Analyzing the pharmacognostical, phytochemical, Anti-hyperuricemic and Xanthine-oxidase inhibitory activities of root of *Operculina turpethum linn* in rat hyperuricemia model

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Abstract

The main objective of present work was to study pharmacognostical and phytochemical evaluation of *Operculina turpethum linn* extract for the treatment of hyperuricemia. The plant *Operculina turpethum linn* (Convolvulaceae), has been traditionally used for the treatment of various diseases and in ayurvedic literature is described as sarvanubhoothi, Trivit. The plant roots were extracted with different solvents including pet ether, ethanol and aqueous in that ethanol extract of plant subjected to phytochemical and pharmacognostical study. Enzyme assay was done by using bovine milk xanthine oxidase (XO). The XO inhibitory activity in vitro was performed by using different doses of root extract and the degree of XO inhibition was expressed as IC_{50}.The antihyperuricemic activity of *Operculina turpethum* were tested in the potassium oxonate-induced hyperuricemic rats for seven consecutive days of oral treatment of 100 mg and 200 mg/kg doses. The results of study show that total flavonoid and phenolic content was estimated. Physico-chemical properties revealed total ash (5.00w/w), acid insoluble ash (1.32), water soluble ash (1.05w/w), water soluble extractive (15.60w/w), petroleum ether soluble extractive (2.00w/w), ethanol soluble extractive (19.20w/w), loss on drying (3.12w/w). The inorganic metal analysis showed the absence of toxic metals and quantification of heavy metals shows the presence of elements within the limit. Fluorescence analysis exhibited considerable variation and preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrate, flavonoids, glycoside, phenol, steroid, tannins and saponins. TLC studies showed that the ethanolic extract showed a single spot in both glycoside and flavonoid solvent system. The ethanolic extract of *Operculina turpethum* has modrate activity of XO inhibition with IC_{50}=92.51 ug/ml. In addition, these results showed that all doses of *Operculina turpethum* root extracts were able to significant reduce serum uric acid levels in the hyperuricemic rats. So, present study confirms the antihyperuricemic activity of *operculina turpethum* root extracts.

Keywords: *Operculina turpethum linn*, Ethanolic Extract, total flavonoid, phenolic content, xanthine oxidase, antihyperuricemic,

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Introduction

Hyperuricaemia or hyperuricemia is an abnormally high level of uric acid in the blood. In the pH conditions of body fluid, uric acid exists largely as urate, the ion form[1-2]. Serum uric acid concentrations greater than 6 mg/dL for females, 7 mg/dL for men and 5.5 mg/dL for youth (under 18 years old) are defined as hyperuricemia[3].It has been reported to affect

almost 10% of the adults at least once in their lifetime[4, 5].

Hyperuricemia is not only a known risk factor for gout, but has been strongly linked with other diseases such as hypertension and kidney failures[6]. From the prospective study, researcher concluded that men with gout had a higher risk of nonfatal myocardial infraction than men without gout, and among men without preexisting coronary heart disease (CHD) the increased mortality risk is primarily a result of an elevated risk of cardiovascular disease (CVD) death[7].

Currently, the pharmacotherapeutic agents for treatment of hyperuricemia and gout are ver y limited. Natural products, mainly of plant ori gin, have long been used in traditional medicin e for the treatment of gout and hyperuricemia[8,9].Opercu lina turpethum (Convolvulaceae) found throughout India and cultivated in Ceylon, tropical America, Mauritius, Philippines, tropical Africa and Australia. This plant is also known as "Trivrit" in Sanskrit and "Nishottara" in Marathi.

Operculina turpethum has been used for centuries as a medicinal plant in ayurvedic medicines because of its resin content (10 %) known as turpethin, jalapine and convolvuline glycoside and essential oil contents. Operculina turpethum is perennial plant with milky juice, root are long, slender, fleshy, much branched. Stems are very long, twining and much twisted together, angled and winged, pubescent, tough and brown when old[10-13].

This present study deals with the preliminary phytochemical screening and detailed pharmacognostical study of root of *Operculina turpethum linn* which includes macro and microscopic studies, determination of physico-chemical parameters of the extract. For pharmacological evaluation study was design to check antihyperuricemic potency of the ethanol extract of *Operculina turpethum* root as compared to allopurinol and examine the XO inhibitory activity of the ethanol extract.

Material and Methods

Drug and chemicals

Xanthine, XO, potassium dihydrogen phosphate, dimethyl sulfoxide (DMSO), potassium oxonate were purchased from Sigma Chemicals Co. Allopurinol was obtained from Ifars Pharmaceutical Laboratories. The UA kit was purchased from sclavo diagnostic. All chemicals and reagents used in this study were of analytical grade

Plant material:

The plant of *Operculina turpetum* was collected in the August in Malshej ghat junnar Maharashtra. The plant were identified and authenticated by the taxonomist of Botanical Survey of India Pune and a voucher specimen (BSI/WRC/Tech./22) was deposited at BSI, Pune.

Macroscopic and Organoleptic studies:

The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug. The fresh root was subjected to macroscopic studies which comprised of organoleptic characters viz., color, odor, appearance, taste, texture etc.

