

# Optimization of Physico-Chemical Parameters for the Extraction of Bio-Flavonoids from Leaves of *Bacopa monnieri*

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**ABSTRACT:** *Bacopa monnieri* species commonly known as water hyssop, is an herb often used in Ayurveda. It is reported to contain various bio chemical compounds such as quercetin, rutin Alkaloids, Glycosides Brahmoside, Brahminoside, Brahmic acid, Isobrahmic acid etc. It shows pharmacological activities like anti-oxidant, anti-cancer, anti-viral and anti-inflammatory activities etc. The present study was intended for the optimization of extraction of Quercetin and rutin from leaves of *Bacopa monnieri* and its various physico-chemical parameters have been studied. For the extraction of quercetin and rutin, the optimum results were observed for the effect of different solvents, soaking time, different solvent percentages, different volumes of hexane with methanol as solvent, extraction time with hexane and effect of pH were methanol, 1day, 100%, 1:1, ratio, 90min and 6.0 respectively. The highest Quercetin and rutin concentrations for optimized conditions were 43 µg/ml and 33 µg/ml respectively.

**KEYWORDS:** *Bacopa monnieri*, quercetin, rutin, extraction,

## 1. INTRODUCTION

*Bacopa monnieri* L. Pennell (family: Scrophulariaceae) is a reputed drug of Ayurveda[3] and commonly known as water hyssop. It commonly grows in marshy areas throughout India, Nepal, Sri Lanka, China, Pakistan, Taiwan, and Vietnam. It is also found in Florida, Hawaii and other southern states of the United States where it can be grown in damp conditions by a pond or bog garden. This plant can be grown hydroponically[4]. The leaves of this plant are succulent, oblong and 4–6 millimeters thick. Leaves are oblanceolate and are arranged oppositely on the stem. The flowers are small and white, with four or five petals. Its ability to grow in water makes it a popular aquarium plant. It can even grow in slightly brackish conditions. It is used in traditional medicine to treat various nervous disorders[5]. It is also used as a stomachic, a digestive, rejuvenate, for promoting memory and intellect, for skin disorders, and as an antiepileptic, antipyretic, and analgesic. This medicinal plant is locally known as Brahmi. Its phytochemical constituents[9] are Alkaloids[8], Glycosides Brahmoside[7], Brahminoside, Brahmic acid, Isobrahmic acid, Stigmasterol, Flavonoids like Quercetin, Rutin, Apigenin and Luteonin, Saponins etc. It shows Pharmacological activities like Antioxidant activity[2], Anti-inflammatory activity[1], Anti-diabetic activity[11], Hepato protective Activity, Anticholinesterase activity, Neuroprotective role[6], Adaptogenic activity, Antiulcerogenic activity, Antibacterial activity etc.

## II. MATERIALS AND METHODS

### Chemicals and reagents:

Methanol, sodium nitrite, sodium hydroxide, aluminium chloride, distilled water, hexane.

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## Collection of the plant material:

The fresh whole plant of *Bacopa monnieri* was collected from Araku Valley, Near Visakhapatnam, Andhra Pradesh, India

## Processing of the plant material:

The leaves are cleaned, dried under shade and powdered. The powder is then screened using 100 mesh size and the fraction obtained are stored in an air-tight container.

## Extract preparation:

1 gm of powder is added to 50 ml methanol (100%) in a conical flask. The solution is soaked for 1 day. After the soaking time [10], the solution is filtered using Whatman filter paper no:1 and the filtrate is heated at 65°C. so that the solvent which is taken in glassware is evaporated. The resultant solution is cooled and make up to 25 ml with distilled water. This solution is added to 25 ml of hexane taken in a separating funnel. Incubate the solution of methanolic extract for 90 minutes.

## Determination of Quercetin:

1 ml of methanolic extract is taken in a test-tube from the extract phase of the separating funnel. To this 0.3ml of 5% sodium nitrite was added. After 5 minutes 0.3 ml of 10%  $AlCl_3$  was added. After 6 minutes 2 ml of 1M sodium hydroxide was added and the mixture was made up to 10 ml by adding distilled water. The absorbance of the reaction mixture was measured at 510 nm using colorimeter. The concentration of quercetin was determined by using calibration curve.

## III. RESULTS AND DISCUSSION

The extraction of quercetin and rutin from leaves of *Bacopa monnieri* and its optimum results were observed for the effect of different solvents, soaking time, different solvent percentages, different volumes of hexane with methanol as solvent, extraction time with hexane and effect of pH were studied.



