

“Isolation of Rutin from *Oxalis psittacorum*”

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD
DEGREE OF BACHELOR OF PHARMACY IN PHARMACY

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ABSTRACT:

General phytochemical screening of *Oxalis psittacorum* revealed the presence of Saponin, tannins, glycoside and alkaloids etc. The aim of this study is to identify and characterize the bioactive principle from the plant. It has wide folk medicinal uses. The isolation and characterization of Phyto-constituents was done from the methanolic extract by the gradient fractionation method. The structure of the isolated compound was established on the basis of physical, chemical test and spectroscopic evidences (IR, UV). A flavonol structure was isolated from the methanolic extract of the plant. The odorless, colorless compound which solubilizes in methanol having m.p. 186.0 C and Rf value 0.58 in mobile phase Methanol: Glacial Acetic Acid: Water (90: 2: 8) was identified by the various spectroscopical methods. So from this study it is concluded that isolated compound may be rutin which is responsible for various pharmacological activities of the plant. Anti – helminthes activity was studied and drug response was good.

I. INTRODUCTION:

Plants were a significant source of novel pharmacologically dynamic synthetics as of not long ago, with a few blockbuster prescriptions got either straightforwardly or by implication from plants. (Salituro et al., 1998) In spite of the current spotlight on engineered science for the purpose of finding and assembling prescriptions, plants keep on assuming a huge part in disease treatment and counteraction. (Sharma et al., 1975) Indeed, even before the turn of the hundred years, 11 percent of the WHO's 252 fundamental and essential drugs were made altogether of blooming plants. Regular products will keep on assuming an essential part in the improvement of restorative medicines. (Osadeba

et al., 2003) Numerous normal items, notwithstanding those that have tracked down direct helpful application as medication elements, can act as substance models or layouts for the plan, amalgamation, and semi-combination of novel synthetic compounds for the treatment of human infections. (Atawodi et al., 2003) In spite of the fact that there are a few new ways to deal with drug disclosure, for example, combinatorial science and PC based sub-atomic displaying plan, and many medications are made by manufactured science, not even one of them can supplant the significant job of normal items in drug revelation and improvement since regular items give the majority of the center designs or platforms for engineered synthetics. (Sharma et al., 2012) *Oxalis psittacorum* (Lam.) Vahl. has been used by ancestral networks in 'INDIA' for a really long time to treat illnesses like torment, psoriasis, mouth ulcers, pallor, obstruction, and diabetes, with logical proof of antipyretic, calming, antibacterial, antiviral, and hostile to cancer growth properties. (Majumdar et al., 2020)

Rutin is a flavanoid naturally present in many plants. It is also known as rutoside. It is slightly soluble in water, but more soluble in alcohols. Rutin has several important pharmacological properties, which are beneficial to health and can potentially be used as an antioxidant, antifungal, antiallergic, antihelminthese

II. MATERIALS AND METHODS: MATERIALS:

The solvents used were of high (analytical grade) and include: methanol, n hexane, chloroform, ethyl acetate and n purchased from Sigma Co. USA; silica gel 60 µm (Qualikems,

India) was used for column chromatography, sephadex LH Healthcare) was used for purification of isolated compound.

Thin layer chromatography (TLC) was carried out on aluminum-backed Kieselgel 60 F254

TLC plate (Merck no. 5554, Darmstadt, Germany) and a Gallenkamp electro thermal melting point apparatus was used to determine the melting point of isolated compound.

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Ziziphus mucronata

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butanol fraction of a methanol an important medicinal plant of the Rhamnaceae family widely used in ethnomedicine to treat inflammation, diarrhoea, cough, sores, asthma, measles, fever and urinary problems. Its isolation was carried out by a combination of column chromatography and gel filtration. The structure of the isolated compound was determined by analysis of its UV, IR, 1D and 2D Proton and 13 NMR spectral data, as well as comparison with reported data. This is the first validated scientifically (Abdullahi et al., 2017).

