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PHARMACOGNOSTICAL AND PHARMACOLOGICAL EVALUATION OF *OXALIS CARNICULATA* LINN. FOR ANTI-DIPSOTROPIC AND ANTI-PYRETIC ACTIVITY

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ABSTRACT

Ayurveda is an ancient system of health care that is native to the Indian subcontinent. The present study was based on the pharmacognostic and pharmacological evaluation of O*xalis carniculata* linn. for anti-dipsotropic and anti-pyretic activity. *Oxalis corniculata* Linn. (Family: Oxalidaceae) is a well-known plant in India and is one of the most versatile medicinal plants having a wide spectrum of biological activity. All the chemicals with specifications were issued from the departmental store. The whole plant of *Oxalis carniculata* was collected from Moradabad area in the month of June & July. The whole plant was collected and washed with water. The whole plant was dried under the shade. The dried material was stored in air tight container. The whole plant was authenticated by Dr. G.P. Sinha Scientist-E & Head Botanical Survey of India Central Regional Centre 10, Chatham Line, Allahabad-211002, UP, India. Macroscopy, microscopy and physicochemical evaluation of *Oxalis carniculata*. The extraction was performed using Soxhlet apparatus then pharmacognostic and analytical evaluation of the herb were done. The extract was also used for acute toxicity to determine the dose. The anti-dipsotropic potential was evaluated through withdrawal anxiety and alcohol consumption model, however antipyretic activity was evaluated through brewer's yeast inducted pyrexia model. In aqueous extract treated animals, the rectal temperature was estimated as 38.63±0.14#ºC and 38.18±0.13#ºC at the dose of 200mg/kg and 400mg/kg, respectively that showed for its anti-pyretic action. In our study on day 11, the ethanol-treated animals spent significantly more time in the ethanol-paired chamber as compared to the saline-paired chamber (P<0.001). EE (200 and 400 mg/kg) administration led to a dose-dependent reversal of withdrawal anxiety as evidenced by significant increase in time spent in the open arm ($P < 0.01$). In concluded that in anti-dipsotropic activity, EE200 and EE400 showed significant increase in the time spent in open arm of plus maze indicating the reduction in anxiety after alcohol withdrawal and reduction in the total ethanol consumption. This indicates that Oxalis *corniculata* Linn. have significant anti-dipsotropic and anti-pyretic activity.

Keywords: *Oxalis carniculata,* phytoconstituents, herbal extract, anti-dipsotropic, anti-pyretic.

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INTRODUCTION

Ayurveda is an ancient system of health care that is native to the Indian subcontinent. It is presently in daily use by millions of people in India, Nepal, Sri Lanka, China, Tibet, and Pakistan. The word "Ayurveda" is a tatpurusha compound of the word a*yus* meaning "life" or "life principle", and the word *veda*, which refers to a system of "knowledge". According to Charaka Samhita, "life" itself is defined as the "combination of the body, sense organs, mind and soul, the factor responsible for preventing decay and death" [1]. According to tradition, Ayurveda was first described in text form by Agnivesha, named *Agnivesh tantra*. The book was later redacted by Charaka, and became known as the Charaka Samhita. Another early text of Ayurveda is the Sushruta Samhita, which was compiled by Sushrut, the primary pupil of Dhanvantri, sometime around 1000 BC. Dhanvantri is known as the Father of Surgery [2].

According to the *Charaka Samhita* (400-200 BC), the Ayurvedic theories of aetiology are based on Nyaya *darsana* (one of the major schools of ancient philosophy in India) that presents four scientific methods of proof [3][4]:

1. The word, based on the experience and intuition of the sages (*shabda*)
2. Direct observation (*pratyaksha*)
3. Inference and deductive logic (*anumana*)
4. Experimentation (*yukti*)

Basic Principles of Ayurveda [4]

Ayurveda is based on the following theories:

- Pancha Mahabhuta Theory (Five Elements)
- Tri-dosha theory (Three Body Humors)
- Sapta-dhatu theory (Seven Body Tissues)

Plant Profile

Oxalis corniculata (Family: Oxalidaceae) is a well-known plant in India and is one of the most versatile medicinal plants having a wide spectrum of biological activity. It is commonly known as creeping wood sorrel, an excellent plant in the nature having composition of all the essential constituents that are required for normal and good health of humans. Herb is a good appetizer, removes kapha, vata, and piles; astringent cures dysentery and diarrhoeas, skin diseases and quarten fevers. [7,8,9] An infusion of the small leaves is externally used to remove warts and opacities of cornea. The leaves are anti-inflammatory, refrigerant and antiscorbutic.

The Indian system of medicine is replete with medicinal plants claimed to its works also for the antialcoholic and antipyretic activity. Plants like *Pueraria lobata* (Fabaceae), *Hypericum perforatum* (Clusiaceae), *Tabernanthe iboga* (Apocynaceae), as well as *Panax ginseng* (Araliaceae) and related species have been investigated for their effect on the alcohol dependence. These plants have been grouped under the general class of rejuvenators i.e., drugs that counter the degenerative changes associated with ageing [10].

