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Pharmacognostic and phytopharmacological investigation of *oxalis corniculata* L. (oxalidaceae)

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ABSTRACT

The pharmacognostical studies of *Oxalis corniculata* L. belongs to family Oxalidaceae was done. In macroscopic studies, it is observed that the leaves are green and flowers are yellow color, pseudo umbels, axially, 1-6 flowered bracts two, linear, bracteole, sepals five lanceolate, petals are oblongata in nature apex and emarginated. Leaves are 3-foliate, leaflets obcordate, Chartaceous, pilose base cunate, margin entire. In this plant fruits are capsule in nature, oblong, abruptly tapering above; puberulous seeds are numerous per locule, ovoid transversely. Physicochemical properties are an important parameter in detecting adulteration on improper handling of the drug. In the evaluation of crude drug, ash values, extractive values are important parameters. The estimation of ash value is useful for detecting low- grade products, exhausted drugs and excess of sandy matter. The determination of extractive values with a range of solvents gives information about extractable non-polar and polar as well as total extractable plant constituents. The pharmacognostical studies of the plant were carried out with a focus on bringing out diagnostic characters will be of immense help in the proper identification and standardization of botanical species of the plant drugs. Which play a major role to establish the particular standards and helps to minimize the adulteration of the plant *Oxalis corniculata* L.

Keywords: *Oxalis corniculata* L., obcordate, Chartaceous, pilose base cunate, botanical species, 3-foliate

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1. Introduction

The plant *Oxalis corniculata* L. (Creeping wood sorrel) also called procumbent yellow sorrel belongs to family Oxalidaceae. It is very popular perennial herb that is distributed throughout the world. It is a somewhat delicate appearing, low growing. It is common in damp shade places, road sides, pastures, plantations, lawns. In the literature survey the plant *Oxalis corniculata* L. is used traditionally by the tribal people and native local healers for the treatments of wounds, fractured bones, body pains, diarrhea, dysentery, convulsions, dementia, and used as hypoglycemic agent, diuretic (Subramaniam sotheswaran

et al., 1998, Madhava chetty et al., 2008). It is used for the convulsions in children and healing for fractured bones (Sridhar et al., 1993). Crushed leaves are used to treat mouth infections in children (Cambie et al., 1994). Leaves infusion is used to treat induration of breasts and warty vaginal discharges (Tewari et al., 1976). The plant studies have reported, that the *Oxalis corniculata* L. showed wound healing (Taranalli et al., 2004), Abortifacient and Antiimplantation (Sharangouda and patil, 2007), Relaxant activity (Achola et al., 1996), Anti diarrhoeal (Pierro watcho et al., 2005) and Anti bacterial (Satish et al., 2008).

2. Methodology

Plant material:

Oxalis corniculata L. (Oxalidaceae) whole plant were collected, washed, cleaned, dried in shade, and pulverized in a grinder-mixer to obtain a coarse powder and then passed through a 40- mesh sieve.

Apparatus:

Test tubes, Test tube holder, Test tube stand, Glass rods, Spatula, Burners, Measuring cylinder, Digital weighing balance, Grinder mixer, Sieve no. 40, Soxhlet apparatus, Heating mantle.

Solvents: Petroleum ether, Chloroform, Ethanol, Methanol, Ethyl acetate, Water, etc.

Chemicals:

Alcohol, Alpha naphthol, Conc.H₂SO₄, Ferric chloride, 5% HgCl₂ solution, 5% Lead acetate solution, Acetic acid solution, Potassium dichromate, 95% Ethanol, Magnesium turnings, Tannic acid, 1% Copper sulphate, Sodium nitroprusside, 10% NH₄OH, Iodine solution, 5% Ammonium sulphate, 5% NaOH, Nitric acid, Acetic anhydride, Picric acid, etc

Reagents:

Millons reagent, Barfoed's reagent, Ninhydrin reagent, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Hager's reagent, Fehling's solution A & B, Benedict's reagent, etc.

Extraction

About 1000 gm of powdered drug was successively extracted with methanol, by using soxhlet apparatus. The extraction was carried out until the extract becomes colorless. The solvent is removed from extract by distillation under reduced pressure. The concentrated extract were kept in a desiccator and used for further experiment.

Macroscopic characters of the oxalis corniculata l.

Macroscopic characters of the plant *Oxalis corniculata* L. (Oxalidaceae) was studied directly in the field, and photographed under original environment.

Microscopical evaluation of oxalis corniculata l.

Microscopical examination of the plant drugs is essential to study the adulterants also indispensable in identification. The microscopical evaluation of powder of *Oxalis corniculata* L. shows the characters as below.



Figure.1: Soxhlet extractor

Anatomical studies

Preparation of specimens:

The plant specimens for the study were collected from Talakona forest, Chittoor dist, care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin- 5ml+acetic acid -5ml+70% ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by Sass, 1940. In filtrations of the specimens were carried by gradual addition of paraffin wax (melting point 58°-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary microtome. The thickness of the sections was 10-12µ. De waxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per method published by O'Brien et al., 1964). Since Toluidine blue is polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc.