butanol extract has also been found to have anti-inflammatory activity (Authors, unpublished). Apart from cyclopeptides identified as chemotaxonomic markers of the rhamnaceae family, there is dearth of information regarding isolation of potentially bioactive principles from the plant. To the best of our knowledge, only the isolation of rutin (., 2017) and biflavonoid (., 2018) were previously reported from the leaves of the plant. In continuation of our study aimed at investigating the bioactive of *Z. mucronata*

we report herein, the isolation of Rutin from the butanol soluble fraction of the methanol General Experimental Procedures and Spectroscopic Characterization

solvents used were of high quality (analytical grade) and include: methanol, n-hexane, chloroform, ethyl acetate and n-butanol

purchased from Sigma Co. USA; silica gel 60-120 µm (Qualikems, India) was used for column chromatography, sephadex LH-20 (GE) for purification of isolated compound. Thin layer chromatography (TLC) on aluminum-backed Kieselgel

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The solvents used were of high quality (analytical grade) and include: methanol, n-hexane, chloroform, ethanol, n-butanol, ethyl acetate were purchased from Sigma-Aldrich, USA. Silica gel 60-120µm (Qualikems, India) was used for column chromatography.

III. METHODS:

Collection and identification of plant materials:

The plant *OLAX PSITTACORAM* was collected from local market Bhubaneswar. And it was identified by pharmacognosy department, School of pharmaceutical department, Bhubaneswar.

Preparation of the extract:

The leaf was dried at room temperature for a long time and size decreased physically utilizing mortar and pestle. The size diminished leaf (1 kg) was separated with methanol by maceration for 72 hours and packed in vacuo. This yielded 16 mg of a

sticky concentrate. The concentrate was suspended in refined water and sifted. The water solvent piece was dealt with progressively with ethyl acetic acid derivation and n-butanol to yield ethyl acetic acid derivation portion (EAF) and n-butanol part (nBF) individually.

General and physical properties:

Appearance, color, taste, odor, solubility and melting point of the isolated constituents will be determined.

Chemical identification of constituents:

Small amount of the isolated constituent are dissolved in methanol and perform the Zinc hydrochloride test.

Zinc-hydrochloride test:

To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. Heat the solution and observe the color.

TLC plate study:

A 10*1.5cm TLC plate was heated at 100°C for 10 minutes and it is allowed to cool down to room temperature and the TLC plate was activated. A pencil line was drawn 1.5cm from one edge of this plate. Methanolic extracts sample was spotted using thin capillary pipettes on to the pencil line. The solvent front was allowed to travel until about 1cm from the top end. TLC plates were removed, and solvent front was marked using soft pencil. The Chromatogram was marked. The Butanol Ethyl Acetate Chloroform (4:4:2, v:v:v) had produced the best separation of the spots.

Spectral characterization of the constituents:

TLC study:

After performing the Thin Layer Chromatography we have got the R_F value of 0.52.

Ultra Violet spectra:

The Ultra Violet spectra of the constituents (isolated from TLC and dissolved in methanol) are taken in double beam Shimadzu spectrophotometer (UV-1700) in between range 200 nm to 700 nm. Methanol was taken as reference solvent.

Infrared Spectra:

The IR spectrum of isolated constituents as KBr disc has been determined on a Perkin – Elmer Infrared Spectrophotometer. The structural assignments have been correlated for the characteristic bands as mentioned in results.

Anti-helminthes study:

The study was performed by using adult Earth worm (*Lumbricina terrestris*). Here five different isolated Rutin concentrations were

prepared (0.25mg/30ml, 0.5mg/ml, 1mg/30ml, 1.5mg/30ml, and 2mg/30ml) in distilled water. Five Earthworms (equal size) were placed in the separate Petridis in different time. The test solution was prepared freshly before starting experiments. Observations were made between time of dipping the earthworm and time of death of the earthworm. Paralysis was noted when no movement of the earthworms.

IV. RESULTS AND DISCUSSION:

General and physical properties:

It is found pale yellow in colors which gradually darken on exposure to light. It is tasteless and odorless. It is soluble in pyridine, methanol and alkaline solution. Melting point of the constituent was founded 189° C.

Chemical identification:

Zinc-hydrochloride test:

It is produced red color after few minutes. And it is indicate that flavanoids are present.

Spectral identification:

Uv spectra:

The UV spectrum of rutin in metabolic solution shows two major absorption bands at 368nm and 258nm.

