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**Regular Article**

# Optimization of Physico-Chemical Parameters for the Extraction of Phenolic Components from *Terminalia chebula* Species

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***Terminalia chebula* is a moderate tree used in traditional medicines. It contains Chebulinic acid, Quercetin, Tannic acid and many other compounds. The objectives of this work were extraction of phenolic compounds like Total Phenolic Content, Chebulinic acid and Quercetin. The present studies on optimization of physico-chemical parameters like effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol and methanol as solvents and pH for the extraction of Total Phenolic Content, Chebulinic acid and Quercetin were studied. For the extraction of Total Phenolic Content, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH were ethanol, 1 day, 1hrs, 125 microns, 50% (v/v), 1:1 ratio and 7.0 respectively. The highest Total Phenolic Content concentration for optimized conditions was 2.25mg/dl. For the extraction of Chebulinic acid, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH were ethanol, 1 day, 1hrs, 125 microns, 50% (v/v), 1:1 ratio and 7.0 respectively. The highest Total Phenolic Content concentration for optimized conditions was 3.4mg/ml. For the extraction of Quercetin, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with methanol as solvent and pH were methanol, 2 days, 1hrs, 125 microns, 80% (v/v), 1:1 ratio and 6.0 respectively. The highest Quercetin concentration for optimized conditions was 0.54mg/cl.**

**Keywords:** Terminalia chebula, Total Phenolic Content, Chebulinic acid, Quercetin

*Terminalia chebula* is species of terminalia and commonly called as Black myrobalan, Ink tree or Chebulic myrobalan. It is belongs to the family *combretaceae*. It is extensively used in unani, ayurveda and homeopathic medicine. This is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant. It is used for the treatment of number of diseases like cancer, paralysis, cardio vascular diseases, ulcers, leprosy, arthritis, gout, epilepsy etc. It has been reported as

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antioxidant, antidiabetic, antibacterial (Kannan et al., 2009), antiviral, antifungal, anticancerous, antiulcer (Raju et al., 2009), antimutagenic, wound healing activities (Choudhary, 2011) etc. *Terminalia chebula* contains the triterpenes arjun glucoside 1, arjungenin and the chebulosides 1&2. Other constituents contains tannins up to 30%, chebulic acid 3-5%, chebulinic acid 30% (Lee et al., 2006) tannic acid 20-40%, ellagic acid, 2,4-chebulyi–β-D-gluco pyranose, gallic acid, ethyl gallate, punicalagin terflavin A , terchebin, some purgative of the nature of anthraquinone, flavonoids like luteolin, rutins and quercetin etc (Surya Prakash et al., 2012).

Total phenolic content: Phenolic compounds (Saleem et al., 2002) are well known as a radical scavengers, metal chelators, reducing agents, hydrogen donors. Phenolic compounds in plants possess strong antioxidant activity and may help to protect cell against the oxidative damage caused by free radicals. These phenolic constituents act against the damage of membrane lipids, DNA, protein and cellular organelles, early aging and etc.

Quercetin is a flavonoid widely distributed in nature and is derived from *quercetum* (oak forest).Foods rich in quercetin include black and green tea, apples, onion, red grapes, citrus fruit, tomato, broccoli and other leafy green vegetables etc. It is water soluble polyphenolic compound, which is extremely common and wide spread in the plant kingdom as their glycosides. Quercetin is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. In laboratory studies Quercetin shows anti-cancer, anti-viral and anti-inflammatory activities etc.

The dried fruits of *Terminalia chebula* is used to produced the dye. The appearance of dye powder is brown and the main colouring component is Chebulinic acid. The molecular formula for Chebulinic acid is $C_{41}H_{32}O_{27}$ and it is easily dissolved in methanol, ethanol, and ethyl acetate but sparingly soluble in water. The extraction of Chebulinic acid is done by Soxhlet extractor and it also be extracted by HPLC. Chebulinic acid helps to remove toxins and unwamted fat from the body. Act as an effective anti-bacterial, anti-fungal, improves skin glow and complexion.

## MATERIAL AND METHODS:

### Chemicals and reagents:

Folin Denis reagent, Sodium carbonate ($Na_2CO_3$), Aluminium chloride (AlCl3), Potassium acetate, Methanol, Ethanol, Ethyl acetate, Hexane, Distilled water.