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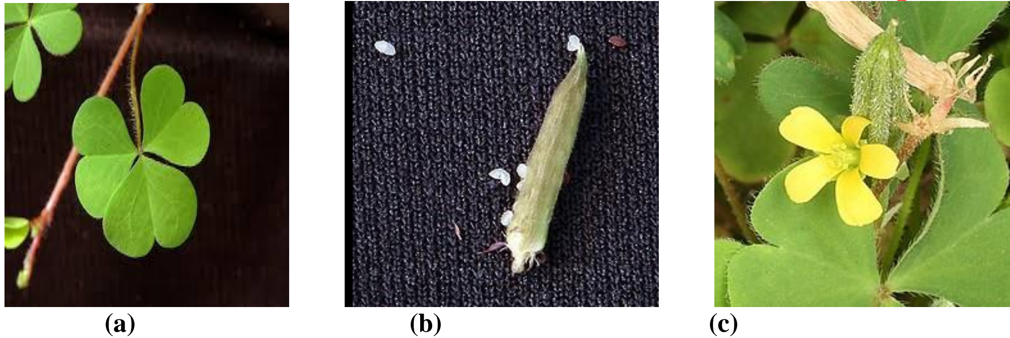

(a) (b) (c)

Fig 1. *Oxalis corniculate* (a) Leaves (b) Fruits (c) Flower

Vernacular Names

Sanskrit: Ambashta, Amlalonika, Amlapatrika, Amlika,
Hindi: Seh-patti, Tinpatiya, Anboti, Chuka tripati, Bhilmori, Khatari
English: Indian sorrel
Urdu: Khatti-buti
Assamese: Changeritenga, Saru tengesi
Bengali: Amrul-sak, Amrul shak, Amrul, Tandi chatom arak, Amrool
Kannada: Huli-huniche, Hulihunice, Pullam-purachi-sappu, Teltuppi
Tamil: Palaikiri, Puliyarail
Telugu: Ambotikura, Pulichintha, Pallachintha
Marathi: Ambali, Chicha
Malayalam: Poliyarala, Puliyaral, Puliyarala, Puliyarila, Pullampurachi
Marathi: Umbuti, Ambuti, Bhinsarpati, Aambotee, Ambata chukaa
Oriya: Sialthur, Siakthur, Ambo chingari
Arabic: Hememdab, Hemda, Homadmad [5]

Classification

Kingdom- Planate
Subkingdom- Tracheobionta
Division- Magnoliophyta
Class- Magnoliopsida
Subclass- Rosidae
Order- Geraniales
Family- Oxalidaceae
Genus- Oxalis
Species- corniculate [6]

Oxalis corniculata is a plant traditionally used to treat dependence on alcohol, treatment of fever, as a rejuvenating drug and also used for urinary tract infections. The whole plant extract of *Oxalis corniculata* has been reported for whole plant is edible and used in salads to be eaten raw. Leaves and flowers are used as remedy for fever, influenza, diarrhea, traumatic injuries, anthelmintic, astringent, antiscorbutic, diuretic, stomachic and febrifuge [11-13]

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Morphology and habitat
It is a somewhat delicate-appearing, low-growing, herbaceous plant abundantly distributed in damp shady places, roadsides, plantations, lawns, nearly all regions throughout the warmer parts of India, especially in the Himalayas up to 8,000 ft –cosmopolitan. It is also distributed in ballast about the eastern seaport town of the United States and becomes quite abundant in Texas and Ontario. These weeds are found throughout Florida. They are common in the southeastern United States; from Newfoundland to North Dakota; and southward to Mexico. *Oxalis corniculata* Linn. is a cosmopolitan weed occurring in the Old World and in temperate and tropical regions of North, Central and South America and the West Indies. [14][15]

Phytoconstituents
Photochemical investigation of plant *Oxalis corniculata* Linn. have revealed the presence of flavanoids, tannins, phytosterol, phenol, glycoseides, fatty acids and volatile oil. The leaves contain flavonoids, iso vitexine and vitexine-2"- O- beta – D- glucopyrunoside. It is rich source of essential fatty acids like palmitic acid, oleic, linoleic, linolenic and stearic acids. They are good source of vitamin C (125 mg/100g), carotene (3.6 mg/100g), but contain a high content of oxalates (12% of dry material). The leaves and stem contain tartaric and citric acid; stem contains malic acid. [16]

Methanolic and ethanolic extracts of this plant show the presence of carbohydrate, glycosides, phytosterols, phenolic compounds, flavanoids, proteins (12.5%), amino acids and volatile oil. Leaves contain carbohydrate (1.36 ± 0.3%), fatty acids (13.2± 0.7%); Palmitic acid 1.8%, mixture of oleic, linoleic and lenolenic acid 3.8%), protein (12.5 ± 0.5%), fiber (6.2 ± 0.3%), tannin (0.62 ± 0.3%), moisture (92.3 ± 2.8%). Whole plant extract suspected to contains phytoestrogens. *Oxalis corniculata* identified several compounds that were characterized by nuclear magnetic resonance, infrared, and mass spectrometry as (i) Oc-1, a mixture of saturated fatty acids C24 to C28; (ii) Oc-2, a mixture of long-chain alcohols C18 to C28; and (iii) Oc-3, a single compound that was a galacto-glycerolipid [17-19].

MATERIALS AND METHODS

Chemicals
All the chemicals with specifications were issued from the departmental store.

Collection and authentication of the plant
The whole plant of *Oxalis carniculata* was collected from Moradabad area in the month of June & July. The whole plant was collected and washed with water. The whole plant was dried under the shade. The dried material was stored in air tight container.[20] The whole plant was authenticated by Dr. G.P. Sinha Scientist-E & Head Botanical Survey of India Central Regional Centre 10, Chatham Line, Allahabad-211002 (Uttar Pradesh) India.

Physicochemical evaluation of herb
The parameters studied were loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values, petroleum ether soluble extractive value, according to the methods outlined by Khandelwal.