Microscopic evaluation

i) Root Microscopy:

The root of Operculina turpethum was collected, fixed in FAA solution (Formalin-Aceto-Alcohol: Formalin, Acetic acid each 5 ml, in 90 ml of 70% ethanol) for 24 hours, dehydrated with graded series of tertiary-butyl alcohol, and cast in paraffin blocks for qualitative microscopic examination. The paraffin-embedded specimens were then cut into sections using a rotary microtome, and the sections were then de-waxed. These sections were safranin-stained, and a compound microscope with projections of 10X and 40X was used to study them[14].

ii) Powder Microscopy:

To study the presence or absence of various types of tissues or structures, the dried of Operculina turpethum root are powdered using electric grinder, passed through sieve No. 60 and then subjected for microscopic studies. The powder microscopy was performed according to the methods of Kokate[15] and Khandelwa[16].

Physicochemical parameters:

Physiochemical values such as the percentage of ash values and extractive values were determined according to the official methods[17,18] and as per WHO guidelines on quality control methods for medicinal plant materials[19, 20].

1. Determination of ash values:

About 3 g of powder was spread in a pre-ignited and weighed silica crucible to determine the ash content of the drug. The crucible was then gently burned to remove all of the carbon from it. The crucible was weighed after cooling to estimate the total ash content, and the ash was then tested to identify the acid insoluble and water soluble ash. The percentage of total ash was calculated by taking the air-dried sample as standard.

2. Determination of extractive values:

Considering the diversity and chemical nature of the drug, three different solvents viz. petroleum ether, ethanol and water were used to determine of extractive values. About 5 g of powdered material was subjected to continuous Soxhlet extraction with 100 ml of petroleum ether and ethanol as solvents while using maceration process water extraction done. Determination of extractive values of a crude drug is beneficial in its evaluation process wherever evaluation of chemical components is applicable. After extraction, the extracts are concentrated in rota vaporizer and dried in a vacuum desiccator. Then the extractive values are calculated as percentage w/w of solvent-soluble extractive with reference to the air-dried drug.

3. Determination of moisture content:

Moisture content was determined by the loss of weight on drying (LOD) method. For this 5gm of the drug (powdered root material) was taken and kept in an oven at 105oC till a constant weight was obtained. The amount of moisture present in the sample was calculated as a reference to the air-dried material.

4. Fluorescence analysis:

Crude drugs show their own characteristic fluorescence when exposed to ultraviolet radiation and are dependent on their chemical constituents. This analysis is useful to identify adulterants during crude drug evaluation. In the present study, one gram of crude drug was taken in a watch glass and subjected to fluorescent analysis as such and after treatment with different reagents.

Qualitative and quantitative estimation of heavy metals and inorganic elements

Plant material plays a vital role in metabolism and osmolity. Presence of elements vary with the soil, climatic conditions etc. There are essential and non-essential elements which may be beneficial or harmful to living things. Non-essential elements like lead, arsenic, cyanide, cadmium and mercury bring about toxic effects resulting in intoxication. Hence qualitative and quantitative estimation of inorganic elements in the plant Operculina turpethum were carried out.

Preliminary phytochemical screening:

Plants are considered as bioreactors or biosynthetic laboratories as they synthesize wide range of characteristic therapeutically important molecules in the form of secondary metabolites. Thus, a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. For preliminary phytochemical extracts were subjected for preliminary screening using standard procedure for identifying various phytoconstituents[21].

Estimation of Flavonoids

Total flavonoid content was determined by the aluminium chloride colorimetric method, using quercetin as a standard[22]. Aliquots of extracts solutions (1µg/ml) were taken and 0.1ml of AlCl3 (10%), 0.1ml Na-K-tartarate and 2.8ml distilled water were added sequentially. The solution mixture was vigorously shaken. Absorbance at 415nm was recorded after 30 min of incubation. A standard calibration curve was generated at 415nm using known concentrations of quercetin (20, 40, 60, 80,100 µg/ml). The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg Quercetin equivalent / g of sample.

Total Phenolic Content

Standard Gallic acid (10mg) was dissolved in 100ml distilled water in a volumetric flask (100µg/ml of stock solution). From the above stock solution 0.5 to 2.5ml of aliquots were pipette out into 25ml volumetric flask. Then 10ml of distilled water and 1.5ml of Folin-Ciocalteu reagent, diluted according to the label specification to each of the above volumetric flask were added. After 5min, 4ml of 1M Sodium carbonate was added and volume was made up to 25ml with distilled water. At the same time, the plant extract (0.5ml of 1:10mg/ml) in methanol were separately mixed with above reagents. After 30minutes, absorbance at 765nm was recorded and calibration curve for standard was plotted as absorbance Vs concentration. From this graph the amount of Phenolic content was determined[22].

Thin layer Chromatography:

TLC is a chromatographic technique which is used for the separation of mixture of compounds. TLC is performed on a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F254 (Merck). Samples prepared with different solvents were spotted onto the TLC plate as a single spot with capillary tubes[23]. TLC plates were first viewed in UV chamber and Rf values were calculated.

