PHYTOCHEMICAL INVESTIGATION OF *RUBIA CORDIFOLIA* EXTRACT OBTAINED BY SERIAL EXHAUSTIVE EXTRACTION

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Abstract : Rubia cordifolia L., (Rubiaceae) commonly known as Indian Madder or Manjistha is an important medicinal plant mentioned in Ayurvedic Pharmacopeia and used in Indian Traditional Medicine for curing various Diseases. *Rubia cordifolia* is known to have wound healing, Antibacterial, Antioxidant, Anticancer, Anti-inflammatory, Analgesic, Hepatoprotective, Anti-platelet activating factor & Anti-acne activity owing to presence of various Biologically Active Compounds or Phytochemicals. The present investigation was carried out to have a Qualitative and Quantitative Data of Phytochemicals present in the Dichloromethane fraction of *Rubia cordifolia* Roots obtained by Serial Exhaustive Extraction Method.

Key Words- Manjistha, Phytochemicals, Dichloromethane, Serial Exhaustive Extraction.

I. INTRODUCTION

Rubia cordifolia commonly known as Indian Madder is an important Medicinal Plant which is used for the treatment of various ailments in Ayurvedic system of Medicine since ancient times. The roots of the Plant known for its Pharmaceutical activity is sold in market under the commercial Name Manjistha. Earlier it was cultivated for a Red Pigment derived from Roots. (Mohammad Abu Bin Nyeemet al., 2018). However, later, the Extracts and Phytochemicals of *Rubia cordifolia* Plants had drawn considerable attention due to their potent Bioactivities. Plants synthesize these Secondary metabolites or Phytochemicals which include Alkaloids, Flavonoids, Saponins, Terpenoids, Steroids, Cardiac glycosides, Tannins, Volatile oils, among others. Several bioactive constituents have been isolated and studied for Pharmacological activity from *Rubia cordifolia* (Anuradha Verma., 2016). Serial Exhaustive Extraction is one of the Maceration techniques where in Successive extraction is done with Solvents of increasing Polarity from a Nonpolar solvent to a more Polar Solvent .This ensures extraction of Compounds with a wide Polarity range. Cold Serial Exhaustive extraction can be done with various solvents from increasing polarity such as Hexane, Chloroform, Ethyl acetate, Acetone, Ethanol and Water. The various Chemical components obtained in each Solvents can be investigated further. The present study comprises of Serial exhaustive extraction of *Rubia cordifolia* root powder followed by Qualitative and Quantitative assessment of Phytochemicals in Dichloromethane extract.

II. MATERIALS AND METHODS

Collection and Preparation of Plant material.

The roots of *Rubia cordifolia* were collected from Matheran area and identified by an expert Taxonomist from KET's V.G.Vaze College, Mulund, Mumbai. The Plant material was washed under tap water followed by distilled water and shade dried for 10 days. They were initially cut into small pieces with the Secator and was then fine powdered with the help of a Mechanical Grinder.

Serial Exhaustive Extraction

Cold Serial Exhaustive Extraction method was used and the order of Solvents selected from Non polar to Polar was Hexane-Dichloromethane-Ethyl acetate-Ethanol. 100 grams of powdered *Rubia cordifolia* in Hexane was kept on a Shaker at 20° C for 8-10 hrs. The filtrate was separated and the powder was air dried completely. This procedure was repeated twice with Hexane for the air dried powder. The filtrate of all three extractions was pooled together. The air dried powder was similarly extracted in Dichloromethane, followed by Ethyl acetate and Ethanol to obtain an extract in respective Solvents. Each Solvent extract was further concentrated in a Vacuum evaporator and further dried in an oven at 60^0C.

High Performance Thin Layer Chromatography.

HPTLC was done on CAMAG HPTLC instrument with WinCATS Software (V 1.4.6 2002). 10x10 cm of Silica Gel Pre-coated plates made of Aluminium base with 60 F_{254} was used for HPTLC.5µl (1mg/ml) of each Solvent extract was loaded with syringe by CAMAG Linomat 5 and Nitrogen as an inert Gas. The mobile Phase used was Toluene: Ethyl Acetate: Formic Acid (85:14:1). (Aisha Siddiqui et al., 2011). Once, the solvent run was completed, the plates were observed under 366 nm.

Qualitative Phytochemical Analysis

A Preliminary Phytochemical Screening was done for DCM (Dichloromethane) extract.

Tannins: (Prabhavathi R. M et al., 2016)
The DCM extract was extracted in 2-3 ml of Methanol and 10 % Alcoholic Ferric Chloride was added to it in the ratio of 1:1. Occurrence of a Blue-Black, Green or Blue-Green precipitate indicates the presence of Tannins.

Alkaloids: **(**Jigna Parekh et al.,2007)
The DCM extract was stirred with 5 ml of 1 % aqueous HCl on water bath and filtered and to 1 ml of this filtrate, few drops of Dragendorff's reagent were added. Occurrence of Orange-Red precipitate indicates presence of Alkaloids.