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Loss on Drying (LOD)

Loss on drying is a widely used test method to determine the moisture content of a sample, although occasionally it may refer to the loss of any volatile matter from the sample. Loss in drying does not usually refer to molecularly bound water or water of crystallisation. The % loss on drying is calculated by the following equation:

% loss on drying at 105 C (LOD)= W_{start} - W_{dry} / W_{start} – W_{tare} × 100

Determination of Total Ash

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred platinum or silica dish at a temperature not exceeding 450ºC until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450º. Calculate the percentage of ash with reference to the air-dried drug. Ash value can be calculated by using formula: -

$$\text{Ash value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Determination of Acid Insoluble Ash

Boil the ash obtained in b for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air-dried drug.

Determination of Water-Soluble Ash

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450º. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air dried drug.[21]

Determination of Alcohol Soluble Extractive

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105º, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Extraction of *Oxalis carniculata*

Pet. Ether extract (PE) was prepared by continues percolation method by using Soxhlet apparatus. Same material was then extracted in ethanol (EE) by same method. Aqueous extract (AE) was prepared with the maceration method.

Analytical evaluation of Extract

a. Thin Layer Chromatography

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through ad sorption and a mobile phase in the form of a

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liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent. Identification can be affected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Selection of mobile phase

Solvent mixture was selected on the basis of the phyto-constituents present in each extract. Solvents were analyzed as its order of increasing polarity. Several mobile phases were tried for the separation of maximum components. After several trials, the best solvent system was selected which showed good separation with maximum number of components.

PHARMACOLOGICAL EVALUATION

Preparation of animals

Wister rats were used for the study. The animals were housed in the animal house of IFTM University. They were given *ad libitum* and free water access. The temp. and humidity were controlled. The protocol of the study was passed by the IACE of the university.

The animals were divided into three groups consisting 6 animals in each group

Acute Toxicity study

Acute oral toxicity was evaluated by OECD guideline 425: Fixed Dose Procedure.

Antipyretic activity

Brewer's yeast induced pyrexia in rats

This antipyretic activity animal model was slightly modified method described by Adams *et al*. Antipyretic activity on Wistar rats was screened with Brewer's yeast induced pyrexia. The rats were divided into eight groups of six each. The basal rectal temperature of the rats. was measured by introducing 1-2 cm of digital thermometer in rectum. After measuring the basal rectal temperature, the pyrexia was induced by intra peritoneal injection, 20% suspension of brewer's yeast in normal saline at a dose of 10 ml/kg of body weight. After 18 hrs of yeast injection, rats which showed a raise in temperature of at least 1°C were taken for the study. Immediately after 18 hrs of yeast injection, animals in the various groups were treated as follows: [22,23].

Group1: Normal saline
Group2: PE (200 mg/kg) suspended in the Twin 80
Group3: PE (400 mg/kg) suspended in the Twin 80
Group4: EE (200 mg/kg) suspended in the Twin 80
Group5: EE (400 mg/kg) suspended in the Twin 80
Group6: AE (200 mg/kg)
Group7: AE (400 mg/kg)
Group8: Paracetamol (100 mg/kg)

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Anti-dipsotropic Activity

Development of conditioned place preference model

Apparatus

The conditioned place preference (CPP) apparatus contains three compartments. The two end compartments (30.5 cm × 26.5 cm × 37 cm) were connected by a central corridor (12.75 cm × 23 cm × 15.25 cm). The compartment on the left had black walls with a perforated stainless-steel floor with round holes on staggered centers. The central corridor was transparent with a smooth plexiglass floor, and the right compartment had white walls with a stainless-steel mesh floor.

Procedure

(1) Preconditioning phase: (first and second day) The animals were placed in the middle chamber and allowed to explore both the chambers for 30 min.

(2) Conditioning phase: (3rd - 10th day) Each mouse was treated for eight consecutive sessions with the alternate oral administration of alcohol and saline. On days 3, 5, 7, and 9, the animals were administered ethanol (2 g/kg body weight; i.p. 10% [v/v]) and placed in one compartment for 30 min. Besides, on days 4, 6, 8, and 10, the animals were administered saline and placed in opposite compartment.

(3) Post conditioning phase: (11th–12th day) Mice were placed in the middle chamber and allowed free access to both chambers for 30 min. Time spent in ethanol and saline-paired chamber was measured.

(4) Treatment protocol: After development of withdrawal (15th day), the following treatment schedule was followed:

Group 1: ethanol
Group 2: saline/ abstinence
Group 3: ethanol + PE (200 mg/kg)
Group 4: ethanol + PE (400 mg/kg)
Group 5: ethanol + EE (200 mg/kg)
Group 6: ethanol + EE (400 mg/kg)
Group 7: ethanol + AE (200 mg/kg)
Group 8: ethanol + AE (400 mg/kg)
Group 9: ethanol + diazepam (1 mg/kg)

The behavioral tests were performed 60 min after oral drug administration and 30 min after intraperitoneal administration.

Behavioral studies to measure alcohol withdrawal anxiety

Elevated plus maze

The model has been validated pharmacologically and currently considered the "gold standard" test of anxiety-related behavior. Elevated plus maze was performed as described by Kokare *et al.*[13] In summary, after drug treatment, individual mice were placed at the center of the maze, head facing an open arm. During the 5 min test period, the number of entries and time spent on the open arm were recorded.

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Chronic-treatment study to measure alcohol intake (Two-bottle choice ethanol drinking)

We used the standard two-bottle choice protocol, which is a widely used animal model to capture aspects of voluntary alcohol consumption in humans.[14] Following 7 days of acclimatization, animals were subjected to an ethanol drinking acquisition regimen. The animals remained in their home cages at all times throughout the study but had their water bottles removed during a 4 h and ethanol presentation period. During this time, animals were exposed to a free choice between ethanol (15% v/v) and water for 20 days but with no drug pretreatment.