Fig.1: (a) leaves of *Bacopa monnieri*



(b) Dried leaf powder



(c) Methanolic extract

## EFFECT OF PROCESS PARAMETERS:

### 1. EFFECT OF DIFFERENT SOLVENTS ON EXTRACTION OF QUERCETIN:

For the extraction of quercetin and rutin from powder of leaves of *Bacopa monnieri* plant, different organic solvents such as methanol, ethanol, water and ethyl acetate are used. Among these, methanol showed the best results and the concentration of quercetin and rutin were 6 µg/ml and 10 µg/ml respectively. The results are shown in the fig.2

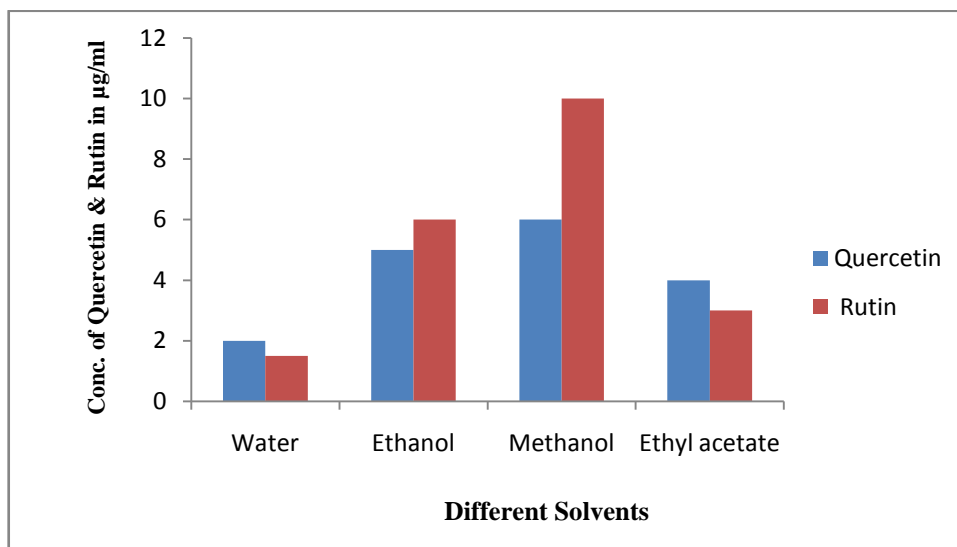


Fig. 2. Effect of different solvents on extraction of Quercetin

### 2. EFFECT OF SOAKING TIME ON EXTRACTION OF QUERCETIN:

For the extraction of quercetin and rutin from powder of Bacopa monnieri leaves, the samples were incubated for different time periods viz., 1, 2, 3 and 4 days. It was observed that, soaking for 1 day[12] gave the best result and the concentration of quercetin and rutin obtained were 19 µg/ml and 19 µg/ml. The results are shown in the fig.3

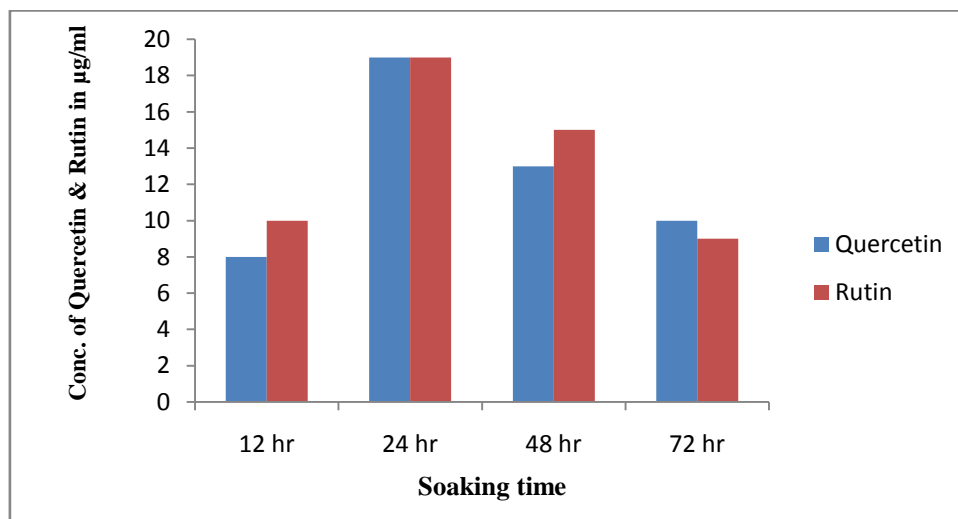


Fig.3. Effect of soaking time on extraction of quercetin

### 3. EFFECT OF DIFFERENT SOLVENT COMPOSTION ON THE EXTRACTION OF QUERCETIN:

Different solvent percentages like 0%, 20%, 40%, 60%, 80%, 100% of methanol solvent are used for extraction of Quercetin and rutin. The optimum solvent percentage was found to be 100% and its concentrations are found to be 19µg/ml and 19µg/ml. The results are shown in the fig.4