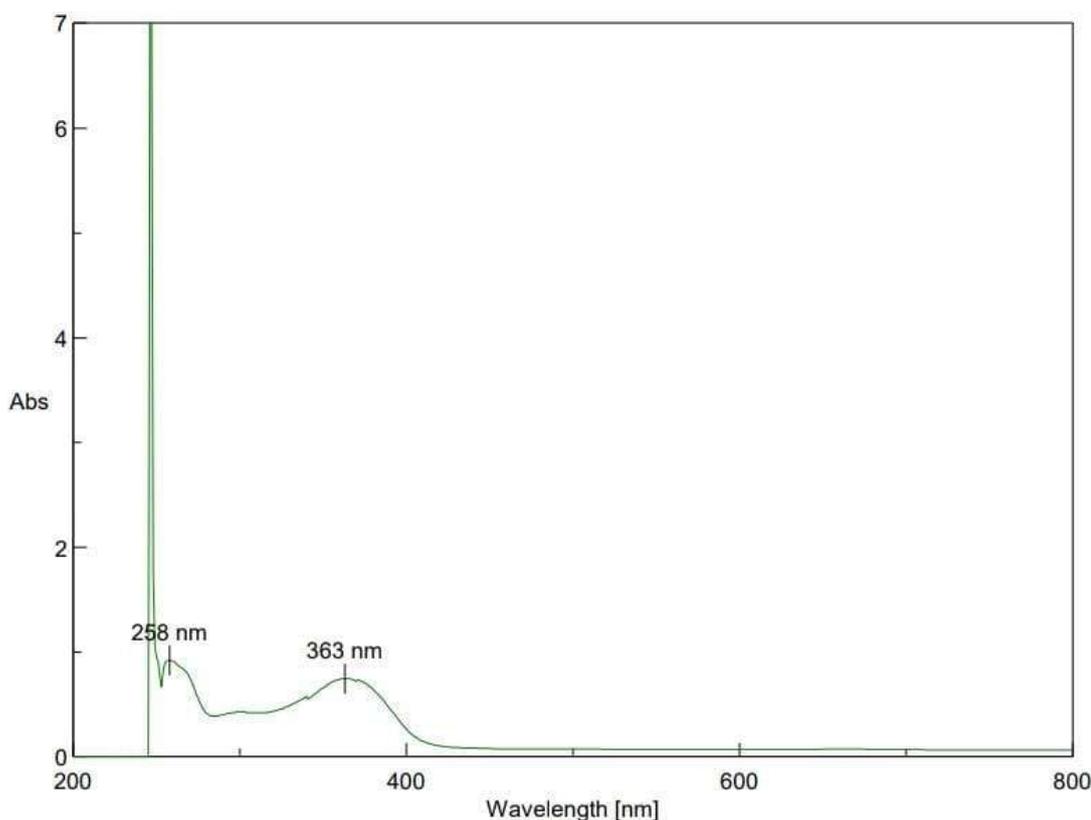


Figure – 1, UV- spectra analysis of isolated fraction

IR values:

shows that peak obtain 1014.37cm⁻¹ peak and this peak contain Alkanes, Alkenes, Mononuclear Aromatic Benzene, Alcohol and Phenols, Acetals, Ethers, Esters, Lactones, Anhydrides, Amines, Thiocarbonyl group, Sulfoxides, Sulfones, Sulfonyl chlorides, Primary sulfonamide(solid), Secondary sulfonamide(solid), Sulfonates, Halogen compounds, Silicon compounds, Phosphorus compounds. 1652.7cm⁻¹

peak contain Alkenes, Mononuclear Aromatics Benzene, Ethers, Ketons, Aldehydes, Carboxylic acids, Esters, Lactones, Acid chlorides, Anhydrides, Amides, Lactams, Amines, Nitrites, Halogen compounds, Phosphorus compounds. 2341.16cm⁻¹ peak contain Alkynes, Amines, Sulfur compounds, Phosphorus compounds. 2948.63cm⁻¹ peak contain Alkanes, Alkenes, Alkynes, Mononuclear aromatic benzene, Alcohols and phenols, Ketones, Aldehydes, Carboxylic

acids, Amides, Lactams, Amines, Phosphorus compounds.