After 20 days of ethanol administration, animals were divided into different groups for 10 days of treatment. Each day, the bottles were weighed before and after 4 h of limited access period and the differences were used to calculate the water and ethanol intake. The mean intake was expressed as g/kg body weight/day of water and g/kg body weight/day of ethanol intake. All animals were given unrestricted food access. Every 2 days, the bottles were switched to eliminate place preference.[15] After 20 days of pretreatment with ethanol (15% v/v), the animals were divided into different treatment groups (n = 7 per group) as follows:

Group 1: (control) received saline 30 days

Group 2: received free choice ethanol (15% v/v)/water 30 days

Group 3: received free choice ethanol (15% v/v)/water and PE (200 mg/kg) 21st–30th day

Group 3: received free choice ethanol (15% v/v)/water and PE (400 mg/kg) 21st–30th day

Group 3: received free choice ethanol (15% v/v)/water and EE (200 mg/kg) 21st–30th day

Group 3: received free choice ethanol (15% v/v)/water and EE (400 mg/kg) 21st–30th day

Group 3: received free choice ethanol (15% v/v)/water and AE (200 mg/kg) 21st–30th day

Group 3: received free choice ethanol (15% v/v)/water and AE (400 mg/kg) 21st–30th day

Group 4: received free choice ethanol (15% v/v)/water and diazepam 21st–30th day.

RESULT AND DISCUSSION

Macroscopy

Root: Dark brownish, thin, about 1-2 mm thick, branched, rough, soft; no odour and taste.

Stem: Creeping, brownish-red, soft, very thin, easily breakable; no odour and taste.

Leaf: Palmately compound, trifoliate; petiole-green, thin, about 3-9 cm long, cylindrical, pubescent; leaflet-green, 1-2 cm long, obcordate, glabrous, sessile or sub sessile; taste, somewhat sour.

Flower: Yellow, axillary, sub-umbellate.

Fruit: Capsules cylindrical, tomentose.

Seed: Tiny, dark brown, numerous, broadly ovoid transversely striate.

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Fig 2. Whole Plant (O. carniculata)

Fig 3. Flower Of O. carniculata

Fig 4. Fruit of *O. carniculata*

Fig 5. Leaf of *O. carniculata*

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Microscopy

Powder (leaf) Microscopy

Microscopic examination indicated the presence of spiral and annular vessels. These vessels were covered by parenchymatous tissues. Parenchyma tissues were composed of simple thin-walled cells with greenish chlorophyll in it. Some crystals and other cellular organelles were present in the powder. Epidermal cells were more or less rectangular with transparent cell contents. These cells had limited and small inter cellular spaces thus closely packed.

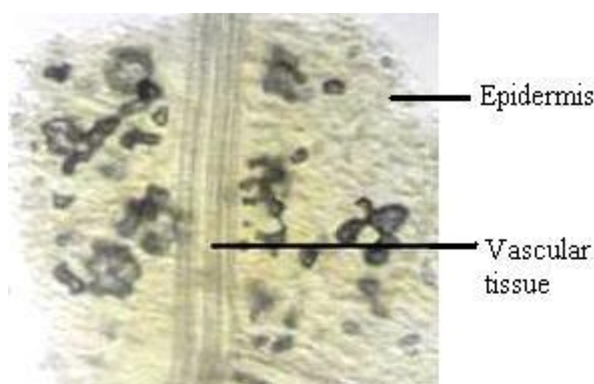

Fig 6. Leaf Microscopy

Transverse section of stem

Cortical tissues and pith region are also light green. There is a layer of thick-walled sclerenchymatous tissues all around the vascular area. The sclerenchyma tissues are also stained with safranin and probably 2–3 layered in thickness. The cells of this layer are moderately thickened. The pith region has largest size cells than other areas. Some of these cells have packed starch grains and crystals. Xylem is towards the center of the section while phloem is outside. Cambium is not fully visible. Few stomata are visible on the epidermis tissue. Guard cells and subsidiary cells are not fully visible. Non glandular trichomes are seen in the form of single layer. They are uniseriate. The transverse section of stem is shown in Figure.

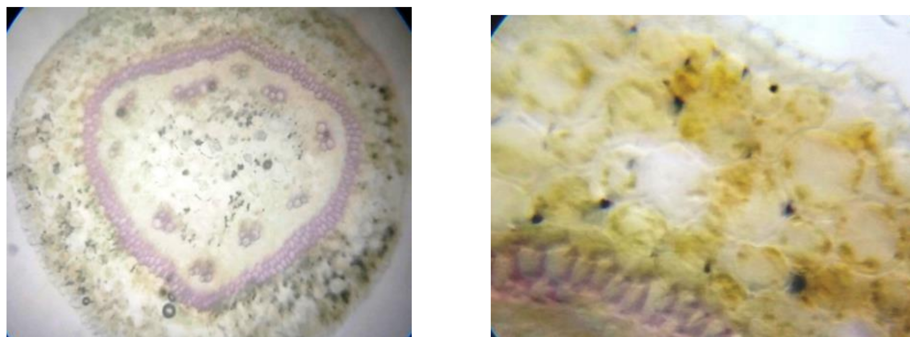

Fig 7. Stem microscopy

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Extraction of *Oxalis carniculata*

Fig 7. Extraction of hreb

Successive extract was performed and pet. Ether (PE), ethanol (EE) and aqueous (AE) extracts were prepared, dried and stored.

Physicochemical evaluation of herb

The results of physicochemical evaluation were compared with the monograph of this drug given in the Ayurvedic Pharmacopoeia of India and were found within the limit. This value ensures the purity and strength of the herb.