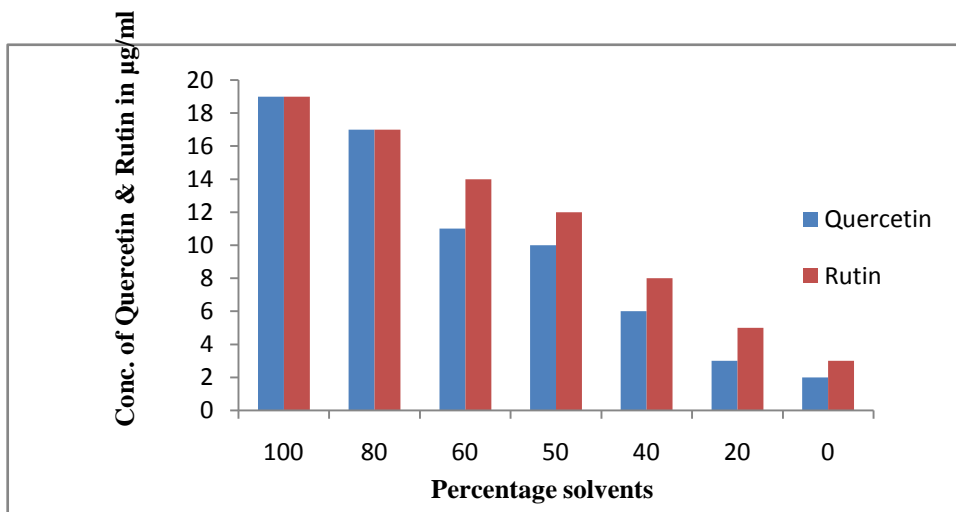


Fig.4. Effect of different solvent composition on the extraction of quercetin

4. EFFECT OF DIFFERENT VOLUMES OF HEXANE ON THE EXTRACTION OF QUERCETIN:

Different volumes of hexane with solvent in different ratios such as 1:1, 1:2, and 2:1 are used for the extraction of bio flavonoids. The optimum value is observed with the ratio 1:1 and the concentration of quercetin and rutin are found to be 25µg/ml and 22µg/ml. The results are shown in the fig.5

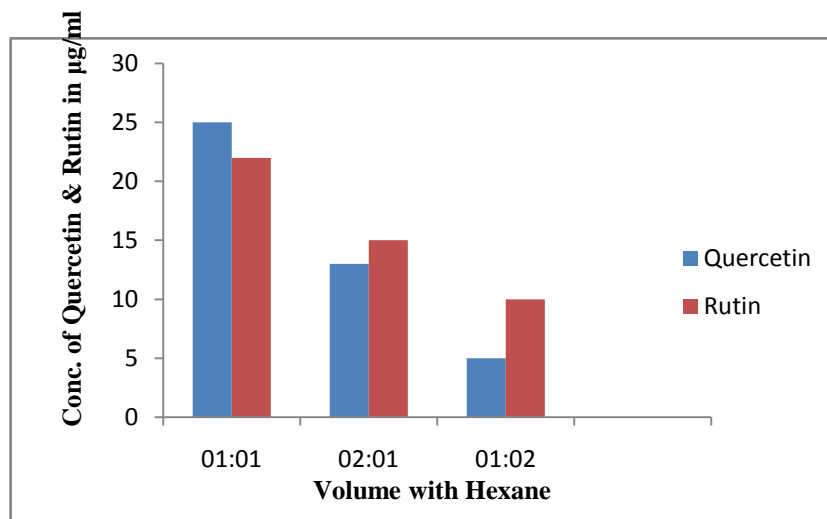


Fig.5. Effect of different volumes of hexane on the extraction of quercetin

5. EFFECT OF EXTRACTION TIME WITH HEXANE ON EXTRACTION OF QUERCETIN:

To investigate the influence of hexane on extraction of quercetin and rutin flavonoids, different time intervals were taken viz., 1, 2,3and 4 hours. Solvent- solvent extraction was done with hexane as one of the solvent. It was observed that, for 90 mins, the concentration of quercetin and rutin were 39 µg/ml and 31µg/ml. The results are shown in the fig.6

