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Research Article

# Comparative study of Antiplatelet Aggregation Activity of *Bacopa monnieri* extracted using Microwave and Ultrasonication

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**ABSTRACT**

Plant extracts having an antiplatelet aggregation activity may be a potential choice for development of anti-inflammatory drugs because of their natural origin. The extraction of bioactive compounds being the most crucial step in the commercial development of natural products it is essential to have rapid and efficient phytochemical extraction methods. Extracts of *B. monnieri* were prepared using different methods such as the Conventional organic solvent extraction method, Microwave Assisted Extraction (MAE) and the Ultrasonication Assisted Extraction (UAE) method. The efficiency of the extraction methods were studied by determining anti-inflammatory activity by the *in vitro* antiplatelet aggregation method. The study revealed a 48.41 % and 45.68 % increase in activity for the extracts prepared using ultrasonication and microwave assisted extraction establishing their superiority compared to the conventional organic solvent extraction method.

**Keywords:** Microwave, ultrasonication, anti-inflammatory, antiplatelet aggregation, *Bacopa monnieri*.

## INTRODUCTION

Traditional extraction methods used for medicinal plants include maceration, infusion, percolation, decoction, soxhlet extraction, counter current extraction, steam distillation, etc. However since the general methods of extraction had several drawbacks in terms of the yield, amount of solvent and time for extraction certain new extraction methods including microwave assisted extraction (MAE), ultrasound assisted extraction (sonication), supercritical fluid extraction etc. are gaining importance[1].

Microwave Assisted Extraction (MAE) of constituents from plant material has shown tremendous research interest and potential in the recent years. Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz. Inside the plant cell when the moisture gets heated due to microwave effect it evaporates generating tremendous pressure on the cell wall due to swelling of the plant cell. The pressure thus generated pushes the cell wall from inside, resulting in its rupture, thereby facilitating the leaching out of the active constituents to the surrounding solvent, thus improving the yield of phytoconstituents[2].

Ultrasonication Assisted Extraction (UAE) uses high frequency sound waves (20–50 kHz) to create minute

pores in the cell wall of plant cells and thereby release the phytochemicals from plant materials. This extraction process is less time consuming compared with traditional laboratory methods because of particle disruption of the plant material[3].This extraction methodology was used for the isolation of essential oils, polysaccharides and bioactive phytochemicals including menthol, cardiac glycosides, pyrethrins and camptothecin[4].

Plants extracts are being applied for relief from pain, fever and inflammation since time immemorial. Due to the inherent side effect problems like severe gastric disorders associated with the current synthetic anti-inflammatory agents, the search for new anti-inflammatory agents from the huge array of medicinal plant resources, is intensifying, in the hope of discovering novel therapeutic agents capable of suppressing, reducing or relieving pain with very little or no side effects.

*Bacopa monnieri*, a member of the *Scrophulariaceae* family was traditionally used as a brain tonic to enhance memory development and to provide relief to patients with anxiety or epileptic disorders. The anti-inflammatory effects of *Bacopa monnieri* are being studied and the effectiveness of extract in inhibiting experimentally induced inflammation was compared with that of indomethacin, a known anti-inflammatory drug. Several compounds are responsible for the pharmacological effects of *Bacopa*. Some of the identified active constituents of *Bacopa* include– the alkaloids Brahmine and herpestine, saponins d-mannitol and hersaponin, monnierin ,betulic acid, tigmasterol, betasitosterol as well as numerous bacosides and bacopasaponins[5].

Anti-inflammatory properties can be studied by several *in vivo* and *in vitro* methods. However there are several ethical issues regarding the use of animals in the early stages of drug discovery for natural anti-inflammatory products. Therefore several *in vitro* methods have been developed which include Anti-Protein denaturation method, Human Red Blood Cell (HRBC) membrane stabilization method, Anti – platelet aggregation activity and measurement and inhibition of pro-inflammatory mediators. The Anti – platelet aggregation activity method is based on the principle that platelets adhere to the damaged regions of the endothelial surfaces forming an aggregation mass. In this method the aggregation of human platelets induced by ADP was used to study the anti-platelet aggregation effect of plant extracts with decrease in absorbance indicating increase in aggregation[6].