Pharmacological evaluation

Experimental animal

The laboratory animal male Wistar rats (150-180 g), obtained from the Animal House of Lakshmi bio farms, Alephata Tal-junnar Dist-Pune were used for the study. Animals were housed at temperature of 25±2 ^{0}C and relative humidity of 45-55%. A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics committee of Ashvin rural ayurvedic college and hospital under proposal no: ACOP/AH/2022/1

In vitro XO inhibitory activity

The xanthine oxidase (XO) inhibitory effect of *Operculina turpetum* extract was assessed spectrophotometrically at 290 nm according to Sunarni et al.[24] and Yumita et al.[25] with minor changes. The mixture assay consists of 0.9 mL of 0.05 M sodium phosphate buffer (pH 7.5 at 25EC), 1 mL of *Operculina turpetum* extract(100 µg mLG1 in DMSO) and 0.1 mL of XO enzyme solution (0.1 unit mLG1 in phosphate buffer, pH 7.5) was prepared in cold buffer directly before using. After a 15 min pre-incubation at 25EC, then the reaction was allowed to start by addition of 2000 µL of freshly prepared solution of substrate (0.15 mM xanthine solution). Next, a further incubation process was achieved for the reaction mixture at 25EC for 30 min. After addition of 1 mL of 1N HCl solution into assay mixture for stopping the reaction, the absorbance was recorded at wave length 290 nm by using UV/Vis spectrophotometer (Chrom Tech, USA) against the blank which is prepared in the same procedure but with replacement of enzyme solution by phosphate buffer. The positive control solution was prepared by using allopurinol (100 µg/ml) in DMSO. The inhibitory activity against the XO was stated as the percentage of inhibition (%)

XO inhibition (%) = {1-

β Where, " represents the activity of XO in absence of the tested substance (*Operculina turpetum* powder) and $ is the activity of XO with presence of *Operculina turpetum* powder. Different concentrations of both *Operculina turpetum* powder and allopurinol (1, 2, 3, 4, 5, 10, 25, 50 and 100 µg mLG1) were used for evaluation of XO inhibitory activities and then the dose-response logarithmic curve was applied to determine the median maximum inhibitory concentration IC50.

Animal model of hyperuricemia in rats and drug administration

Hyperuricemic rat model was prepared by giving potassium oxonate as uricase inhibitor. The experiment was performed according to the Liu's method [26] with minor modification in potassium oxonate dose. Rats were divided randomly into normal control, hyperuricemic control, standard (allopurinol), and sample groups (n=6 each). Allopurinol and extract of

Operculina turpetum were prepared in suspension dosage form using 0.3% carboxymethylcellulose sodium (CMC-Na). The *Operculina turpetum* extract at dose of 100 mg/kg body weight (bw) and 200 mg/kg bw was given orally to rats. As a standard, allopurinol was administered in the same manner at a dose of 10 mg/kg bw. The volume of the suspension which was administered based on body weight of rat. The rats were fasted 1 day before being used in the experiment. Water was withdrawn from the animals 1 hr prior to drug administration. The animals were transferred to the laboratory at least 1 hr before the potassium oxonate-induced hyperuricemia experiment. Before potassium oxonate administration, blood was collected for determining serum UA level on 0 h. Briefly, rats were injected intraperitoneally with potassium oxonate (250 mg/kg bw suspended in CMC-Na 0.3%) to increase the serum UA level, except normal control group, 1 hr before the drug administration. Blood samples were collected from rats by tail vein bleeding in duration 1-3rd h for obtaining UA level on 1st, 2nd, and 3rd h after drug administration. The blood was allowed to clot for 30 minutes at room temperature and then centrifuged at 10.000 × g for 7 minutes to obtain the serum. The serum was stored at −20°C before UA level determination. Serum UA level was determined by enzymatic-colorimetric method, using a standard diagnostic kit, according to manufacturer's instructions.

Statistical analysis:

Results showed the mean ± standard deviation. The significant difference was statistically calculated by the analysis of variance (one way) followed by Tukey post hoc test. Statistical significant was set at $p<0.1$, 0.05, and 0.01

Results and discussion

A systematic approach is necessary in pharmacognostic study which helps in confirmation and determination of identity, purity and quality of a crude drug. This detailed and systematic pharmacognostic study will give valuable information for future research work.

Macroscopic and Organoleptic Studies: The root is thick and wood. The surface of the root is irregularly fissured; the fissures are shallow. The thickness of the periderm varies along the circumference as shown in Fig.2.

Fig. 1: *Operculina turpethum L* plant

Fig. 2: Part used- Root

Fig. 3: Powdered drug

Microscopic Studies:

Root Microscopy:

1. Cork- Thin cork, consisting of 3- 5 rows of brown Cells. Phelloderm 4-5 layers of tangentially elongated cells shown in Fig. 4A.