### Collection of Plant material

The fruit of *Terminalia chebula* collected from local market in Visakhapatnam, Andhra Pradesh, India.

### Processing of the Plant material

These fruits were cut into small pieces and powdered. The total powder done in to different mesh sizes from 44 to 120. The different size powders were stored in the air tight small covers.

### Extract preparation

Weigh the amount of 2g of powder and add ethanol (25%) and methanol (25%), in different flasks and makeup this solution up to 50 ml. Soak the solution for 1 day and 2 days respectively. After the soaking time filtrate the solution by using Whatman No.1 filter paper and heat the filtrate solution at $78^0$c and $65^0$c respectively. So that the solvent which is taken in

the glass wear is evaporated and make up this solution up to 25 ml with distilled water to this solution add 25 ml of hexane solvent (Harpreet Walia et al.,2011), mix the solution thoroughly. Pour the entire mixture in the separating funnel by using glass funnel. Incubate the solutions of ethanolic and methanolic extract for 1hr and 2hrs respectively.

**Determination of Total Phenolic Content (TPC) and Chebulinic acid (CBA) by Colorimeter**

By Folin-Denis Method:

1ml of ethanolic extract was withdrawn in a 10 ml volumetric flask separately. To each flask 0.5ml of Folin Denis reagent and 1ml of Sodium carbonate were added and volume is made up to 10ml with distilled water. The mixtute was allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 700 nm using colorimeter. The Total Phenolic Content and Chebulinic acid were determined by using calibration curve.

**Determination of Total Flavonoids (Quercetin)**

0.5ml of methanolic extract taken in a test tube and 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water were added. The mixture was allowed to stand for 30 min at room temperature. The absorance of the reaction mixture was measured at 415 nm using colorimeter. The Quercetin was determined by using calibration curve.

**Results and Discussion:**

**Effect of Different Solvents for Extraction of Total Phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin:**

Different organic solvents such as methanol, ethanol (Suchalaths S et al., 2005), ethyl acetate and water were used to extract the optimum yield of Total Phenolic Content, Chebulinic acid and Quercetin from Terminalia chebula species. For Total phenolic Content and Chebulinic acid the solvent ethanol shows best results and the concentrations were 1.0mg/dl and 1.5mg/ml respectively. For Quercetin the solvent methanol shows best result and its concentration was 0.30 mg/cl. The results were shown in fig 1.

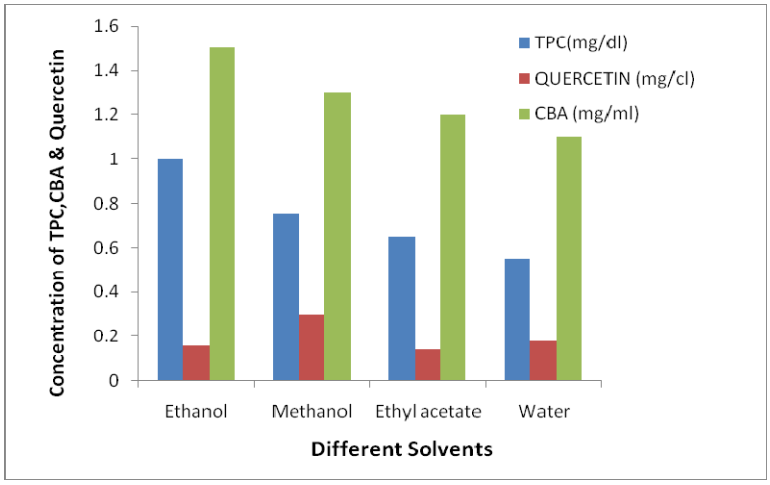


**Fig 1: Effect of Different Solvents for Extraction of Total phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin**

**Effect of Soaking Time for Extraction of Total Phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin:**

The samples were incubated under proper conditions at different time intervals viz., 1, 2, 3 and 4 days to investigate the influence on extraction of Total phenolic Content, Chebulinic acid and Quercetin. It was observed that first day was the best soaking time for the extraction of Total Phenolic Content and Chebulinic acid and the concentrations were 2.05mg/dl and 2.8mg/ml. Second day was suitable for the extraction of Quercetin and its concentration was 0.355mg/cl. The results were shown in fig 2.