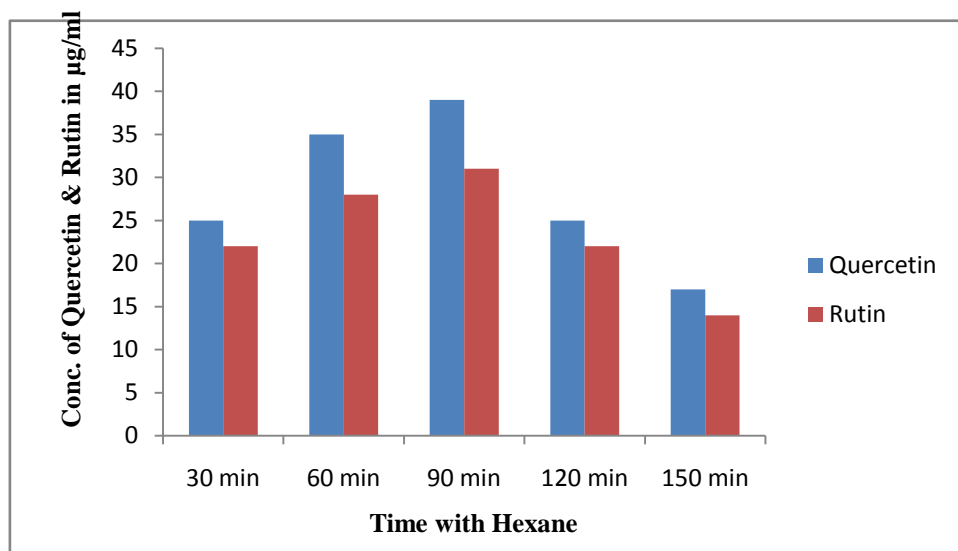


Fig.6. Effect of extraction time with hexane on extraction of Quercetin

#### 6. EFFECT OF pH ON EXTRACTION OF QUERCETIN:

To determine the effect of pH on extraction process different pH values namely 5, 6, 7, 8 and 9 were used. The optimum concentration of quercetin and rutin were 43 µg/ml and 33 µg/ml observed at pH 6. The results are shown in the fig.7

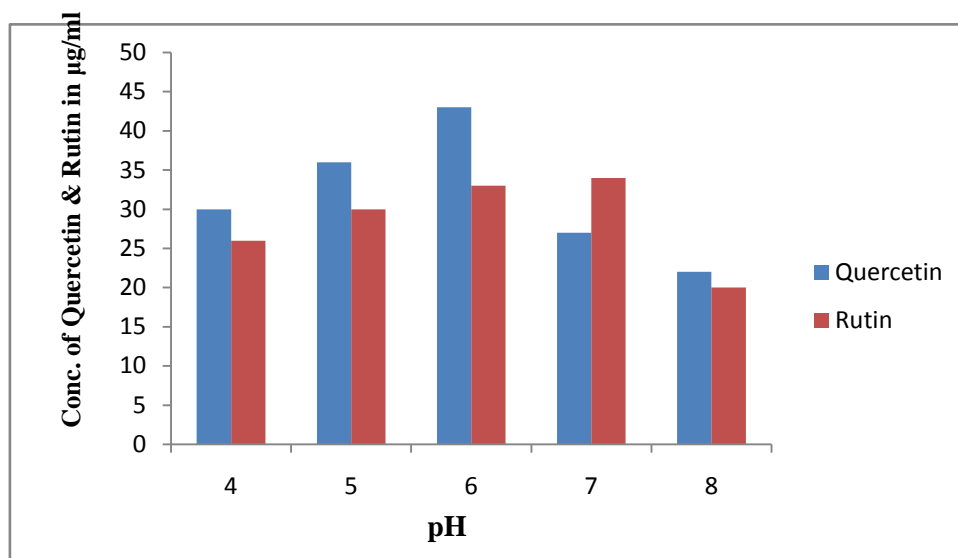


Fig.7. Effect of pH on extraction of quercetin

#### IV. CONCLUSION

Quercetin and rutin are flavonoids widely distributed in nature. It is rich in foods including fruits, vegetables, leaves and grains. These flavonoids are shows pharmacological activities like anti-oxidant, anti-cancer, anti-viral and anti-inflammatory activities etc. The present study was intended for the optimization of extraction of Quercetin and rutin from leaves of *Bacopa monnieri* and its various physico- chemical parameters have been studied. For the extraction of

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