Saponins: **(**Jigna Parekh et al., 2007)
1 g of DCM extract was boiled with 5 ml of Distilled water, filtered and to the filtrate 3 ml of distilled water was added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of Saponins.

Flavonoids: **(**DN Onwukaeme et al., 2007)
The DCM extract was treated with dilute NaOH followed by Dilute HCl. A yellow solution with NaOH which turns colourless on addition of dilute HCl was treated as positive for presence of Flavonoids.

Terpenoids: (P.Dhaval., 2017)
2 mg of extracts was treated with 2 mL of chloroform and concentrated H_2SO_4 to form a layer. A reddish brown colour formation at the interface confirms the presence of terpenoids.

Anthraquinones: **(**DN Onwukaeme et al., 2007)
1 ml of dilute (10 % Ammonia) was added to 2 ml of DCM extract. Pink Red Colour in Ammonical layer confirms presence of Anthraquinones.

Phenol: (Rakesh.K.Sindhu., 2012)
5 % Ferric chloride solution was added to the extract. Colour change to Blue-Black precipitate confirms presence of Phenols.

Cardiac Glycosides: **(**DN Onwukaeme et al., 2007)
50 mg of DCM was dissolved in 2 ml of Chloroform. A layer of Sulphuric acid is added. Brown ring at interphase is indicative of presence of Cardiac Glycosides.

Steroids: **(**Jigna Parekh et al., 2007)
To 1 ml of DCM extract 2 ml each of Acetic anhydride concentrated sulphuric acid H_2SO_4 was added. Colour change to Blue or Green indicates presence of Steroids.

Proteins: (Radha Gupta et al., 2017)
1 ml of DCM extract was treated with Ninhydrin and heated. Formation of Red/Blue to Violet colour indicates presence of Proteins

Quantitative Phytochemical Analysis

Total Phenolic Content (Alhakmani et al., 2013)

The DCM extract was prepared 1mg/ml in Methanol. 0.2 ml of this was added to 0.8 mL of Folin-Ciocalteu reagent. 2.0mL of 7.5 % Na_2CO_3 was added along with 7 ml of distilled water. Gallic acid (0.05-0.3 mg/ml) was used as standard for deriving a calibration curve. All the tubes were incubated in dark for 2 hours. Absorbance was read at 765 nm on UV-Vis spectrophotometer. The calibration curve was plotted with absorbance values of Gallic acid dilutions on y- axis and

concentration on X- axis. Regression line equation was obtained as (y=2.768x + 0.7138). The absorbance of sample was substituted in the equation to give concentration equal to Gallic acid from graph (C). Amount of phenol present in the extract was calculated by the formula,

Total Phenol Content= C x V/ W (GAE/g)

V = volume of sample added
W= weight of extract in gram

Determination of Alkaloids (Chukwuma S. Ezeonu et al., 2016)

Quantitative determination of Alkaloid was according to the method given by Harborne. 40 mL of 10% acetic acid in ethanol was added to 0.5 g of extract and allowed to stand for 4 hours. The sample was filtered through Whatmann Filter paper No1. The extract was concentrated on a water bath at 80 ºC to one-fourth of its original volume. Concentrated Ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The supernatant was discarded and the precipitates were washed with 20ml of 0.1M of Ammonium hydroxide and then filtered through Whatmann Filter paper No 1.The dried residue was weighed. The total Alkaloid content was derived using the following formula

$$\% \text{ Alkaloid} = \frac{Weight\ of\ Alkaloid}{Weight\ of\ Extract} \times 100$$

Total Flavonoid Content (Madike.L.N., 2017)

Total flavonoid content was estimated by Aluminium Chloride colorimetric method. To 1.5 mL of $AlCL_3$ 500 µL of plant extract (1mg/mL) was added. Blank was prepared by adding 500 µL of distilled water instead of plant extract. Quercitin was used as a standard (20-100 µg/mL). All the tubes were incubated at RT for 60 min and absorbance was read at 420 nm. The total flavonoid content expressed as quercetin equivalent (QE) was calculated based on the calibration curve, using y = 0.018x -0.094 b, where x is the absorbance and y is the concentration (mg QE) of the methanolic quercetin solutions.

Total Flavanoid Content= C x V/ W (GAE/g)

Determination of total Protein Content (Santhosh Kumar N., 2018)

Standard used was Bovine Serum Albumin (0.1-0.5 mg/ml). 50 µl of sample was added to 1.5 ml of Bradfords reagent and the tubes was incubated in dark for 5 min. Distilled water was used as blank. Absorbance was read at 595 nm. The concentration of proteins present in the plant extract was estimated from the calibration plot.