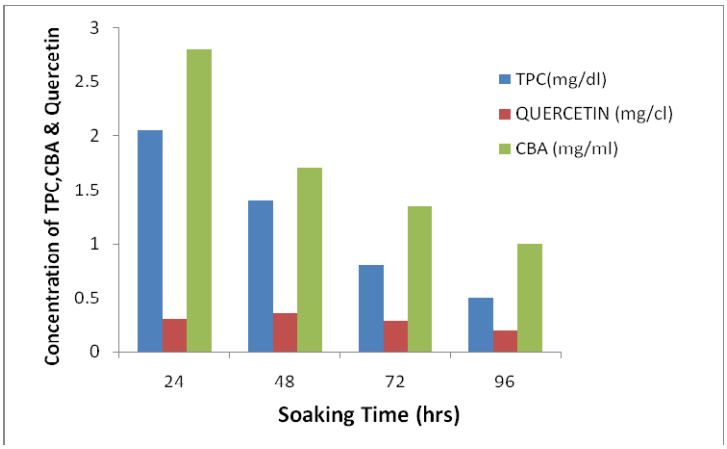


**Fig 2: Effect of Soaking Time for Extraction of Total phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin**

**Effect of Extraction Time with hexane for extraction of Total Phenolic Content (TPC), Chebulinic acid and Quercetin:**

To investigate the influence of hexane on extraction of Total Phenolic Content, Chebulinic acid and Quercetin different time intervals were taken viz., 1, 2, 3 and 4 hrs. Solvent-Solvent extraction was done with hexane as one of the solvent. It was observed The results envisaged from fig 3 shows that optimum concentrations were observed at first hour. The concentrations were 2.1mg/dl, 3.0mg/ml and .38mg/cl respectively.

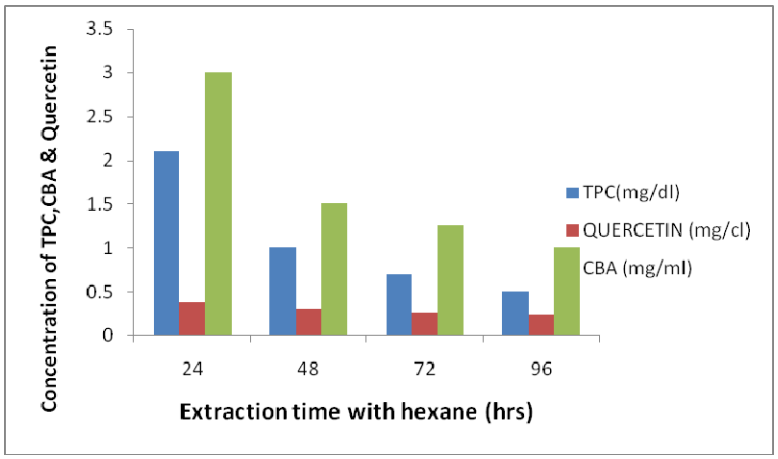


**Fig 3: Effect of Extraction time with hexane for Extraction of Total phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin**

**Effect of Different Particle size for the Extraction of Total Phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin:**

Different particle size viz., 354, 328, 250, 205, 149, 125 and 74 microns were used to find out the optimum concentrations of Total Phenolic Content, Chebulinic acid and Quercetin. The present investigation suggests that the extraction of Total Phenolic Content, Chebulinic acid and Quercetin at different particle sizes indicates that the optimum particle size (Anil D. Mahajan et al., 2011) was 125 microns for extraction of Total Phenolic Content, Chebulinic acid and Quercetin. The optimum concentrations were 2.15mg/dl, 3.2mg/ml and 0.4mg/cl. The results were shown in fig 4.

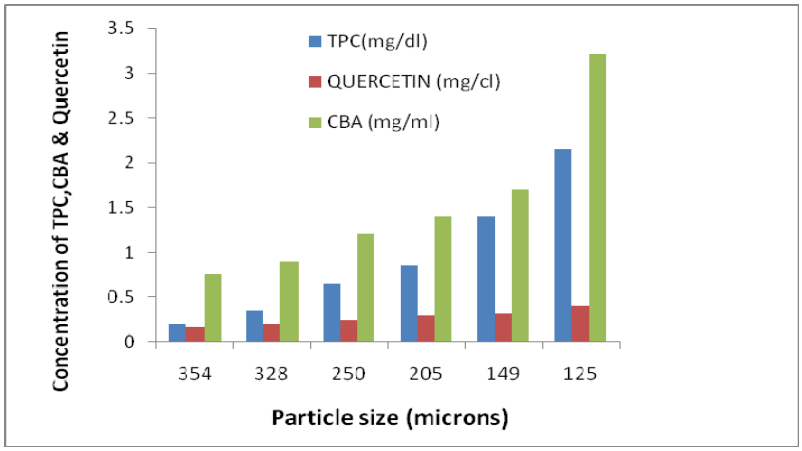


**Fig 4: Effect of Different Particle size for the Extraction of Total phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin**