Wherever necessary sections were also stained with safranin and Fast-green and IKI for starch (O'Brien et al., 1964). For studying the stomatal morphology venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of the leaf) as well as clearing of leaf with 5% Sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycine mounted temporary preparations were made for macerated/cleared macerations. Powdered materials of the different parts were cleared with NaOH and mounted in glycerine medium after staining different cell component were studied and measured.

Photomicrography

Microscopic descriptions of tissues are supplemented with micrograph, wherever necessary photographs of different magnifications were taken with NIKON lab photo 2 microscopic units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esau, 1964).

Physicochemical Parameters

The evaluation of a 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 physicochemical parameters were evaluated to obtain the qualitative information about the purity and quality of *Oxalis corniculata* L. Ash value aids in the determination of quality of crude drug in powder form.

The ash content of a crude drug is generally considered as a residue remaining after maceration. Ash contains inorganic salts like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium are adhere to it or may also be added to for the purpose of adulteration. There is a considerable difference (varieties with narrow limits) in the case of same individual drug. Hence ash determination furnishes a basis for judging the identity and quality of the drug gives information to its adulteration with inorganic matter. Ash standards have been established for a number of the drug in the pharmacopoeias (Khandelwal, 2006).

Ash Values

Total ash:

Heat a silica or platinum crucible to red heat for 30 min, Allow to cool in desiccator and weigh. Weigh accurately about 1gm of the substance being examined and evenly distributed in the crucible. Dry at 100°C to 105°C for one hour and ignite to constant weight in the muffle furnace at 600° ± 25°C. Allow the crucible to cool in desiccator after each ignition. The material should not catch fire at any time drying the procedure. If after prolonged ignition a carbon free ash cannot be obtained, exhaust the charred mass with hot water. Collect the residue on an ash less filter paper, incinerate the residue and filter paper until ash becomes white or nearly so. Calculate the percentage of the drug with reference to air dried drug.

Water-insoluble ash

The total ash is boiled with 25ml water and filtered through the ash less filter paper (whatman-41). It is followed by washing with hot water. The filter paper is ignited in the silica crucible, Cooled and the water insoluble matter was weighed. The water soluble ash is calculated by subtracting the water insoluble matter from the total ash.

Acid-insoluble ash

The total ash obtained is boiled for five minutes with 25ml of 2M hydrochloric acid and filtered through an ash less filter paper. The filter paper is ignited in the silica crucible, Cooled and then acid insoluble ash is weighed.

Extractive Values: Extraction values are useful for determination of crude drugs and it gives an idea about the nature of the chemical constituents present. The solvent used for the extraction should be in position to dissolve the quantities of desired substances.

Alcohol soluble extractives

About 5g of the powder is macerated with 100ml of the specified strength in a closed flask for 24 hours. Shake frequently during first 6hrs and allow it for standing to 18 hrs. It is filtered rapidly taking precautions against loss of alcohol and 25ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish. Dried at 105°C and weighed. The percentage of alcohol soluble extractive is calculated with reference to the air dried powder.

Water soluble extractives:

About 5 g of the powder drug is macerated with 100 ml of distilled water in a closed flask for 24hrs. Shake frequently during 6 hrs and allow the same for standing for 18 hrs. It is filtered rapidly and 25 ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive is calculated with reference to the air dried powder.

Chloroform soluble extractives

About 5 g of the powder drug is macerated with 100 ml of chloroform in a closed flask for 24hrs. Shake frequently during 6 hrs and allow the same for standing for 18 hrs. It is filtered rapidly and 25 ml of the filtrate is evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of chloroform soluble extractive is calculated with reference to the air dried powder.