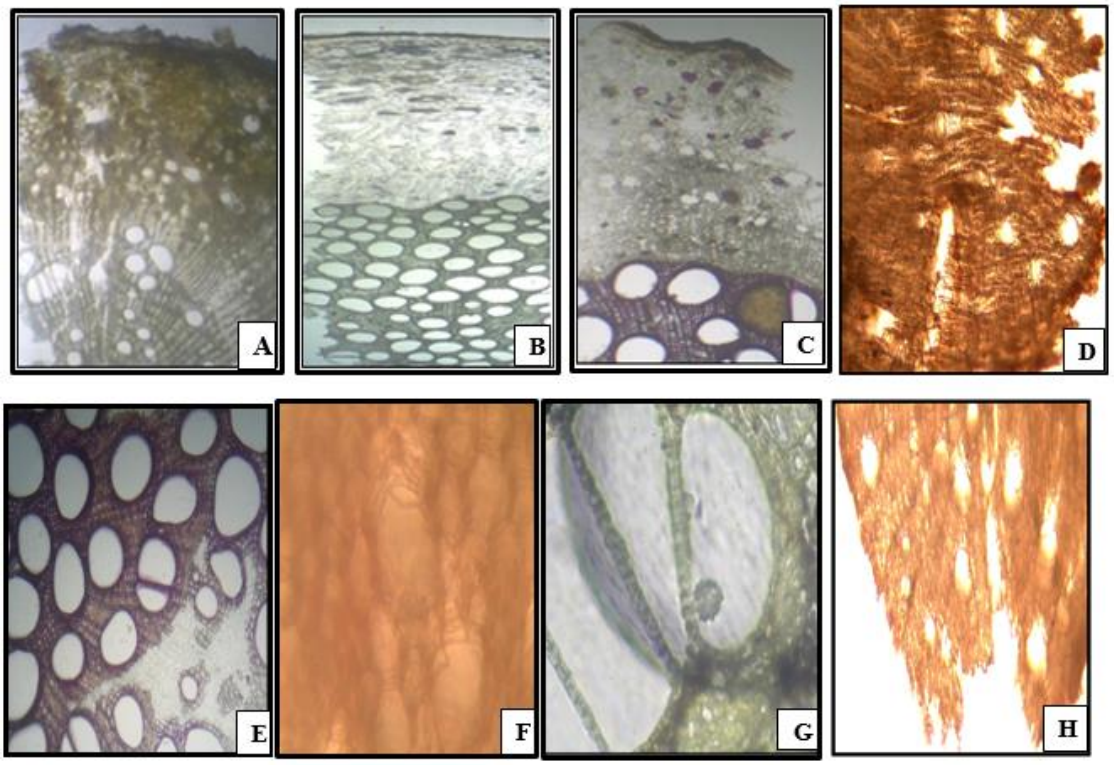
2. Cortex: The cortical zone gradually gets transformed into secondary phloem and there is no border or distinct boundary between the cortex and secondary phloem. The cortical cells are densely loaded with starch grains and sparsely distributed calcium oxalate crystals shown in Fig. 4B. The starch grains are either simple; concentric or exocentric. These are also compound starch grains.

3. Xylem: 3-5 radiating arms with Intraxylary phloem shown in Fig. 4E

4. Secondary xylem: Secondary xylem is the thick and dense central cylinder comprising wide, circular thin walled vessels and thick walled lignified fibers. The vessels are solitary and diffuse in distribution. There are also xylem parenchyma cells. The xylem parenchyma cells are thick, tangential layered and they are apotracheal layered type. The parenchyma layers are away from the vessels Fig. 4C.

5. Sec. Phloem: Thick, consist sieve elements shown in Fig. 4D and phloem parenchyma.

Fig. 4: Transverse section of *Operculina turpethum* root showing

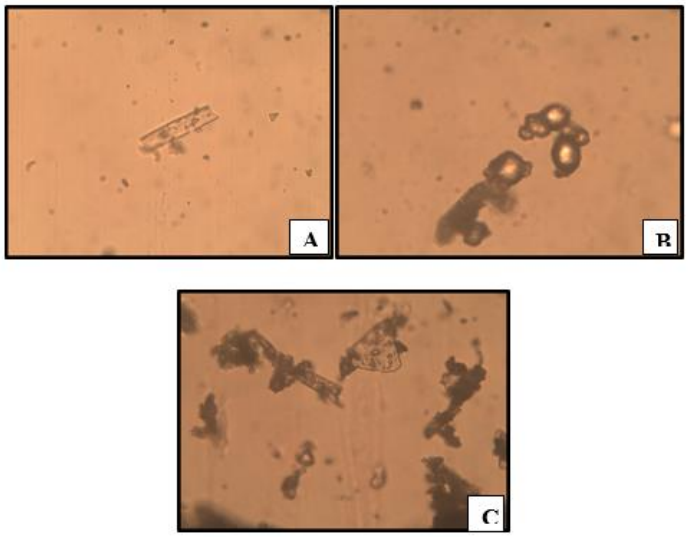


A.Cork. B. Cortex C. Xylem D. Sieve cell E. Interxylary phloem. F. Large Xylem vessels G. Intervascular pitting H. Large lignified xylem and secondary phloem

Powder microscopy:

Secondary xylem is the thick and dense central cylinder comprising wide, circular thin walled vessels and thick walled lignified fibers. The cortical cells are densely loaded with starch grains and sparsely distributed calcium oxalate crystals. The starch grains are either simple; concentric or exocentric

Fig.5 Powder characteristics of *Operculina turpetum* root



A. Xylem vessel B. Pericyclic fibers C. Sieve cells

Physicochemical constants:

Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting of proper standards. Various physicochemical parameters like ash values, extractive values, moisture content and fluorescence on reaction with various chemical reagents were investigated and the results are presented (Tables 1-3).

