more polar solvent (Ethanol).

Phytochemical analysis

Preliminary qualitative tests [13]

The extracts were subjected to preliminary qualitative phytochemical investigation. The various tests and reagents used are given below.

Carbohydrates

1. Molish's Test (General Test):- To 2-3 ml. Aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids.
2. Fehling's Test:- Mix 1 ml. Fehling's A and 1 ml. Fehling's B solution, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow, then brick red ppt is observed.
3. Benedict's Test:- Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.
4. Barfoed's Test:- Test solution treated with Barfoed's reagent and after boiling on water bath, it showed brick red colour precipitate.

Proteins

1. Biuret Test (General Test):- To 3 ml. Test solution add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour appears.
2. Million's Test:- Mix 3 ml. Test solution with 5 ml. Million's reagent. White ppt. Warm ppt turns brick red or the ppt dissolves giving red coloured solution.
3. Xanthoprotein Test:- Mix 3 ml. Test solution with 1 ml. Conc H_2SO_4. White ppt is formed. Boil precipitate turn's yellow. Add NH_4OH, ppt turns orange.

Amino Acid

1. Ninhydrine Test (General Test):- Heat 3 ml. Test solution and 3 drops 5% Ninhydrine solution in boiling water bath for 10 min. Purple or bluish colour appears.
2. Test for Tyrosine:- Heat 3 ml. Test solution and 3 drops Million's reagent. Solution shows dark red colour.
3. Test for Tryptophan: - To 3 ml. Test solution and few drops glyoxalic acid conc. H_2SO_4. Reddish violet ring appears at junction of two layers.
4. Test for Cysteine:- To 5 ml. Test solution and few drops of 40% NaOH and 10% lead acetate solution. Boil. Black ppt. of lead sulphate formed.

Fats and Oils

1. Place a thick section of drug on glass slide. Add a drop of Sudan Red III reagent. After 2 min., wash with 50% alcohol. Mount in glycerin. Observe under microscope. Oil globules appear red.
2. Solubility Test:- oil are soluble in ether, benzene and chloroform, but insoluble in

90% ethanol and in water. (Exception- castor oil, soluble in alcohol).

3. Filter paper gets permanently stained wit oils.

4. Extracts give red colour with 2-3 drops of tincture alkana.

5. Saponification Test:- Evaporate extracts to get 10 ml. Oil add 25 ml. NaOH. Boil in boiling water bath for 30 min. Cool. Add Na_2SO_4 solution. Soap forms and rise to the top filter. To filtrate add H_2SO_4. Evaporate, collect residue, it contains glycerol. Dissolve residue in ethanol. With ethanolic solution, perform following test:

a) To ethanolic solution, add few crystals of $KHSO_4$. Heat vigorously. Pungent odour of acrylic aldehyde is produced.

b) To ethanolic solution, add few drops of $CuSO_4$ and NaOH Solutions. Clear blue solution is observed.

Steroid

- Salkowski Reaction:- To 2 ml. of extract add 2 ml. of chloroform and 2 ml. conc. H_2SO_4. Shake well. Chloroform layers appears red and acid layer shows yellow fluorescence.
- Liebermann- Burchard Reaction:- Mix 2 ml. extracts wit chloroform. Add 1-2 ml. acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube. First red, then blue and finally green colour appears.
- Liebermann's Reaction:- Mix 3 ml extract with 3 ml. acetic anhydride. Heat and cool. Add few drops conc. H_2SO_4. Blue colour appears.

Volatile Oils

Hydrodistillate material. Seprate volatile oil from distillate and perform the following tests:

- Volatile oil have characteristic odour.
- Filter paper is not permanently stained with volatile oil.
- Solubility Test:- Volatile oil are soluble in 90% alcohol.