The present investigation aims at bridging the gaps in the applications of newer extraction techniques to medicinal plants widely used in India by investigating the effect of methods like the application of microwave radiation and ultrasonication on the extraction of phytochemicals from *Bacopa monnieri* and to study its anti platelet aggregation activity.

## MATERIALS AND METHODS:

### Collection and Authentication:

The sundried plant of *Bacopa monnieri* was provided by Konark Herbals and Healthcare, Mumbai and authenticated by the Botany Department of the College. The plant material was powdered and the extracts were prepared.

### 1. Extraction Methods:

**Conventional organic solvent extraction[7]:**

50g of dried and powdered plant material of *Bacopa monnieri* was added to 500ml of methanol and left overnight. The extract was filtered using Whatman no.1 filter paper and used for further studies after evaporating the solvent under vaccum in a rotary evaporator. The residue was dissolved in DMSO and stored at 4 $^{o}$C.

**Novel methods of extraction [8]:**

**Microwave assisted extraction (MAE)**

50g of dried and powdered plant material was exposed to microwave energy for a time duration of 5 minutes at 100% power and then added to 500ml of the methanol and left overnight.

**Ultrasonication assisted extraction (UAE)**

50g of dried and powdered plant material was added to 500ml of methanol and sonicated for time duration of 5 minutes and left overnight.

The extract of *Bacopa monnieri* prepared using MAE and UAE was then filtered using Whatman no.1 filter paper. The post treatments of the extracts were same as that of the conventional extract.

### 2. Analysis of phytochemicals in extracts:

**Determination of Total phenolics[9]:**

Extracts of *Bacopa monnieri* prepared using the conventional organic solvent extraction, MAE and UAE methods were analysed for their total phenolic content by the Folin- Ciocalteau method. The diluted extracts (3ml) were pipetted into different test tubes to which 0.5mL of Folin- Ciocalteau reagent and 2 mL of 20 % (w/v) $Na_2CO_3$ solution were added. The tubes placed in a boiling water bath for exactly 1 min and then were cooled under running tap water. The absorbance of the resulting blue solution was measured at 650 nm with a spectrophotometer. The amount of phenolics present in the sample was determined from a standard curve prepared with

catechol and was expressed in mg per gram of the extract.

**Total triterpenoid Saponin estimation by Vanillin–Sulfuric acid assay [10]:**

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

**3. Determination of Anti-inflammatory activity**

The *in-vitro* anti-inflammatory activity of the extracts was determined by the Anti-platelet aggregation activity[11]:

The platelet rich plasma (PRP) (obtained from Nanavati hospital, Mumbai) for each assay was re-suspended to get 0.9 X $10^7$ platelets per 2ml of isosaline (pH 7). Platelet aggregation was determined by measuring the change in the optical density of stirred PRP after addition of the aggregating agent to the cuvette. Platelet aggregation occurs only if the PRP in the cuvette is stirred. The colorimeter was standardized by placing the PRP sample representing 0% transmittance. The increase in light transmittance/decrease in absorbance indicates platelet aggregation. To determine the *in vitro* inhibition of platelet aggregation, 0.2ml of plant extracts prepared in isosaline were used. The platelet aggregation was induced with ADP at a concentration of 1.2mg/ml, which was used as control. The Aspirin 100µg/ml was used as a standard. The absorbance value was recorded at the end of 10min at 620nm and percentage inhibition of platelet aggregation calculated.

**Statistical Analysis**:

All experiments were performed in triplicates and the results are expressed as mean ± standard deviation.

**RESULTS AND DISCUSSION**

**Total phenolic content:**

The phenolic content of the conventional methanolic extract was found to be 6.72±0.27 mg/g (catechol equivalents, CE). However the microwave and ultrasonication extract yielded a higher phenolic content compared to the conventional extract. *Bacopa monnieri* yielded maximum phenolic content of 11.51±0.28 mg/g (CE) and 9.17±0.15 mg/g (CE).