Table 1. Physicochemical evaluations

Physicochemical parameters	Values
Loss on drying	5.83±0.45% w/w
Total ash	11.4±1.24% w/w
Acid insoluble ash	2.65±0.08% w/w
Water soluble ash	2.80±0.02% w/w
Alcohol Soluble Extractive	12.39±1.42% w/w
Water Soluble Extractive	04.23±0.94% w/w

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Thin Layer Chromatography

Preparation of TLC Plate: 42 g of silica gel was dissolved in 25 ml chloroform, 25 ml methanol. Prepared the TLC plates by spreading the gel on it.

Marking the TLC Plate: The silica gel TLC plates were marked by using pencil.

Activation of TLC Plate: Placed the TLC plate in an oven at 50-600C for 15-20 min to "activate it". Activation involves driving of water molecules that bond to the polar sites on the plate.

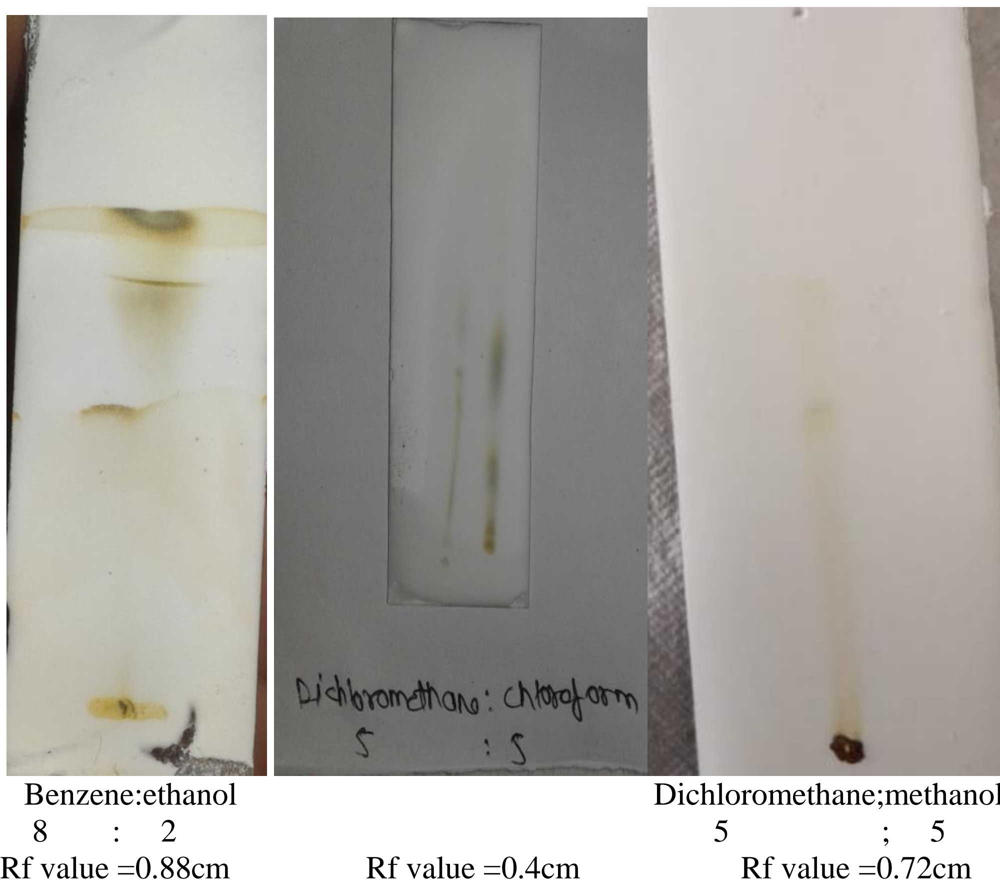
Spotting the TLC Plate: The narrow end of capillary was placed into the extract. When extract rises into the capillary then touch the capillary on the silica plate very carefully. Allowed the solvent to completely evaporate from the spot.

Developing the TLC Plate: The TLC plate was placed very carefully in the developing bottle containing mobile phase solvent system. Left it for some time so that solvent front can move.

Drying the Plate: Placed the slide in an oven at Temperature 50-60 to evaporate the solvent [60,61,62].

Maximum numbers of spots were observed in Benzene: ethanol in the ratio of 8:2

Developer - 15% H_2SO_4 in n-butanol



Benzene:ethanol 8 : 2		Dichloromethane;methanol 5 ; 5
Rf value =0.88cm	Rf value =0.4cm	Rf value =0.72cm

Fig 8. Images of developed TLC

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Phytochemical screening

It refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

Table 2. Phytochemical screening of ethanol and aqueous Extract

Phytochemicals	Ethanol	Pet. ether	Aqueous
Alkaloids	+	-	+
Flavonoids	+	-	+
Terpenoids	-	-	-
saponins	-	-	+
Carbohydrates	+	-	+
tannins	-	-	-
proteins	+	-	-
Steroids & phytosteroids	+	+	-
Glycosides	+	-	-
Phenol	-	+	-

Pharmacological evaluation

a. Acute oral toxicity (OECD guideline 425: Fixed Dose Procedure):

In this study the changes were observed in animals for 14days. No changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic, central nervous systems activity and behaviour pattern was observed. Special attention was made of observations of tremors, convulsions, salivation, diarrhoea, lethargy, coma or death. None of such symptoms were observed in animals at the dose of 2000 mg/kg body weight so it can be concluded that the LD50 for extract of *O. carniculata* L. is more than 2000mg/kg body weight.

