



## MICROWAVE AND ULTRASONICATION ASSISTED EXTRACTION OF PHYTOCHEMICALS FROM *B. monnieri* AND STUDY OF ITS ANTIOXIDANT ACTIVITY

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### ABSTRACT

Plant extracts having an inhibitory effect on aging may be a good choice for cosmetic applications because of their natural origin. The extraction of bioactive compounds from plants is one of the most critical steps in the commercial development of natural products. Therefore rapid and efficient phytochemical extraction methods are essential. The present study involves the use of microwave and ultrasonication as methods to extract phytochemicals from *B. monnieri*. The efficiency of the extraction methods were studied by determining the saponin content and the antioxidant activity of the extracts by the Vanillin sulphuric acid and the DPPH method respectively. The study revealed a 5.01% increase in the yield of saponins and 12.61% increase in the antioxidant activity of the microwave extract. The extracts prepared using ultrasonication yielded a 1.47% and 9.08% increase in saponin content and antioxidant activity respectively. Thus the study revealed the superiority of Microwave and Ultrasonication assisted extraction compared to the conventional extraction method.

**KEYWORDS:** Microwave, Ultrasonication, Saponin, Antioxidant, *B. monnieri*



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## INTRODUCTION

Plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxide radicals, hydroxyl radicals and peroxyxynitrite<sup>1</sup>. Natural antioxidants have been widely used in different fields because of their ability to protect against cardiovascular diseases, cancer, deficiencies in immune response, age-related problems, etc. The use of the antioxidants from plant extracts in the cosmetic products improves their quality, leads to the cell rejuvenation and skin health<sup>2</sup>. In this reason many leading cosmetic firms over the world use natural plant extracts in their products. Therefore, the study of plants as a resource of antioxidants has become important in the context of present global scenario where oxidative stress is found to be one of the major causes of health hazards. Growing interest in different plant secondary metabolites has prompted the need to review the traditional extraction technologies and develop new economical, efficient and rapid extraction technologies for enhancing the concentration of phytochemicals. Traditional organic solvent-based extraction often suffers from low extraction yields and long extraction times. Hence, Microwave assisted extraction (MAE) and ultrasonication assisted extraction (UAE) technique of bioactive materials from medicinal plants is a potential alternative to the conventional extractions. Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz. Plant cells contain minute microscopic traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect evaporates and generates tremendous pressure on the cell wall leading to its rupture<sup>4</sup>. This facilitates the faster release of the phytochemicals in larger quantity. Ultrasonication uses high frequency sound, creating pores in the cell wall thereby liberating phytochemicals from plant cells<sup>5</sup>. *Bacopa monnieri*, a member of the Scrophulariaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. Traditionally, it was used as a brain

tonic to enhance memory development, learning, and concentration and to provide relief to patients with anxiety or epileptic disorders. *Bacopa*'s antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer. Compounds responsible for the pharmacological effects of *Bacopa* include alkaloids, saponins and sterols. Many active constituents – the alkaloids Brahmine and herpestine, saponins d-mannitol and hersaponin, acid A, and monnierin – were isolated in India over 40 years ago. Other active constituents have since been identified, including betulinic acid, stigmastanol, beta-sitosterol, as well as numerous bacosides and bacosaponins<sup>6</sup>.

## MATERIALS AND METHODS

The sun dried leaves of *Bacopa monnieri* were powdered and used for extraction of phytochemicals.

### Extraction

#### **Conventional organic solvent extract<sup>6</sup>**

50g of powdered *Bacopa monnieri* was added to 500ml of methanol and kept overnight. The extracts were filtered using Whatman no.1 filter paper. The solvent was evaporated under vacuum in a rotary evaporator. The residue was dissolved in DMSO and used for further studies.

#### **Microwave Assisted Extraction**

50g of powdered *Bacopa monnieri* was exposed to microwave radiation at 100% power (1200W) for 1 min. This was added to 500ml of methanol and kept overnight. The extracts were filtered using Whatman no.1 filter paper. The solvent was evaporated under vacuum in a rotary evaporator. The residue was dissolved in DMSO and used for further studies.

#### **Ultrasonication Assisted Extraction**

50g of powdered *Bacopa monnieri* was added to 500ml of methanol and sonicated for 1min. The extracts were kept overnight and filtered

using Whatman no.1 filter paper. The solvent was evaporated under vacuum in a rotary evaporator. The residue was dissolved in DMSO and used for further studies.