Table 1: Physiochemical parameter of *Operculina turpethum* root

Sr.No.	Physiochemical parameter	%W/W
1	Total Ash	5
2	Acid insoluble ash	1.32
3	Water soluble ash	1.05
4	Loss on drying	3.12

Table 2: Solvent extractive values percent (W/W) of *Operculina turpethum* root

Name of extract	Method of extraction	Colour	Consistency	Percentage yield
Pet ether extract	Soxhlet	Light yellow brown	Slight sticky	2.00w/w
Ethanol extract		Brown	Sticky	19.20w/w
Water extract	Maceration	Greenish brown	Sticky	15.60w/w

The Fluorescence analysis of the powder and the various extracts are given in Table 3 and 4.

Table 3: fluorescence analysis of *operculina turpethum* root powder

Treatment	Day light	UV light	
		254nm	366nm
Powder	Faint yellow	Yellowish	White
Powder + water	Pale green	Pale green	Green
Powder + 1N HCl	Dark Yellow	Black	Dark Yellow
Powder+ HNO3	White	Faint yellow	Faint yellow

Powder+1N NaOH	Dark brown	green	Faint yellow
Powder+ 1N KOH	Whitish yellow	Dark Yellow	Faint yellow
Powder+H2SO4	White	Brown	Faint yellow
Powder + Ethanol	Faint yellow	White	Yellow
Powder + Acetone	White	Faint yellow	White
Powder + Methanol	Faint yellow	Yellowish	White

Table 4: Fluorescence analysis of various extracts of the leaves of Operculina turpethum

S.NO	**EXTRACTS**	**DAY LIGHT**	**UV LIGHT**	
			SHORT 254nm	**LONG 365nm**
1	Pet ether	Greenish black	Dark green	Greenish black
2	Ethanol	Dark green	Green	Green
3	Aqueous	Brown	Pale brown	Pale yellow

There was no characteristic fluorescence seen with either the powder or the extracts.

Estimation of inorganic elements and heavy metals

Qualitative and quantitative estimation of inorganic elements and heavy metals were analysed in the leaf powder and the results were tabulated in Table 5.

Table 5: Qualitative analysis of heavy metals and inorganic elements

S.NO	**ELEMENTS**	**OBSERVATIONS**
1	Aluminium	+
2	Arsenic	-
3	Borate	-
4	Calcium	+
5	Carbonate	-
6	Chloride	-
7	Copper	+
8	Iron	+
9	Lead	-
10	Magnesium	+
11	Mercury	-
12	Nitrate	-
13	Phosphate	-
14	Potassium	+
15	Silver	-
16	Sulphate	+

+ ive indicates presence, - ive indicates absence

The powder shows the presence of Aluminium, Copper, Iron, Magnesium, Potassium and Sulphur. The quantitative estimation of these elements was carried out and the results are tabulated in Table 6.

Table 6: Quantitative estimation of inorganic elements

S.NO	INORGANIC ELEMENTS	CONTENT (μg/mg)
1	Aluminium	0.951
2	Copper	0.186
3	Calcium	0.254
4	Iron	1.718
5	Magnesium	0.053
6	Potassium	0.117
7	Sulphate	0.659

The quantitative estimation of heavy metals (As, Cd, Pb) showed that all these were within the specified limits. The results were tabulated in Table 7.

Table 7: Quantitative estimation of heavy metals

S.NO	ELEMENT	RESULTS (ppm/ml)	SPECIFICATION
1	Arsenic	0.002	Not more than 5.0 ppm
2	Cadmium	0.024	Not more than 0.3 ppm
3	Lead	0.070	Not more than 10 ppm

Preliminary phytochemical screening:

Table 8: phytochemical analysis of *operculina turpethum* root extract

Chemical constituents	Chemical test	Pet. Ether extract	Ethanol extract	Water extract
Alkaloids	Dragondorff's test	-	+	-
Amino acid	Millon's test	-	-	-
Carbohydrate	Molish test	-	+	+
Flavonoids	Shinoda test	-	+	+
Glycoside	Killer- Killiani test	-	+	-
Protein	Biuret test	-	-	-
Saponines	Froth formation test	-	+	+
Steroids	Libermann-Burchard test	-	+	-
Tannins	Ferric chloride test	-	+	+

- Absent ; + Present

Preliminary phytochemical screening: Extracts obtained by continuous Soxhlet were subjected for standard qualitative phytochemical tests to identify the presence of chemical constituents (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. (Table 8)

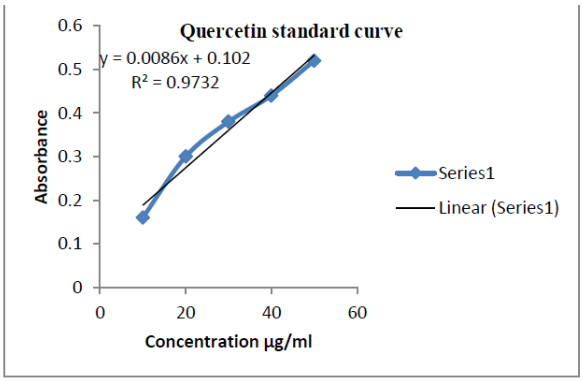
Flavonoid estimation:

The estimation of Flavonoids was carried out by the colorimetric method

Table-9: Total Flavonoid content of *Operculina turpethum* root extract

Concentration	Absorbance 415nm
10	0.16
20	0.30
30	0.38
40	0.44
50	0.52
Ethanol extract	0.42

Fig. 6: Calibration curve for the standard Quercetin



The concentration of Flavonoid present in Ethanol extract was found to be 24.34µg equivalent to Quercetin in 1 mg (2.43%).