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b. Antipyretic activity
Brewer's yeast induced pyrexia in rats

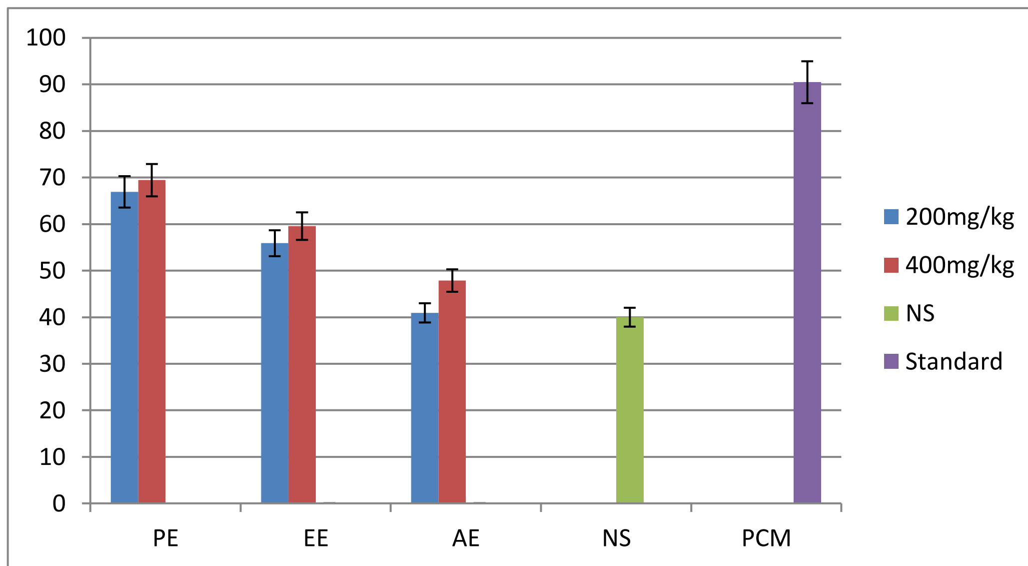

Fig 9. Effect of the extract on the pyrexia (fever)

In aqueous extract treated animals, the rectal temperature was estimated as 38.63±0.14#ºC and 38.18±0.13#ºC at the dose of 200mg/kg and 400mg/kg, respectively that showed for its anti-pyretic action.

Table 3. Effect of extract on the rats in 4 hrs

Group	Dose (mg/Kg)	Initial rectal temp. (ºC)	Rectal temp. (ºC) 18 hrs after Brewer`s yeast induction	Rectal temp.(ºC) after treatment with extract			
				1hr	2hrs	3hrs	4hrs
Control	-	37.07±0.25	39.35±0.22	39.62±0.38	39.57±0.36	39.45±0.36#	39.33±0.37#
PE	200 400	37.10±0.26 37.10±0.29	39.25±0.25 39.20±0.14	38.93±0.39 ** 38.97±0.37 **	38.62±0.40 # 38.37±0.21 #	38.30±0.29# 38.05±0.23#	37.98±0.37# 37.77±0.16#

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EE	200 400	37.10±0.26 37.08±0.25	39.28±0.13 39.30±0.28	39.07±0.18 * 39.12±0.35 *	38.85±0.14 # 38.90±0.39 #	38.62±0.16# 38.50±0.32#	38.45±0.23# 38.08±0.21#
AE	200 400	37.07±0.18 37.08±0.17	39.32±0.29 39.35±0.33	39.12±0.22 * 39.02±0.15 **	38.70±0.11 # 38.85±0.14 #	38.63±0.14# 38.18±0.13#	38.30±0.23# 37.85±0.16#
PCM	100	37.10±0.18	39.27±0.18	38.58±0.26 #	38.22±0.19 #	37.50±0.29#	37.32±0.19#

C. Anti-dipsotropic Activity

Development of conditioned place preference model

In CPP, the animal's choice to spend more time in either environment provides a direct measure of the conditioned reinforcing effect of a drug. Animals were found to prefer the ethanol-paired chamber over the saline-paired chamber. In our study on day 11, the ethanol-treated animals spent significantly more time in the ethanol-paired chamber as compared to the saline-paired chamber ($P < 0.001$). Animals spent about 70% of total time in ethanol-paired chamber versus the saline-paired. For the control group (saline-treated), the time spent in both the chambers were comparable. The CPP results suggested that the animals got addicted to alcohol.

Table 4. Time spent in ethanol and saline paired chamber

Group	**Time spent in seconds**	
	Ethanol paired chamber	**Saline paired chamber**
Ethanol treated group	222±45.60	76±36.29
Saline treated group	105±68.85	194±26.78

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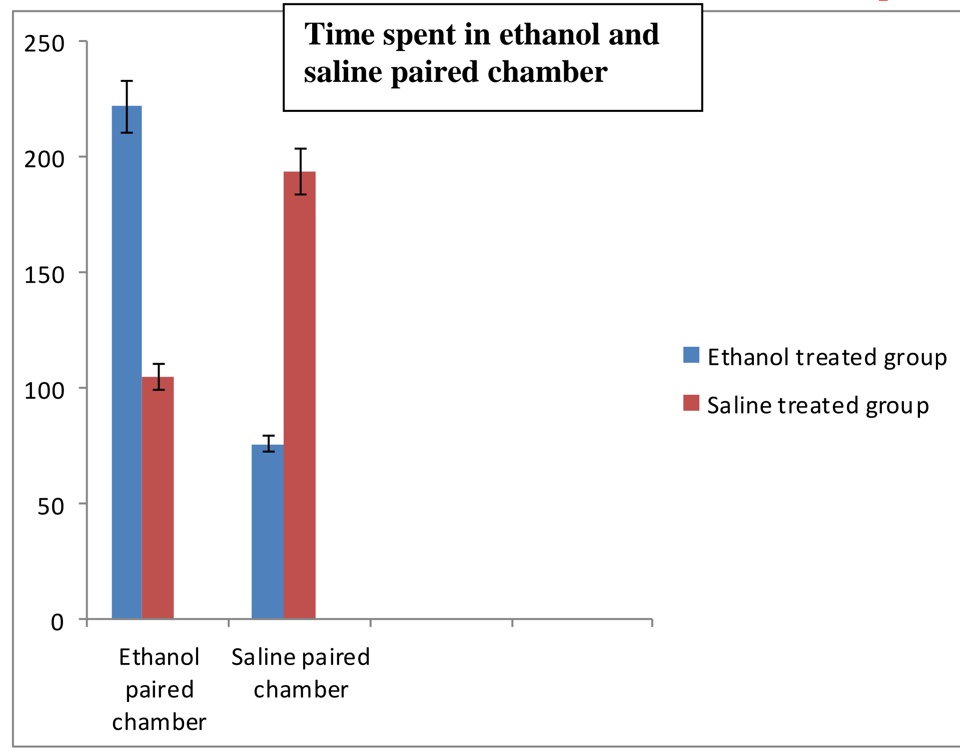