Glycosides

Preparation of test solution: - the test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

a) Test for cardiac Glycosides :

- Baljet's Test:- A thick section shows yellow to orange colour with sodium picrate.
- Legal's Test:- To aqueous or alcoholic extract, add 1 ml. pyridine and 1 ml. sodium nitroprusside. Pink to red colour appears.
- Keller – Killiani Test:- To 2 ml. extract, add glacial acetic acid , one drop 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown colour appears at junction of the two liquid layer and upper layer appears bluish green.

b) Test for Anthraquinone glycosides :

- Borntrager's Test for Anthraquinone Glycosides: - To 3 ml. extract, add dil. H_2SO_4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink to red.
- Modified Borntrager's Test for C-glycosides: - To 5 ml. extract, add 5 ml. 5% $FeCl_3$ and 5 ml. dil HCl. Heat for 5 min. In boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer. Add equal volume of dilute ammonia. Ammoniacal layer shows pinkish red colour.

c) Test for Saponin Glycosides :

- Foam Test:- Shake the drug extracts or dry powder vigorously with water. Persistent foam observed.
- Heamolytic Test**:-** Add drug extract or dry powder drug to one drop of blood placed on glass slide. Haemolytic zone appears.

d) Test for Cyanogenetic Glycosides :

- Grignard Reaction or Sodium Picrate Test:- Soak a filter paper strip first in 10% picric acid. Then in 10% Sodium carbonate. dry. In a conical flask place moistened powdered drug. cork it. Place the above filter paper strip in the slit in the cork. The filter paper turns brick red or maroon.
- To dry powder or extract, add 3% aqueous mercurous nitrate solution. Metallic mercury forms.
- Dip a piece of filter paper in guaiacum resin and moist it with dilute copper sulphate solution. Expose it to freshly cut surface of drug. Blue stain is produced.

e) Test for Coumarin Glycosides :

- Coumarin glycosides have aromatic odour.

- Alcoholic extracts when made alkaline, shows blue or green fluorescence.
- Take moistened dry powder in test tube. Cover test tube with filter paper soaked in dilute NaOH. Keep in water bath. After Sometimes expose filter paper to U.V. light. It shows yellowish – green fluorescence.

f) Test for Flavonoids :

- Shinoda Test:- To dry powder or extracts , add 5 ml. 95% ethanol. Few drops conc. HCl and 0.5 g magnesium turnings. Pink colour observed.
- To small quantity of residue. add lead acetate solution. Yellow coloured precipitate is formed.
- Addition of increasing amount of sodium hydroxide to the residue shows yellow colouration, which decolourises after addition of acid.

Alkaloids

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute HCl. Shake well and filter. With filtrate, perform following test :-

- Dragendroff's Test:- to 2-3 ml. filtrate, add few drops Dragendroff reagent. Orange brown ppt is formed.
- Mayer's Test:- 2-3 ml. filtrate with few drops Mayer's reagent gives ppt.
- Hager's Test:- 2-3 ml. filtrate with Hager's reagent gives yellow ppt.
- Wagner's Test:- 2-3 ml. filtrate with few drops Wagner's reagent gives brown ppt.
- Murexide Test for Purine Alkaloids:- To 3-4 ml. test solution, add 3-4 drops of conc. HNO_3. Evaporate to dryness. Cool and add 2 drops of NH_4OH. Purple colour is observed.

Test for Tannins and Phenolic Compound

To 2-3 ml. of aqueous or alcoholic extracts, add few drops of following reagents :

- 5% $FeCl_3$ Solution:- Deep blue –black colour.
- Lead Acetate Solution:- White ppt.
- Bromine Water:- Decolouration of bromine water.
- Acetic acid Solution:- Red colour solution.
- Potassium Dichromate:- Red ppt.
- Dilute iodine Solution:- transient red colour.
- Dilute HNO_3:- Reddish to yellow colour.
- One drop NH_4OH, excess 10% $AgNO_3$ solution. Heat for 20 min. In boiling water bath. White ppt observed. The dark silver mirror deposits on wall of test tube.

Test for Triterpenoids

Preparation of test extracts solution:- The test extract solution was prepared by dissolving extract in the chloroform.