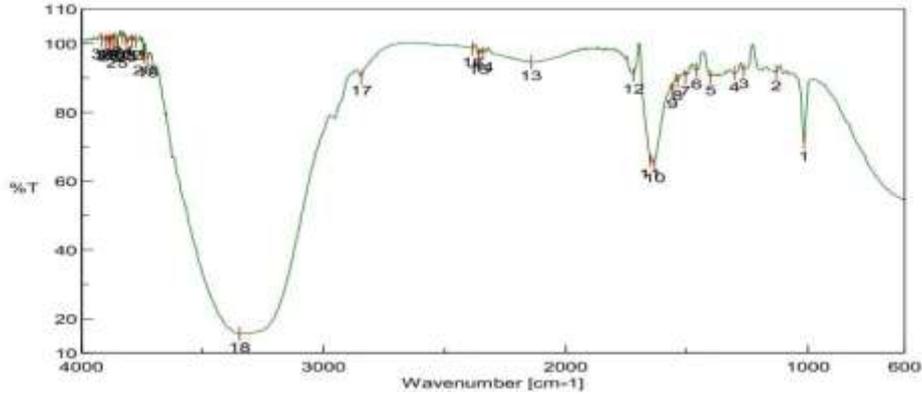


Figure – 2, ftir analysis of isolated fraction

Anti-helminthes study:

shows that earthworm was treated with isolated Rutin fraction which was extracted from olax psittacorum leaves. From which time the

earthworm was dipping and the death time was noted down. And 2mg/30ml dose shows the good resposes.

Table -1, Anti-helminthes

Drug dosage(mg)/30ml	Timing of helminthes being alive(Hrs)
0.25	10
0.5	8
1	7
1.5	6.29
2	5.3

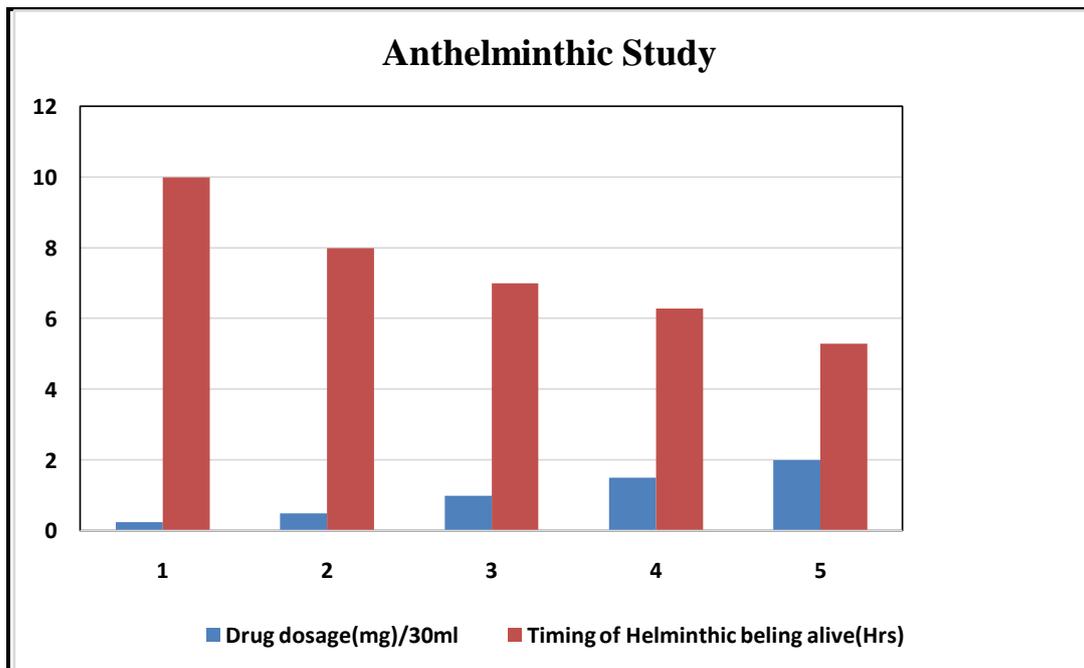


Figure- 3, Anti-helminthes doses responses



Figure-4, Anti-helminthes study

V. CONCLUSION:

From the above experiment by using the methanolic extract of *Olax psittacorum* leaves concluded that collected fraction was Rutin, which was confirmed by using TLC, UV-VIS, FTIR. And it has good anti-helminthics activity.

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