Fig 10. CPP on ethanol administration

Time spent in alcohol and saline administration chamber on day 11 by control and ethanol-treated group. Ethanol-treated animals showed significant increase in ($P < 0.001$) time spent in ethanol-paired alcohol compared with saline-paired chamber. Values represent mean ± standard error of the mean.

Effect on withdrawal anxiety

In the present study, according to the CPP model described by Thanos *et al*., with minor modifications, acute ethanol withdrawal anxiety was developed and measured using elevated plus maze test. Five days of abstinence followed 10 days of conditioning phase in which alternate dose of ethanol and saline was given for 10 days. After 5 days of abstinence from ethanol, animals showed a significant decrease in time spent ($P < 0.01$) in the open arm of the elevated plus maze as compared with the control, suggesting withdrawal anxiety. EE (200 and 400 mg/kg) administration led to a dose-dependent reversal of withdrawal anxiety as evidenced by significant increase in time spent in the open arm ($P < 0.01$). Both, EE (200 and 400 mg/kg) and diazepam showed comparable anxiolytic potential against ethanol withdrawal anxiety.

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Table 5. Effect on withdrawal anxiety

Group	Time spent in open arm (Sec ±SEM)
ethanol	36±9.50
saline/ abstinence	18±3.46
ethanol + PE (200 mg/kg)	32±4.20
ethanol + PE (400 mg/kg)	38±4.33
ethanol + EE (200 mg/kg)	48±6.45
ethanol + EE (400 mg/kg)	76±6.56
ethanol + AE (200 mg/kg)	29±5.43
ethanol + AE (400 mg/kg)	35±5.47
ethanol + diazepam (1 mg/kg)	82±7.86

Table 5.8(c)

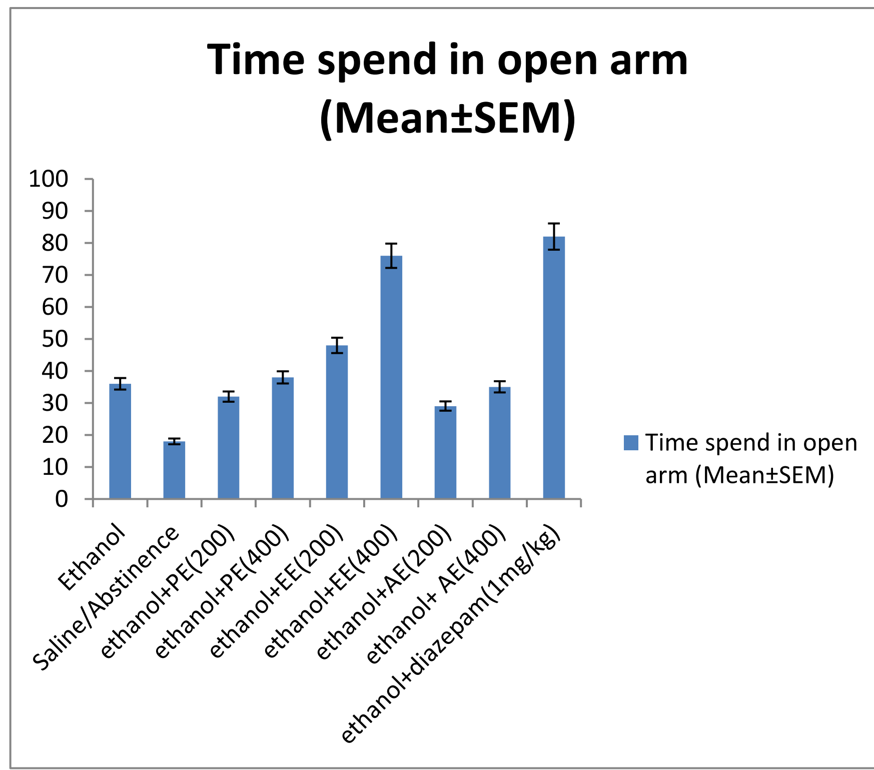

Fig 11. Effect on withdrawal anxiety

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Effect on alcohol consumption

Shankhpushpi-treated animals showed a significant ($P < 0.001$, $n = 7$) decrease in ethanol and water intake as compared with the control group after day 24 or 4 days postshankhpushpi therapy. This was comparable with diazepam-treated animals, who also showed a significant decrease ($P < 0.001$, $n = 7$) in ethanol intake. However, animals' administration with $GABA_A$ blocker followed by shankhpushpi failed to show a decrease in ethanol and increase in water intake till day 30. The above results suggest that shankhpushpi prevented chronic ethanol intake,