- Salkowski Test:- few drops of concentrated sulphuric acid were added to the test solution, shaken on standing lower layer turned golden yellow.
- Libermann- Burchard Test:- To the test solution of the extracts, few drops of acetic anhydride were added and mixed well. Then 1 ml. of concentrated sulphuric acid added from side of the test tube, a red colour was produced in the lower layer indicating presence of tri terpenes.

Chromatographic studies [14-18]

Thin Layer Chromatography (TLC) studies were carried out for various extracts to confirm the presence of different phytoconstituents in these extracts. TLC is a mode of liquid chromatography, in which, the extracts is applied as a small spot or band at the origin of thin sorbent layer supported on a glass / plastic / metal plate. The mobile phase migrates through the stationary phase by capillary action. The separation of solutes takes place due to their differential adsorption/partition coefficient with respect to both mobile and stationary phases. Each separated component has same migration time but different migration distance.

The mobile phase consists of a single solvent or a mixture of solvent. Although, a number of sorbents like silica gel, cellulose, polyamide, alumina, chemically modified silica gel etc. are used, Silica gel (type 60) is most commonly used sorbent. Handmade plates are prepared by using techniques like, pouring, dipping or spraying. Now- a-days, readymade precoated plates are also available. The plates need to be activated at 110^0C for 1 h. This removes water/ moisture loosely bound to silica gel surface.

Qualitative TLC analysis

When PE, CE, AlcE and AqE were studied for antiurolithiatic study, it was found that the AlcE showed potent activity. As per the literature, Saponins disintegrate the mucoproteins thereby prevent CaOx retention and deposition. The preliminary phytochemical investigation of PE, CE, AlcE and AqE of roots of *Hedychium coronarium* J. Koening revealed the presence of Saponins,

glycosides, Fats and Volatile oil. Qualitative TLC of AlcE was performed using different solvent system and specific visualizing reagents to check the presence of these phytoconstituents. The AlcE and AqE showed the presence of Saponins. The Detail of TLC is as follows:-

Adsorbent: Silica gel GF 254 (activated)
Thickness: 0.4 mm
Plate Size: 10 x 20 cm
Activation temp: 110^0C for 1 hr
Volume of Spot: 20µl
Solvent system: n- Butanol : Glacial Acetic acid : water (0.5 : 0.1 : 0.4)

The spots were observed in iodine chamber (yellow to brown spot) as well as under UV light (254 nm) after spraying with Anisaldehyde- H_2SO_4 reagent a blue or violet colour.

Preparative TLC analysis: The alcoholic extract of grain exhibit significant antiurolithiatic activity, hence, it was selected foe detailed phytochemical analysis. Preparative TLC plates of (Approx.) 0.5 mm layer thickness were prepared using slurry of Silica Gel GF_{254} by pouring technique. The plates were activated at 110^0C fir 1 h. Sample of alcoholic extract of roots was prepared in Ethanol and applied as a band on plate. Sample Overloading was avoided to reduce tailing effect. Then the plates were dried in air and developed in the pre-saturated developing chamber using the same solvent system as used for qualitative TLC. The substance separated as distinct bands.

PHARMACOLOGICAL INVESTIGATION

Evaluation for antiurolithiatic activity (*In-Vitro*) [19]: Alcoholic extracts and aqueous extracts of roots were evaluated for antiurolithiatic activity by an invitro model using calcium oxalate stones. Formulation cystone was used as a reference standard.

Preparation of experimental kidney stones (calcium oxalate stones) [20]: Equimolar solution of calcium chloride dehydrates in distilled water and sodium oxalates in 10 ml of (2 N sulphuric acid) were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. The precipitate freed from traces of sulphuric acid by ammonia solution. Washed with distilled water and dried at 60^0C for 4 hours.

Preparation of semi- permeable membrane from egg: The semi- permeable membrane of egg lies between the outer calcified sheel and the inner contents like albumin & yolk. Shell was removed chemically by placing the egg in 2M HCL for overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the content squeezed out completely from decalcified egg. Washed thoroughly with water and stored in refrigerator at a pH of 7-7.4.