**Effect of Different Solvent percentages for the Extraction of Total Phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin:**

Percentage of the solvent is also plays a vital role for the extraction of components. The study on different solvent (Ethanol, Methanol) percentages like 0%, 20%, 40%, 50%, 60%, 80% and 100% shows significant variations. Fig 5 shows that optimum solvent percentages were found to be at 50% ethanol for both Total phenolic content & Chebulinic acid and 60% methanol for Quercetin and the concentrations were 0.55mg/dl, 3.3mg/ml and 0.15mg/cl respectively. The results were shown in fig 5.

**Effect of Different volumes of Hexane for the Extraction of Total Phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin:**

To determine the volume of hexane (Harpreet Walia et al., 2011) for the extraction of Total phenolic content, Chebulinic acid and Quercetin different volumes of hexane with solvent (ethanol, methanol) were considered such as 2:1, 1:1, 1:2 and 1:3. These different volumes and solvent (ethanol, methanol) shows a significant effect on the extraction. The optimum extraction of Total Phenolic Content and Chebulinic acid were achieved at 1:1 with ethanol as a solvent. The optimum concentrations were 2.2mg/dl, 3.35mg/ml. For Quercetin the optimum extraction was also recorded at 1:1 with methanol and the concentration was 0.525mg/cl. The observed results were shown fig 6.

**Effect of pH for the Extraction of Total Phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin:**

To determine the effect of pH on the extraction process different pH values were considered such as 5, 6, 7, 8, and 9. It was observed that the extraction of Total Phenolic Content and Chebulinic acid were found to be optimum pH at 7.0 and optimum concentrations were 2.25 mg/dl, 3.4 mg/ml and optimum pH at 6.0 (Lokeswari N et al., 2006) for Quercetin, optimum concentration was 0.54 mg/cl. The results were shown in fig 7.

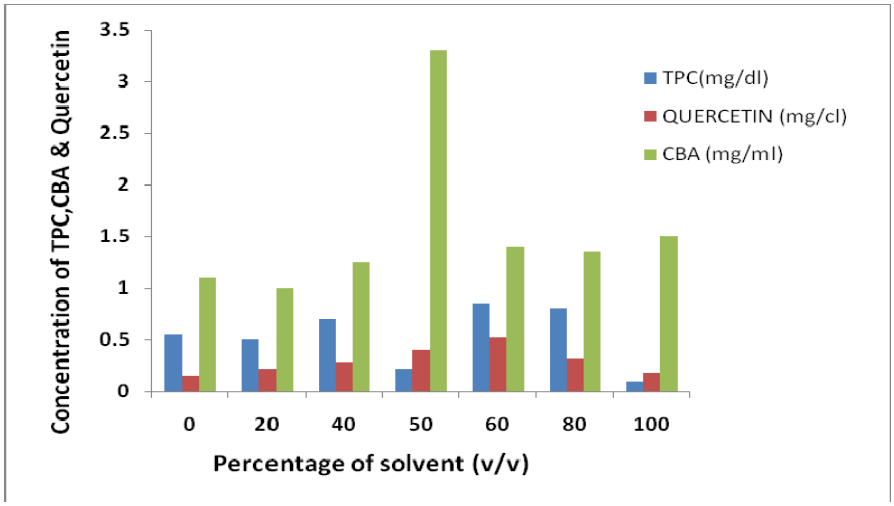


**Fig 5: Effect of Different Solvent percentages for the Extraction of Total phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin**