#### **Estimation of Saponin content by Vanillin-Sulfuric acid assay<sup>7</sup>**

For quantification of triterpenoid saponins, 0.25ml of diluted plant extract was taken, 0.25ml vanillin solution (10%) and 2.5ml of sulphuric acid (72%w/v) were added to it and thoroughly mixed in an ice water bath. The mixture was warmed in a water bath at 66°C for 10 min and then cooled in ice-cold water bath. The absorbance at 535 nm was recorded against the blank. The Total Triterpenoid saponin (TTP) content was determined from a standard plot and was expressed as saponin

g% of the extract.

#### **Determination of antioxidant activity by DPPH Radical Scavenging Assay<sup>8,9</sup>**

DPPH free radical scavenging assay was performed to determine the antioxidant activity of the extracts. DPPH (0.002%) was used as free radical. Equal volume of extracts and DPPH were mixed and the tubes were incubated at room temperature in dark for 10 minutes. The optical density was measured at 517nm using UV-Visible Spectrophotometer. The degree of stable DPPH decolourization to DPPH (reduced form of DPPH) which is yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH was calculated using the following equation.

$$\text{Scavenging activity (\%)} = (A - B) / A \times 100$$

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

## **RESULTS AND DISCUSSION**

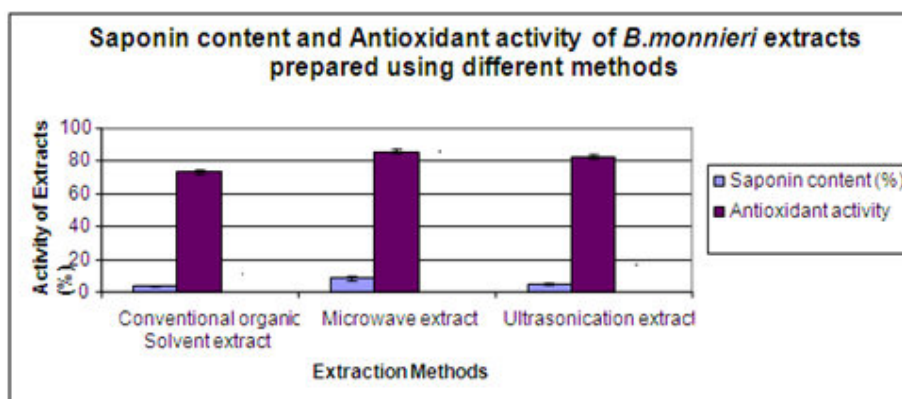
#### **Estimation of Saponin content**

The efficacy of extraction procedure was checked by estimating phytochemical content using a chemical method. Saponin content of *B.monniери* extracts was determined using Vanillin - Sulphuric acid assay. The saponin content of the conventional, microwave and ultrasonication extract was found to be 4.63±0.15 g%, 9.91± 0.14 g% and 6.33±0.25g% respectively (Graph 1).

#### **Antioxidant activity by DPPH Radical Scavenging Assay**

In the DPPH assay the antioxidant activity of the conventional, microwave and ultrasonication extracts was found to be 73.27± 1.49%, 85.88±1.23 % and 82.35±1.3%. The results indicated that the microwave extract exhibited highest antioxidant activity followed by the ultrasonication extract compared to the conventional extract of *Bacopa monniери* (Graph 1).

**Graph 1**  
**Saponin content and Antioxidant activity of *B. monniери* extracts prepared using different methods**



## CONCLUSION

The current study establishes the superiority of the microwave assisted extraction (MAE) and ultrasonication assisted extraction (UAE) methods in yielding *B. monnieri* extracts with higher saponin content and increased antioxidant efficacy. This can be attributed to the rupture in cell wall of the plant cells under the influence of these treatments. Thus the modern techniques of MAE and UAE can also be used for the extraction of phytochemicals from various medical plants. The plant extracts prepared using these novel technologies can be used in various cosmetic formulations like detoxifying creams, anti-stress day/night creams, anti-pollution creams, anti-aging

creams etc. ensuring a multi-phase protection to skin by enhancing the capacity of skin to resist oxidative stress, hyper pigmentation etc.

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### **Conflict of Interest**

Conflict of interest declared none.

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