Estimation of Calcium oxalate by Titrimetry [21]: Weighed exactly 1 mg of calcium oxalate and 10 mg of the extract/standard and packed it together in semi-permeable membrane by steering as shown in model design Fig No. 08. this was allowed to suspend in a conical flask containing 100 ml of TRIS buffer. One group served as negative control (contained only 1 mg of calcium oxalate). Placed the conical flask of all groups in an incubator, pre heated to 37^0C for 2 hours, for 7-8 hours. Removed the contents of semi-permeable membrane from each group into a test tube. Added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N $KMnO_4$ till a light pink color end point obtained. 1 ml of 0.9494 N $KMnO_4$ equivalents to 0.1898 mg of calcium.

RESULT AND DISCUSSION

Result of the preliminary and comparative phytochemical tests carried out on the dried powdered roots of *Hedychium coronarium* J. Koening and its extracts are presented in Table No.-05. The preliminary phytochemical tests revealed that the roots contained Saponin glycosides, Fats and Volatile oil. Aqueous extracts contained Saponin glycosides and Volatile oil.

Qualitative TLC: The qualitative TLC analysis resulted in separation of different phytoconstituent in different solvent system and they were identified by their characteristic coloured band with corresponding visualizing reagent. The AlcE of roots showed Presence of Saponin glycosides and Volatile oil. Aqueous extracts contains Saponin glycosides and Volatile oil. The AqE & AlcE showed the presences of Saponin glycosides but AlcE were taken into consideration for isolation because of more potent activity shown in pharmacological investigation. The Qualitative TLC analysis of isolated saponins showed a blue coloured band at R.F. value of 0.65.

Invitro activity: The alcoholic extracts obtained by roots at 10 mg concentration produced highest dissolution of calcium oxalate stones in comparison to aqueous extracts. AlcE of roots of *Hedychium coronarium* J. Koening was found to be equally effective as standard Cystone.

CONCLUSION

In the present study, roots of *Hedychium coronarium* J. Koening were subjected to extraction using 70% v/v alcohol and chloroform water I.P. Some part of both extracts was reserved for preliminary phytochemical investigation and rest was utilized for pharmacological screening. The preliminary phytochemical test revealed that the roots contained Saponin glycosides, Fats and Volatile oil. Aqueous extract contains Saponin glycosides and Volatile oil. The AqE & AlcE showed the presences of Saponin glycosides but AlcE were taken into consideration for isolation because of more potent activity shown in pharmacological investigation. The qualitative TLC analysis of isolated glycoside showed the presence of Saponins. However further detailed study is necessary. The pharmacological screening included evaluation of antiurolithiatic activity of different extracts of plant *Hedychium coronarium* J. Koening. The AlcE extracts of the roots were more potent than any other extract in dissolving the experimental kidney stones. From the study results is observed that alcoholic extracts of roots produced highest dissolution of calcium oxalate stones in comparison to the other extracts. The dissolution capacity of roots extracts can be further enhanced by purification. The in-vitro study has given lead data and shown that roots extract of plant *Hedychium coronarium* J. Koening is quite promising for further work in this regard.

ACKNOWLEDGEMENT

Author are thankful to his holiness Shri Mahant Devendra Dass Ji Maharaj, Chairman, Shri Guru Ram Rai Education Mission, Dehradun, Uttarakhand, for providing the facility required for research work. We are also very much thankful to Dr. Praveen Kumar & Mr. Chandra Shekhar Tailor as knowledge bank that we have utilized during research work.

Table 1: Organoleptic evaluation of roots of *Hedychium coronarium* J. Koening

Parameter	**Observation**
Nature	Course Powder
Colour	Dark Brown
Odour	Aromatic
Taste	Bitter

Table 2: Qualitative TLC analysis for separation of different phytoconstituents

Phytoconstituents	**Solvent system**	**Visualizing reagent**	**Colour**
Alkaloids	Chloroform-Methanol (95:5)	Dragendroff's reagent	Red
Flavanoid Glycosides	Ethyl acetate : Formic acid : Glacial acetic acid : water (100:11:11:27)	Polyethylene glycol reagent	Orange or Yellow- Green or Dark Green
Triterpinoids Saponins	n-Butanol – Glacial acetic acid – water (50:10:40) [Upper layer]	Anisaldehyde- H_2SO_4 reagent	Blue or violet
Carbohydrate	n-Butanol – Glacial acetic acid – water (50:10:40) [Upper layer]	Aniline hydrogen phthalate reagent	Brown