**Total triterpenoid saponin content:**

The total triterpenoid saponin content of the extracts, prepared using methods of MAE and UAE was estimated and compared with the conventional organic solvent extract. The saponin content of the conventional, microwave and ultrasonication extract was found to be 4.63± 0.15%, 9.91 ±0.14 % and 6.33 ±0.25%. However, the use of novel extraction methods of microwave and ultrasonication resulted in a 5.3±0.2% and 1.7± 0.12% increase in the yield of saponins.

**Antiplatelet aggregation activity:**

The antiplatelet aggregation activity was studied to determine the anti-inflammatory potential of the extracts. The conventional, microwave and ultrasonication extract exhibited an activity of 42.72±0.75%, 88.4±0.2% and 91.13±0.30% respectively at 100µg/ml of extract (Fig 1). Thus, the ultrasonication extract exhibited highest activity for anti-platelet aggregation. The study revealed a 48.41 % and 45.68 % increase in activity for the extracts prepared using ultrasonication and microwave assisted extraction respectively establishing their superiority. Thus on studying the anti-inflammatory mechanism it can be summarized that the ultrasonication extracts exhibited greater efficiency in anti-inflammation by preventing platelet aggregation. *B. monnieri* is known to contain different phytochemical compounds such as Bacosides A & B, Bacogenins A1, A2, A3, A4 and many more[13]. The difference in the anti-inflammatory efficiency by the platelet aggregation mechanism may be attributed to the difference in phytochemical composition and concentrations in the extracts due to the variation in the kind of technology used.

**CONCLUSION**

Platelet aggregation plays a major role the development of atherothrombotic disorders. As a result, inhibitors of platelet aggregation have become increasingly important. The dissatisfaction in the potential undesirable side effects of the current anti-platelet agents has fuelled the search for new generation of effective agents from natural sources. In recent years natural products have proven to be a reliable template for the development of new scaffolds of drugs. Several studies have reported phytochemicals such as alkaloids, phenolic compounds, flavonoids and a diterpene isolated from different plant sources, showing potent anti-platelet activity. Recently a number of naturally occurring triterpenes and their derivatives have also been reported to possess remarkable anti-platelet aggregation[14,15].

This study suggests that the efficiency of the bioactive molecules is greatly influenced by the extraction technology involved. On a comparative evaluation of extraction using microwave, ultrasonication and conventional organic solvent based extraction method it can be concluded that ultrasonication assisted extraction of *B. monnieri* proved to be most effective in increasing its potential for anti- platelet aggregation activity. The extract prepared using microwave also exhibited higher potential in anti-platelet aggregation activity but it was not as superior as the ultrasonication extract. However the characterization and isolation of phytochemicals present in the extract prepared using ultrasonication and microwave which are responsible for anti-platelet aggregation and their subsequent molecular mechanisms involved needs to be studied. Thus the present preliminary work reveals that ultrasonication and microwave assisted extraction technology can be exploited to prepare herbal therapeutics with greater potency based on the applications required in terms of the antioxidant, anti-inflammatory, anti-tyrosinase activity etc.

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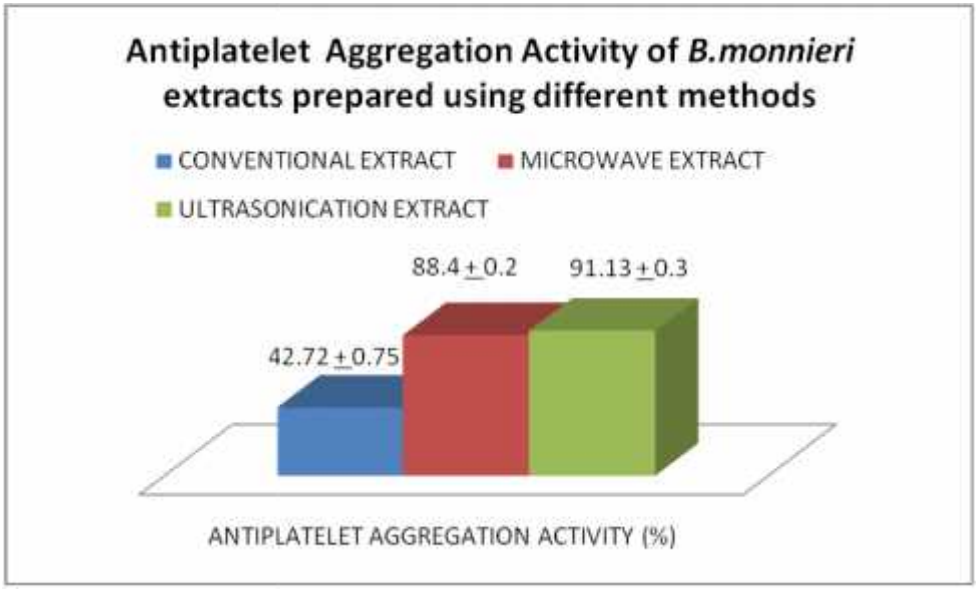