Table 6. Effect on alcohol consumption

Day Group	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Control	2.6±0.5	4.7±0.8	5.4±0.5	6.5±0.5	7.3±0.6	7.9±1.2	8.1±0.9	8.0±0.7	8.4±0.5	8.4±0.4	8.5±0.5	8.6±0.4	8.8±0.4	8.8±0.7	8.9±0.6
PE 200	2.7±0.8	4.1±0.3	5.1±0.4	6.2±0.6	7.3±0.3	7.7±1.0	8.3±0.7	8.5±0.4	8.5±0.9	8.5±0.5	8.6±0.6	8.6±0.5	8.2±0.4	7.8±0.3	7.5±0.3
PE 400	2.1±1.0	4.6±0.7	5.4±0.5	6.1±0.2	7.4±0.2	8.1±1.1	8.0±0.4	8.3±0.5	8.5±0.4	8.6±0.5	8.6±0.7	8.4±0.7	8.0±0.2	7.7±0.8	7.6±0.8
EE 200	2.6±0.6	4.3±0.4	5.0±0.4	5.9±0.5	7.1±0.2	8.2±0.8	8.2±0.5	8.3±0.7	8.4±0.8	8.5±0.4	8.5±0.6	7.8±0.5	7.1±0.3	6.7±0.5	6.4±0.4
EE 400	2.9±0.8	4.6±0.5	5.1±0.9	6.5±0.7	7.2±1.0	7.9±0.7	8.5±0.5	8.4±0.8	8.5±0.7	8.5±0.5	8.6±0.6	7.2±0.7	6.8±0.6	6.3±0.4	6.0±0.6
AE 200	2.4±0.4	4.9±0.3	5.4±0.3	6.4±0.8	7.4±0.8	8.4±1.2	8.4±0.9	8.4±0.8	8.3±0.3	8.5±0.6	8.8±0.4	8.6±0.6	8.2±0.7	7.8±0.5	7.5±0.5
AE 400	2.8±0.6	4.3±0.7	5.3±0.6	6.0±0.7	7.5±0.7	8.3±0.6	8.2±0.4	8.4±0.6	8.5±0.7	8.6±0.5	8.5±0.7	8.1±0.3	7.8±0.6	7.6±0.4	7.2±0.3
Diazepam	2.8±0.9	4.5±0.4	5.6±0.5	6.1±0.2	7.3±0.6	8.0±1.2	8.1±0.6	8.6±0.9	8.6±0.7	8.6±0.5	8.7±0.4	7.7±0.4	7.0±0.8	6.5±0.8	5.6±0.7

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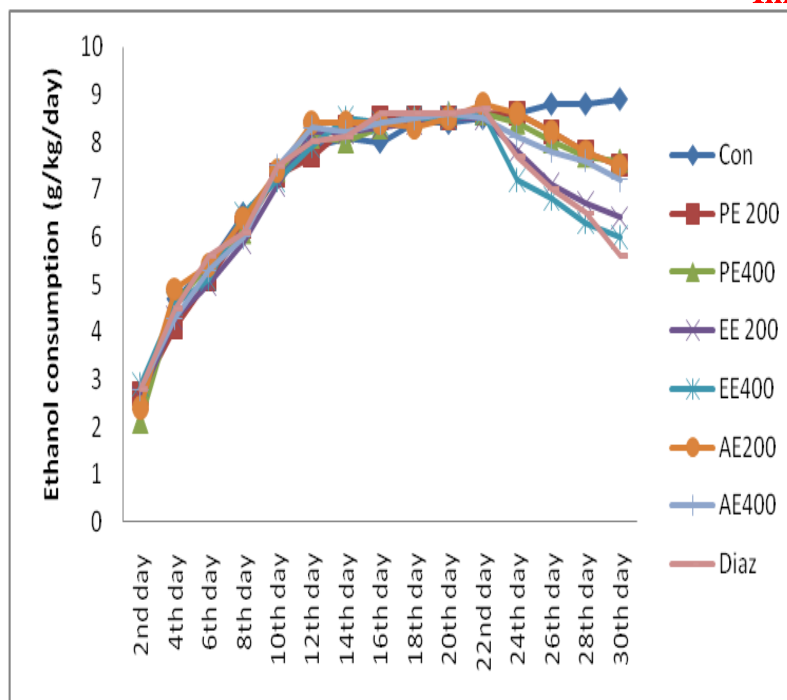

Fig 12. Effect on alcohol consumption

CONCLUSION

The aim of the study was to evaluate *Oxalis corniculata* Linn. for anti-dipsotropic and anti-pyretic activity. For this, whole plants of *Oxalis corniculata* Linn. were collected from Moradabad Area and authenticated. The macroscopical and microscopical features were studied. Physicochemical parameters were evaluated. After this, successive extraction was done in petroleum ether, ethanol and water to get respective extracts PE, EE and AE. Thin layer chromatography and preliminary phytochemical screening was done. Acute toxicity studies were performed and extracts were found safe upto the dose of 2000mg/kg boy weight. After this, Pharmacological evaluation was performed for Antipyretic activity and anti-dipsotropic activities. For this, six test groups of animals were formed and treated with PE200, PE400, EE200, EE400, AE200 and AE400 respectively. In antipyretic studies PE400 group showed significant reduction in body temperature compared to control group. In concluded that in anti-dipsotropic activity, EE200 and EE400 showed significant increase in the time spent in open arm of plus maze indicating the reduction in anxiety after alcohol withdrawal and reduction in the total ethanol consumption. This indicates that Oxalis *corniculata* Linn. have significant anti-dipsotropic and anti-pyretic activity.

FUNDING

Nil.

CONFLICT OF INTEREST

Authors declared for none ‘conflict of interest’.

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