Table 3: Chemicals used for Pharmacological Investigation

Chemicals Used	
• Tris buffer powder • Calcium chloride dehydrate • Sodium oxalate • Cystone Tablets • Ammonia Solution	• Oxallic Acid • $KMnO_4$ • HCl • H_2SO_4

Table 4: Observation of Physicochemical Parameters of the roots of *Hedychium coronarium* J. Koening.

S. No.	Organoleptic Evaluation		Observation
1.	Parameters	Nature	Coarse Powder
		Colour	Dark Brown
		Odour	Aromatic
		Taste	Bitter
2.	Physicochemical Evaluation	% Loss on drying	7.5%
		% Total ash Value	14.33%
		% Water Soluble ash value	4.33%
		% Acid Insoluble ash value	7.67%
3.	Extractive Values	% Alcohol soluble extractive value	6.4%
		% Water soluble extractive value	8.8%

Table 5: Observation of phytochemical investigation of various extracts

S. No.	Phytochemical Constituents	Chemical Test	Extracts			
			PEE	CE	AlcE	AqE
1.	Carbohydrate Test	Molish's Test	-	-	-	-
		Fehling's Test	-	-	-	-
		Benedict's Test	-	-	-	-
2.	Proteins Test	Million's Test	-	-	-	-
		Biuret's test	-	-	-	-
		Xanthoprotein Test	-	-	-	-
3.	Amino Acid	Ninhydrine Test	-	-	-	-
		Tyrosin Test	-	-	-	-
		Cystein Test	-	-	-	-
4.	Fats and Oil	Filter Paper Stain Test	+	-	-	-
		Solubility Test	+	-	-	-
		Saponification Test	+	-	-	-
5.	Steroid Test	Salkowaski Reaction	-	-	-	-
		Liebermann- Burchard Reaction	-	-	-	-
		Liebermann's Reaction	-	-	-	-
6.	Volatile Oil's	Characterstic odour	-	+	+	+
		Filter paper not stain	-	+	+	+
		Solubility Test	-	+	+	+
7.	Glycosides					
	Cardiac Glycosides	Legals Test	-	-	-	-
		Keller- Killiani Test	-	-	-	-
	Anthraquinone Glycosides	Borntrager's Test	-	-	-	-
		Modified Borntrager's Test	-	-	-	-
	Saponin Glycosides	Foam Test	-	-	+	+
		Heamolytic Test	-	-	+	+
	Cynogenetic Glycosides	Sodium Picrate Test	-	-	-	-
	Coumarin Glycosides	Aromatic odour	-	+	+	-
		Flourescence test	+	-	-	-
		Filter paper Soaked in NaoH Test	-	-	-	-

	Flavonoids Test	Shinoda Test	-	-	-	-
		Lead Acetate Test	-	-	-	-
		Sodium hydroxide Test	-	-	-	-
8.	Alkaloids Test	Dragendroff's Test	-	-	-	-
		Mayer's Test	-	-	-	-
		Wagner's Test	-	-	-	-
9.	Tannins and Phenolic Compound	Lead Acetate Test	-	-	-	-
		Acetic Acid Test	-	-	-	-
		Potassium Dichromate Test	-	-	-	-
		Pot. Permagnate Test	-	-	-	-

'+' = Present and '-'= Absent

PEE= Petroleum Ether (60-80^{0}C) Extract
CE= Chloroform Extract
AlcE= Alcoholic Extracts
AqE= Aqueous Extracts