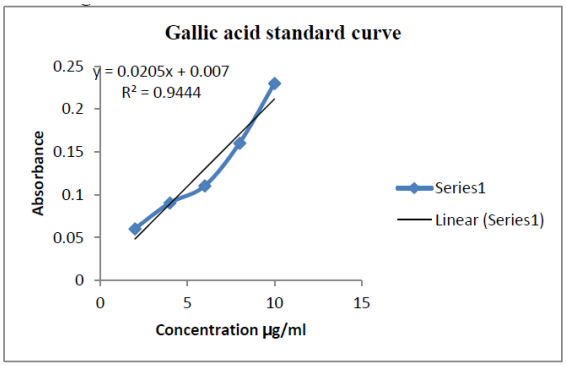
2. Phenol estimation

The estimation of Phenols was carried out by the colorimetric method

Table 10: Total Phenolic content of *Operculina turpethum* root extract

Concentration	Absorbance 415nm
2	0.06
4	0.09
6	0.11
8	0.16
10	0.23
Ethanol extract	0.18

Fig. 7: Calibration curve for the standard Gallic acid



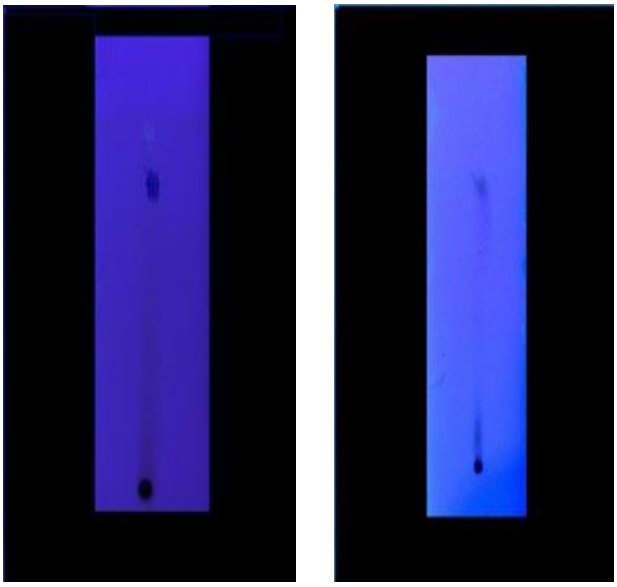
The concentration of Phenols present in Ethanol extract was found to be 8.30µg equivqlent to Gallic acid in 1 mg (0.80%).

Thin layer chromatography of ethanolic extract of *operculina turpethum*

The TLC of the ethanolic extract of root of *Operculina turpethum* was carried out to detect the presence of glycosides and flavonoids and the Rf values are tabulated in Table-22.

Table 11: Detection of Glycoside and flavonoids

EXTRACTS	MOBILE PHASE	Rf VALUE (UV)
Ethanol	Ethyl acetate : Methanol : Water (81:11:8)	0.48
Ethanol	Toluene : Ethyl acetate : Diethylamine (70:20:10)	0.43



A B

Fig. 8: Thin layer chromatography of *Operculina turpethum* root extract

Fig. 8.A: (Glycoside) Fig. 8.B :(Flavonoid)

TLC studies showed that the ethanolic extract showed a single spot in both glycoside and flavonoid solvent system.

PHARMACOLOGICAL EVALUATION

In vitro XO inhibitory activity:

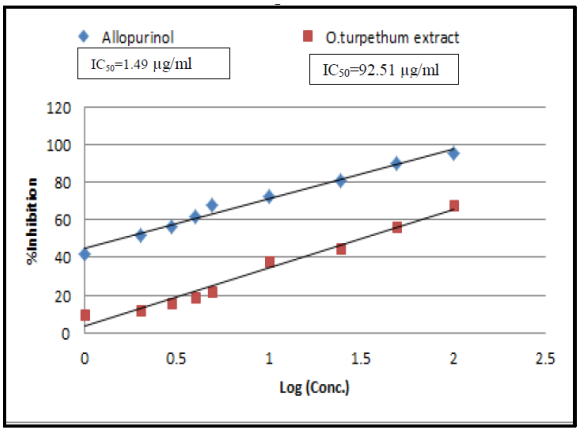
The inhibitory effects of Operculina turpethum extract and allopurinol on bovine milk xanthine oxidase at various concentrations are shown in Table 12. Each showed more than 50% inhibition of XO at a concentration of 100 µg mlG1. At the highest concentration of 100 µg mLG1, *Operculina turpetum* resulted in 68% XO inhibitory activity, while the standard XO inhibitor, allopurinol, showed 95% XO inhibitory activity at the same concentration. The xanthine oxidase inhibitory activity for both *operculina turpethum* extract and allopurinol was also expressed as an IC50, which represents the concentration of the standard drug or sample tested required for 50% inhibition of the activity of xanthine oxidase under the same experimental conditions.