**Figure 1**

**Antiplatelet Aggregation of *Bacopa monnieri* extracts prepared using different methods**

**REFERENCES**

1. Huie CW. A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. Analytical and Bioanalytical Chemistry, 2002; 373: 23-30.
2. Vivekananda M, Yogesh M and Hemalatha S. Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research. Pharmacognosy Reviews, 2007; 1: (1) 7 -18.
3. Mukherjee PK, Quality Control of Herbal drugs: An approach to evaluation of botanicals. Business Horizons, 2002.
4. Fulzele DP and Satdive RK. Comparison of techniques for the extraction of anticancer drug camptothecin from *Nothapodytes foetida*. Journal of Chromatography *A* 2005; 1063(1-2): 9-13.
5. Gohil KJ, Patel AJ, Biswak, DB, Sharma PK, Kumar N, Dudhe R and Bansal V. Review of *Bacopa monnieri*: current research and future prospects. International Journal of Green Pharmacy 2010; 4(1):1-9.
6. Lavanya R, Maheshwari SU, Harish G, Bharath RJ, Kamali S, Hemamalani D, Varma B and Reddy CU. Investigation of *In-vitro* anti-Inflammatory, anti-platelet and anti-arthritic activities in the leaves of *Anisomeles malabarica* Linn. Research Journal of

Pharmaceutical, Biological and Chemical Sciences, 2010; 1: 745-753.
7. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H: Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia, 2011; 1(1):98-106
8. Thomas R, Kamat SD and Kamat DV: Microwave and ultrasonication assisted extraction of phytochemicals from *B. monnieri* and study of its antioxidant activity. International Journal of Pharma and Biosciences, 2014; 5(1): 66 - 69
9. Sadasivam S and Manickam A.Biochemical methods. New Delhi: New Age International publishers, 2005;203-204.
10. Shiau IL, Shih TL, Wang YN, Chen HT, Lan HF, Lin HC, Yang BY and Murase Y Quantification for Saponin from a Soapberry (Sapindus mukorossi Gaertn)in Cleaning Products by a Chromatographic and two Colorimetric Assays. J. Fac. Agr., 2009; 54 (1): 215–221.
11. Desai JV, Thomas R, Kamat SD and Kamat DV. Comparative Studies on In-Vitro Anti-Inflammatory Activity of *Andrographis paniculata* and *Bacopa monnieri*. Journal of Advanced Pharmaceutical Research, 2012; 2(1): 5- 8.
12. Zhou L and Schmaier AH. Platelet Aggregation Testing in Platelet-Rich Plasma. American Journal of Clinical Pathology, 2005; 123:172-183.
13. Rauf K, Subhan F, Al-Othman AM, Khan I, Zarrelli A and Shah MR.Preclinical profile of bacopasides from *Bacopa monnieri* (BM) as an emerging class of therapeutics for management of chronic pains. Current medicinal chemistry, 2013; 20(8):1028-37.
14. Ibrahim T Babalola, Francis O Shode, E A Adelakun , Andy R Opoku , Rebamang A and Mosa. Platelet-Aggregation Inhibitory Activity of Oleanolic Acid, Ursolic Acid, Betulinic Acid, and Maslinic Acid, 2013;1( 6) :54 -60.
15. Yadav Anu and Mendhulkar VD. *In vitro* anti-platelet aggregation activity of the extracts of *Camellia sinensis.* Research in Biotechnology, 2015; 6(3): 10-16.