Table 6: Calcium Oxalate Dissolution

Groups	**Vol. of Standard $KMnO_4$**	**Wt. Of Calcium Estimated**	**Wt. Of Calcium Reduced**	**Percentage Dissolution**
Control	4.6	0.8730 mg	----------	----------
Standard (Cystone)	2.8	0.5314 mg	0.3416 mg	39.12
AlcE of roots*	2.8	0.5314 mg	0.3416 mg	39.00
AqE of roots*	3.0	0.5694 mg	0.3036 mg	34.77

***correspond to 10 mg**

Fig. No. 01: Showing leaves and flower of Hedychium coronarium J. Koenig

Fig. No. 02: In-Vitro Experimental model setup to evaluate antiurolithiatic activity

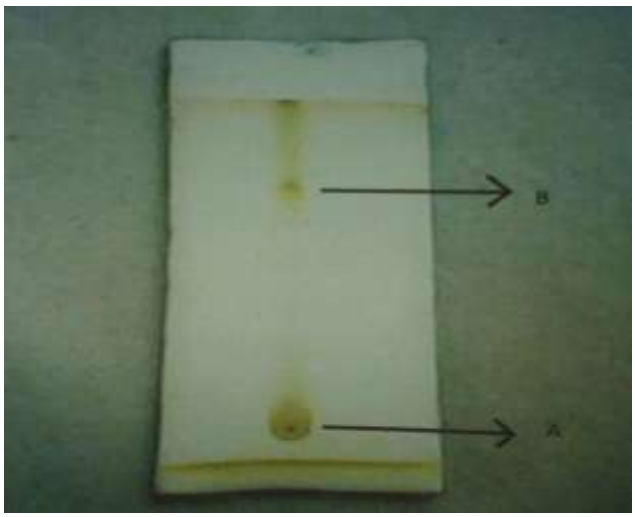

Fig. No. 03: Photograph showing TLC profile.

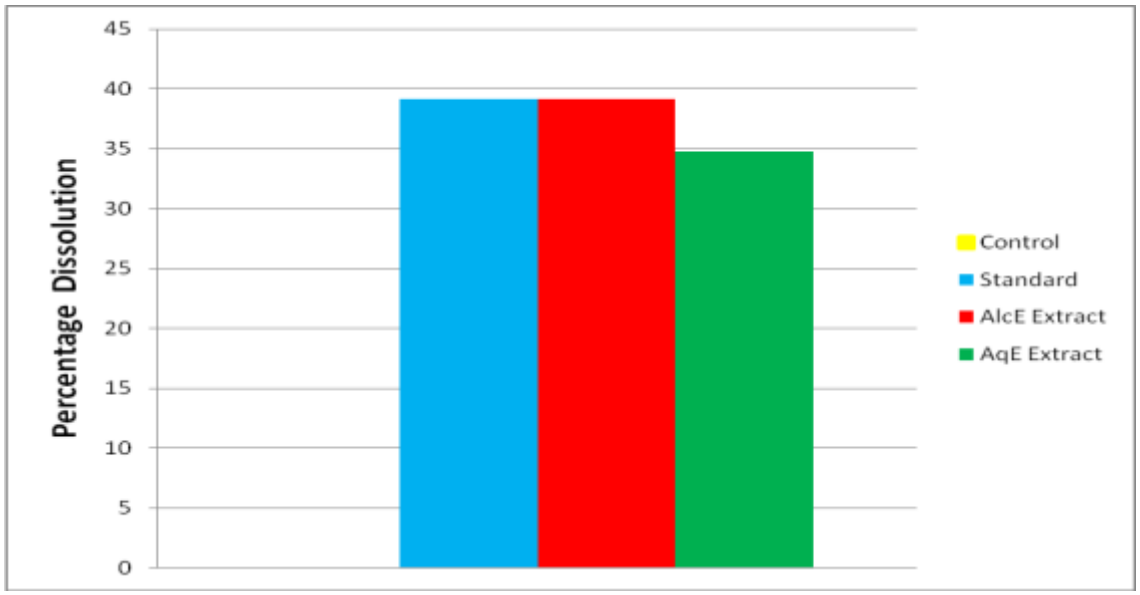

Fig. No. 4 :- Graphical Representation of various extracts of *Hedychium coronarium* J. Koenin

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