The IC50 values were calculated according to the logarithmic dose-response curve using the program GraphPad Prism V 6.05 (GraphPad Prism Software, Inc., USA), the value was corresponding to 1.49 µg mlLG1 for allopurinol and 92.51 µg mLG1 for operculina turpethum extract.

Table 12: Xanthine oxidase inhibitory activity of *operculina turpethum* and allopurinol at different concentrations

Concentration (µg mLG1)	Concentration (µg mLG1)	
	Allopurinol	*Operculina turpethum* extract
100	95±1.5	68±2.2
50	90±0.6	56±0.7
25	81±1.1	45±1.8
10	72±2.1	38±1.4
5	68±2.0	22±0.3
4	62±0.4	19±0.2
3	56±0.6	16±1.1
2	52±1.6	12±0.8
1	42±1.2	10±1.2

Fig. 9: Xanthine oxidase inhibitory activity and IC_{50} values of *Operculina turpethum* extract and allopurinol



Anti-hyperuricemic activity:

The antihyperuricemic activity of *Operculina turpethum* ethanol extract on hyperuricemic rats using potassium oxonate were shown in Table 13 and Fig. 10. Potassium oxonate is uricase inhibitor which can induce hyperuricemia condition in rats. The rat which is induced by potassium oxonate can serve as a useful hyperuricemia animal model to evaluate sample that affect serum UA levels and also to evaluate possible therapeutic agents in certain disorders associated with abnormal UA levels. Treatment with potassium oxonate cause hyperuricemia in rats, as indicated with the drastic increasing in

UA levels compare to normal rats.

Table 13 and Fig. 10 show the antihyperuricemic activity of Operculina turpethum ethanol extract in hyperuricemic rats treated with potassium oxonate. Potassium oxonate is a uricase inhibitor that can cause hyperuricemia in rats. The rat induced by potassium oxonate can be used as a hyperuricemia animal model to test samples that affect serum UA levels, as well as to test potential therapeutic agents in certain disorders associated with abnormal UA levels. Treatment with potassium oxonate causes hyperuricemia in rats, as evidenced by a significant increase in UA levels when compared to normal rats.

UA level in all of treatment groups before potassium oxonate treatment at 0 h was not different from each other as well as with normal control rats. Each animal in normal control group which was received vehicle only (CMC-Na) showed no different in serum UA level. The orally pre-treatment using allopurinol as standard at a dose of 10 mg/kg bw elicited significantly reduction in UA level in hyperuricemia rat at 1-3rd hr after treatment ($p<0.05$). The UA level of allopurinol group 1-3rd h after orally treatment was not different compared to normal control group. This data indicated that treatment with allopurinol had completely recovery and could reduce serum UA level until normal condition. The profile UA level at 3rd hrs after sample administration still increased in hyperuricemic group, whereas the serum UA level in fraction group at 1st hr and 2nd hrs were significantly different compared to hyperuricemic group ($p<0.05$) and only at 1st hr showed no different with normal control. These data indicated that the ethanol extract had potential antihyperuricemic activity, however need longer time to return to normal condition.

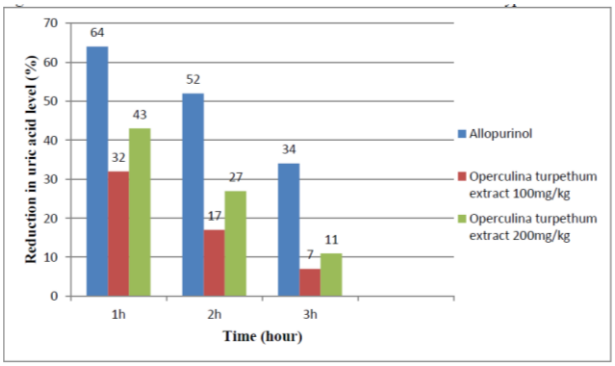
Table 13: Effect of *Operculina turpethum* root extract on serum uric acid level

Group	Dose (mg/kg)	Serum uric acid level (mg/dl			
		0h	1h	2h	3h
Normal control	-	2.63±0.33	2.42±0.37ry	2.34±0.28r	2.46±0.39
Hyperuricemic control	-	2.54±0.26	3.86±0.25cz	4.00±0.39cz	3.35±0.25cz
Allopurinol	10	2.46±0.36	1.82±0.40br	1.93±0.16r	2.20±0.51r
Operculina turpethum	100	2.74±0.36	2.78±0.19rz	3.30±0.28crz	3.21±0.28a
Operculina turpethum	200	2.52±0.20	2.63±0.16rz	3.23±0.23crz	3.09±0.65

Values are expressed as Mean±SEM for 6 rats.

a, b,c: Compared to normal control ($p<0.1$, 0.05, 0.01), p,q,r: Compared to hyperuricemic control ($p<0.1$, 0.05, 0.01), x,y,z: Compared to allopurinol group ($p<0.1$, 0.05, 0.01),

Fig. 10: Reduction in serum uric acid level after root extract treatment in hyperuricemia rat



Conclusion

Based on the findings of the present study, the *Operculina turpethum* root extract significantly reduced the uric acid levels in the blood of the potassium oxonate-induced hyperuricemic rats via xanthine oxidase inhibitory activity. The possible mode of action of the root extract could be related to the highly polyphenolic compounds contained in the ethanolic extract of the plant.