3. Results and Discussion

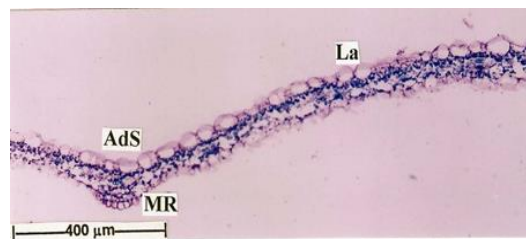


Figure.2. T.S of through midrib with lamina

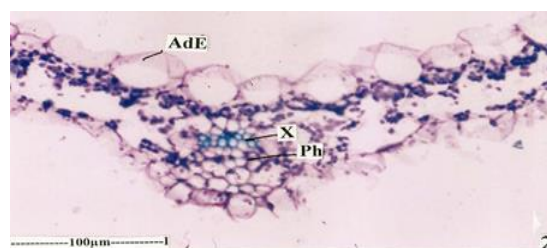


Figure.3.T.S of midrib with lamina enlarged

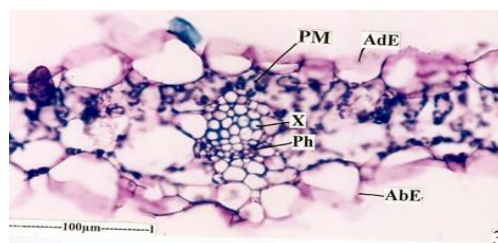


Figure 4. T.S of midrib with lamina enlarged

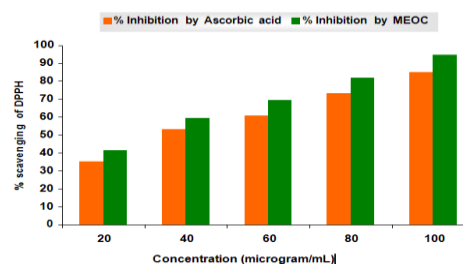


Figure 5. Antioxidant activity by DPPH method

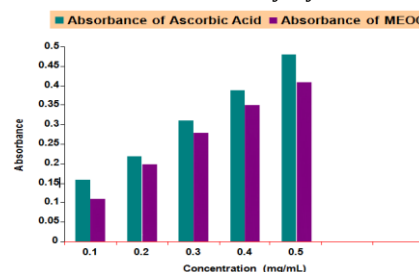


Figure 6. Antioxidant activity by reducing power method



Figure 7. Animal placed in metabolic cage



Figure 8. Collection of urine from animal

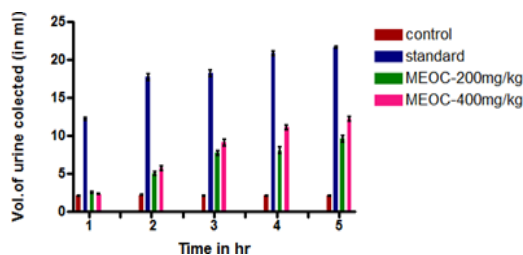


Figure 9. Effect of *Oxalis corniculata* L. on excretion of urine

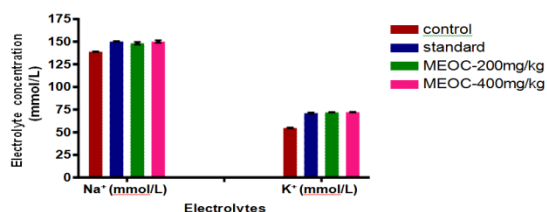


Figure 10. Urinary electrolyte concentration of *Oxalis corniculata* L.

Discussion

The aim of this study was to investigate the diuretic activity of methanol extract of *Oxalis corniculata* L. According to previous ethnopharmacological survey carried out is the south Indian region. The plant materials are used in various diseases like dyspepsia, cancer, piles, convulsions, and dementia and traditionally used as diuretic, but no previous pharmacological clinical study has been carried out to test the diuretic activity of this plant. In this study the methanol extracts was tested at 200mg/kg, 400mg/kg respective doses. The diuretic response was compared with that

produced by furosemide, a widely used loop diuretic in clinical practice. The effect on electrolyte balance was also determined along with diuretic response. The methanol extract of *Oxalis corniculata* L. (200mg/kg) showed lesser diuretic activity compared to MEOC 400mg/kg during the 5h of the test duration (Diuretic action (4.53 and 5.75) when compared to control the MEOC 200mg/kg MEOC 400mg/kg both doses showed more significant diuretic activity but less than that of standard furosemide (Diuretic action 10.21). Urine output continued to be enhanced throughout the study period and the cumulative urinary excretion was significantly higher compared to that of the control.

Furosemide is reported to increase urinary output and urinary excretion of sodium by inhibiting Na⁺/K⁺/Cl⁻ transports system in this thick ascending of henley (Jackson, 1996). The MEOC 200mg and MEOC 400mg/kg both doses significantly increases the urinary excretion of Na⁺ and K⁺ ions was observed was when compared to control.

The secondary metabolites such as flavonoids, saponins are known to responsible for diuretic activity (Sood et al., 1985). In this, plant having three major C-glycosy flavones are reported (Hiroki Mizokami et al., 2008). The diuretic activity of this plant may be due to these flavonoids. The probable mechanism of action of tested extracts may be similar to the furosemide. The diuretic activity MEOC 400mg/kg shown higher level of diuretic action compared to MEOC 200mg/kg. However the exact constituents responsible for the diuretic activity of the extracts studied needs to be evaluated in the future studies.

4. Conclusion

It is concluded from this study, that the methanol extract of *Oxalis corniculata* L. possess significant diuretic activity and may prove to be effective for the treatment of many life-threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension and pregnancy toxemia. The diuretic action may be presence of flavonoids. However further studies required to elucidate the exact mechanism of action for develop its as potent diuretic drug.

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