III. RESULTS AND DISCUSSIONS

The yield of *Rubia cordifolia* Roots in the solvents was calculated after complete evaporation of Solvents. Yield was highest in Dichloromethane (5.6 gm %) followed by Hexane (1.5 gm %), Ethyl acetate (1.2 gm%) and Ethanol (1.1 gm %).This showed that the maximum components were extracted in non -polar solvent. The yield of extract was comparative lesser in polar solvents. HPTLC profile at 366 nm showed presence of 13 components in Dichloromethane extract compared to 4-5 in Hexane, Ethyl Acetate and Ethanol extracts. A similar profile is observed in other studies. (Ramesh S. Deoda et al., 2011) This indicated that the maximum components were extracted in Dichloromethane. Hence, the DCM extract was chosen for further Phytochemical Analysis. The Preliminary Phytochemical tests were carried out for The DCM extract of *Rubia cordifolia* powder. The extract showed presence of Alkaloids, Flavonoids, Steroids, Proteins, Cardiac Glycosides, Anthraquinones, Phenols and Saponins. The total phenol content was found to be 13.5 mg of GAE/gm of DCM extract (Fig 1). The total Alkaloid content was found to be 30% the DCM extract.The total flavonoid content was found to be 10.11 mg of QE/ gm of DCM extract (Fig 2).From the calibration curve obtained by standard BSA (Fig 3), the amount of protein present in the sample was found to be 33.45 mg/ g of sample.

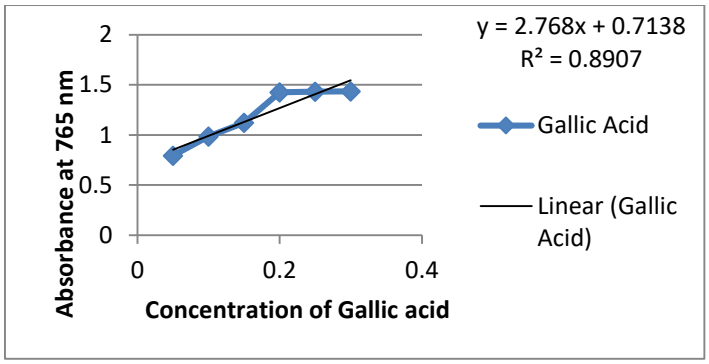

Fig 1-Total phenol content Estimation

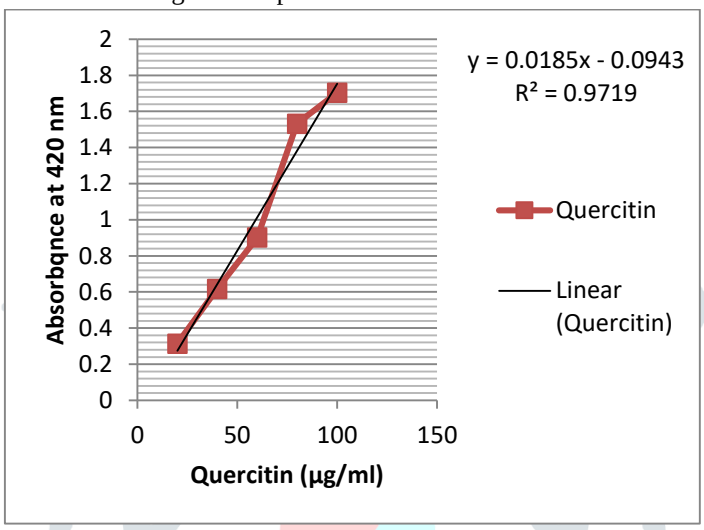

Fig 2-Total Flavanoid Content Estimation

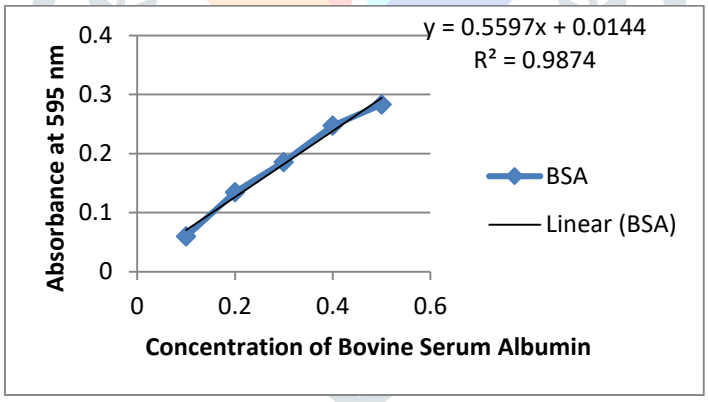

Fig 3-Total Protein content

IV. CONCLUSION

Phytochemical investigation of *Rubia cordifolia* have shown the presence of Flavonoids, Alkaloids, Saponins, Anthraquionones, Phenols, Cardiac glycosides, Proteins and Steroids which may be responsible for therapeutic properties of the plant. Today clinical investigations of herbal formulations and their market preparations, both are on demand because of better safety and efficacy without or minimal side effects. The plant can be further studied for the isolation, identification and quantification of important Bioactive components for the development of new specific therapeutic drugs. Serial Exhaustive Extraction technique can ease the further extensive efficacy testing of these phytochemicals since the selection and separation of Bio-active components is easier than other extraction procedures. These preliminary investigations of the Phytochemistry of *Rubia cordifolia* Roots can further lead to potential invention of Plant based drugs for pharmaceutical usages.

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