References

1. Al-Ashkar, Feyrouz (2010). "Gout and pseudogout". Disease Management Project. Cleveland Clinic. Retrieved 26 December2014.
2. Choi, Hyon K.; Mount, David B.; Reginato, Anthony M. (2005). "Pathogenesis of gout". Annals of Internal Medicine. 143 (7): 499–516
3. Gois, Pedro Henrique Franca, Souza, Edison Regio de Moraes (2020-09-02). "Pharmacotherapy for hyperuricaemia in hypertensive patients". The Cochrane Database of Systematic Reviews. 9: CD008652.
4. Dincer HE, Dincer AP, Levinson DJ. Asymto matic hyperuricemia: To treat or not to trea t. Cleveland Clin J Med 2002; 69:594-608.
5. Ghei M, Milhailescu M, Levinson D. Pathoge nesis of Hyperuricemia: Recent Advances. C urr Rheumatol Rep 2002; 4:270-4.
6. Mazzali M, Hughes J, Kim YG, Jefferson A, Ka ng DH, Gordon KL et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension 2001; 38: 1101-6.
7. Choi HK, Curhan G. Independent Impact of Gout on Mortality and Risk for Coronary He art Disease. Circulation 2007; 116: 894-900.
8. Owen PL, Johns T. Xanthine oxidase inhib itory activity of northeastern North Amer ican plant remedies used for gout. J Ethn opharmacol 1999; 64: 149-60.
9. Kong LD, Cai Y, Huang WW, Cheng CHK, Tan RX. Inhibition of xanthine oxidase by some Chinese medicinal plants used to t reat gout. J Ethnopharmacol 2000; 73: 199-207.
10. Nadkarni KM. Indian Materia Medica. Edition 3, Vol. I, Popular Prakashan, Bombay, 2007: 691-694.
11. Kirtikar KR, Basu BD, Indian Medicinal Plants. Vol.VII, International Book Distributors, Dehradun, 2005: 1730- 1732.
12. Sharma V & Singh M, Operculina turpethum as a panoramic herbal medicine: a review, International Journal of Pharmaceutical Sciences and Research 2012; 3: 21-25.
13. Kohli KR, Nipanikar SU, Kadbhane KP, A comprehensive review on trivrit [Operculina turpethum syn. Ipomoea turpethum], International Journal of Pharma and Bio Sciences, 2010; 1: 443-453.
14. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright-Scientechnica, Bristol, 1975:4-9.
15. Kokate CK. Practical Pharmacognosy. Edition 1, Vallabh Prakashan, New Delhi, 1994; 15-30.
16. Khandelwal KR. Practical Pharmacognosy. Edition 18, Nirali Publication, Pune, 2007; 10-14.
17. Government of India. Indian Pharmacopeia. Edition 4, Ministry of Health and Welfare, Controller of Publications, New Delhi, 1996; A53-A54.
18. Government of India. The Ayurvedic pharmacopoeia of India. Edition 1, Ministry of Health and Family Welfare, Department of Indian Systems of Medicines and Homeopathy, New Delhi, 1996; A53-A55.
19. WHO. Quality Control for Medicinal Plant Material. AITBS Publishers, New Delhi, 1998; 46- 47.
20. WHO. Quality Control Methods for Medicinal Plant Material. Geneva, 1992; 22-34.
21. Pravin Sopan Borhade* 1 , Tushar Atmaram Deshmukh 1 , Vijay Raghunath Patil 1 and Kishanchnad Radhesham Khandelwal Pharmacognostic and phytochemical investigations of operculina turpethum linn. root International Journal of Pharmaceutical Sciences and Research 2014; Vol. 5(6): 2387-2392.
22. Udaysing Hari Patil, Dattatraya K. Gaikwad. Seasonal dynamics in the nutritional and antinutritional status of stem bark of Anogeissus latifoli. International Journal of Applied Biology and Pharmaceutical Technology. 2011; 2(1): 370-378.
23. Boham AB, Kocipai AC. Flavonoid and condensed tannins from leaves of hawaian vaccininum vaticulum and viacalycinium. Pacific Science. 1994; 448: p.458-463.
24. Sunarni, T., I. Fidrianny, M.I. Iwo and K.R. Wirasutisna, 2017. Constituent and

antihyperuricemic activity of Stelechocarpus burahol leaves subfractions. Asian J. Pharm. Clin. Res., 10: 435-439.

25. Yumita, A., A.G. Suganda and E.Y. Sukandar, 2013. Xanthine oxidase inhibitory activity of some Indonesian medicinal plants and active fraction of selected plants. Int. J. Pharm. Pharm. Sci., 5: 293-296.
26. Liu X, Chen R, Shang Y, Jiao B, Huang C. Lithospermic acid as a novel xanthine oxidase inhibitor has anti-inflammatory and hypouricemic effects in rats. Chem Biol Interact 2008;176(2-3):137-42.