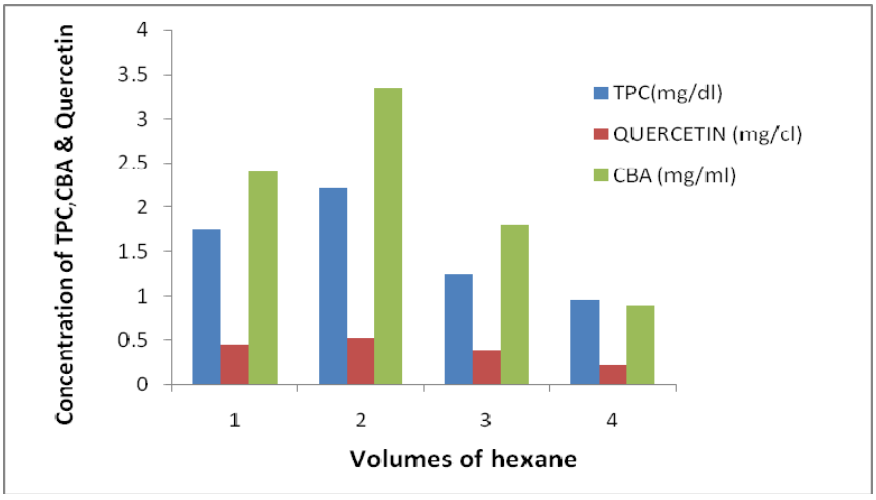


**Fig 6: Effect of Different Volumes of Hexane for the Extraction of Total phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin**

**Conclusion:**

The present study was to intend to optimization of extraction of Total Phenolic Content, Chebuolinic acid and Quercetin and its various physico-chemical parameters have been studied.

For the extraction of Total Phenolic Content, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH were ethanol, 1 day, 1hrs, 125 microns, 50% (v/v), 1:1 ratio and 7.0 respectively. The highest Total Phenolic Content concentration for optimized conditions was 2.25mg/dl.

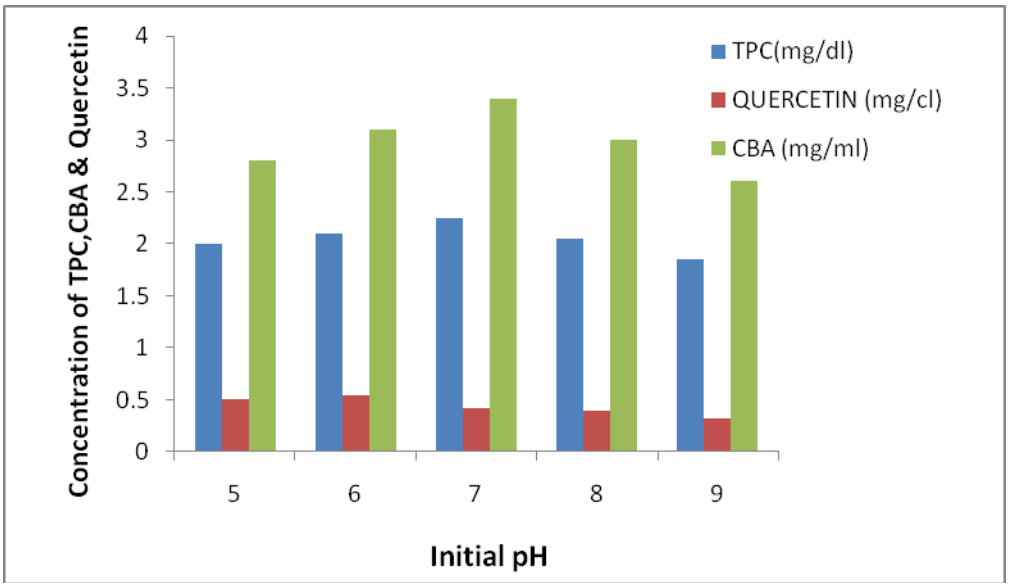


**Fig 7: Effect of pH for the Extraction of Total phenolic Content (TPC), Chebulinic acid (CBA) and Quercetin**

For the extraction of Chebulinic acid, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH were ethanol, 1 day, 1hrs, 125 microns, 50% (v/v), 1:1 ratio and 7.0 respectively. The highest Total Phenolic Content concentration for optimized conditions was 3.4mg/ml.

For the extraction of Quercetin, the optimum results were observed for the effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with methanol as solvent and pH were methanol, 2 days, 1hrs, 125 microns, 80% (v/v), 1:1 ratio and 6.0 respectively. The highest Quercetin concentration for optimized conditions was 0.54mg/cl.

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