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Rapid and perceptive technique of protein as a marker in *Bacopa monnieri* L. in dementia

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Abstract
The aim of the study is to find out the whether an extract of brahmi leaves might be highly putative in dementia type Alzheimer's disease. *Bacopa monnierri* L. is used traditionally as alternative and complementary medicine for memory deficits/enhancer. Different solvent extraction is used for protein quantitation and assessing total phenolic content in brahmi leaves. The two different ways of extraction using phenol-chloroform and TCA which also predicts to find out the protein n correlation with total phenolic content in brahmi leaves shows exceedingly raise as 86.7±5.3 mgGAE/g, this is due to the effects of polyphenolic and flavoanoid contents present in leaves of brahmi has natural antioxidants role to treat memory deficits in aging falls. Further, to characterize low molecular weight protein present in brahmi leaves isolated gel was quantified by mass spectrophotometry represent β-amyloid precursor protein, tau and GABA predominantly present in Bacopa Monnieri L. as a putative biomarker for dementia type Alzheimer's disease.

Keywords: *Bacopa monnieri* L. phenolic content, beta amyloid, antioxidants proteins, dementia

Introduction
Bacopa monnierri L. belongs to the family Scrophulariaceae is a slink, glabrous, luscious herb grows in marshy areas all over India. It has been customarily using to severe dementia, annoyance, nerve pain, insominia [1, 2, 3]. The herb from brahmi leaves, also termed as water hyssop or bacopa, is whispered to whet the brain by defensive cells and rising the chemicals which is associated with learning and memory. Brahmi leaves used to treat Alzheimer's disease, anxiety, and affective deficit hypertrophy diseases (ADHD), along with combating stress and improving memory [1, 2]. Brahmi leaves useful in the treatment of epilepsy and helps in cognitive decline levels for memory impairment [4]. Bacopa has been shown to decrease β-amyloid precursor protein deposits particularly in treating chemo preventive model in mice possess more competence in aging falls on dementia type Alzheimer's disease [5, 6]. Brahmi leaves has the putative effects on memory enhancer, it also improved acquisition of learning tasks [6]. Natural antioxidants from brahmi leaves was also depict as more reliable content in poly phenols, flavanoids, saponins, amino acids like glutamine, GABA and tannins progressively raise in leaves extract which is used for avert the lack of memory deficits and antiaging [7, 8, 9]. In brain cells, brahmi leaves imply the protection of free radicals scavenging activities of protein play a important role in age related falls. Antioxidants are the substance with the aim of help protect in opposition to cell damage causes by means of potentially detrimental molecules called free radicals scavengers [8, 9]. A pilot study of antioxidant proteins in brahmi leaves and phytochemical analysis also quantized using different solvent extraction methods. The putative protein can be characterized by protein quantification, PAGE analysis and mass spectrometry for confirming the low molecular weight protein in brahmi leaves. Further, the present study is to determine whether an extract of brahmi leaves could be useful in dementia type Alzheimer's disease.

Materials and Methods
All other chemicals and Analar reagent were purchased from Nice, India and Precision Scientific chemicals, Coimbatore. Meteorological information was obtained from the nearest weather station in Dhanalakshmi srinivasan Agriculture college campus, Perambalur on August 9th 2017 from 07:30 to 21:00 hours: Temperature: 18.8 – 30.9 °C; Relative humidity: 50 – 99%; Wind direction: NE, W, WN; Wind speed: 0.5 – 2.5 m/s. *Bacopa monnieri* leaves Wettst was collected for chemical characterization of proteins and its antioxidant intended to use as a putative biomarker for nerve diseases.

Preparation of plant samples

a. **Green leaf extract:** Methanolic extract of fresh *Bacopa monnieri* leaves (5g) was prepared by vigorous grinding in 80% alcohol and distilled water by using pestle and mortar. The mixture was centrifuged at 10000 rpm for 20 min. The supernatant was collected and used for analysis.
b. **Dry leaf extract:** Same procedure was carried by using dry leaf extract powder (Calabrese *et al*, 2008).

Sample preparation for biochemical analysis

Immediately after collection, samples were stored in liquid nitrogen. Afterwards, they were transferred into the freezer at -80 °C. Tissue samples were subsequently lyophilized and ground. The prepared samples were then stored in airtight vials at -20 °C prior to biochemical analysis.

Total phenolic Content

Total phenolic concentrations in green leaf *Bacopa monnieri* medicinal plants were spectrophotometrically determined using the Folin-Ciocalteu method [10, 11, 12, 13].

Determination of proteins by spectrophotometric methods

Preparation of reagent/chemicals

The phosphate buffers were prepared by mixing 16.0 ml of (i) and 84.0 ml of (ii) and adjust the volume to 200 with distilled water. A 0.2 M monobasic sodium phosphate by taking 27.8 g of NaH_2PO_4 in a volumetric flask and the volume adjusted to 1 liter with distilled water.

A 0.2 M dibasic sodium phosphate Na_2HPO_4. $7H_2O$ by taking 53.6 g of Na_2HPO_4 volumetric flask and the volume adjusted to 1 liter with distilled water (ii). Alkaline Na_2CO_3 reagent was prepared by taking 2.0 g of Na_2CO_3 and dissolved in 0.1 N NaOH and volume adjusted to 100 ml. (0.1 M, pH-7.6)

Procedure for Lowry's method

Added 1.0 ml of supernatant to 5.0 ml of alkaline copper sulfate reagent mix thoroughly. It allows standing for 10 mins and then adding 0.5 ml of Folin's reagent. In order to develop color, this is kept standing for 30 minutes. This was followed by recording absorbance in a spectrophotometer at 660 nm, against a blank. The blank is prepared by taking 1.0mL of 0.5 M NaOH in place of the sample in a cuvette. Bovine serum albumin is used to draw a sigmoidal curve and the number of proteins in different solvents extracts were estimated [11]. SDS-PAGE pattern of low molecular weight protein was detected in *Bacopa monnieri* leaves confirmed by mass spectral data analysis. The SDS-PAGE was performed in a Biorad gel electrophoretic system according to the method of Laemmli (1970) [14, 15, 16]. Further, the leaf protein from *Bacopa monnieri* leaves quantification was also determined [16] to exemplify by using MALDI- TOF mass spectrometry analysis followed by Reeve and Buddie, (2018) methods [14].

Statistical analysis

Biochemical analysis of Total phenolic and protein values are expressed as Means ± S.D of five experiments. Student's t-test was used for statistical significance using SPSS (statistical presentation system software) for windows version 10.0.1 software, Inc. New York. A probability level of $P< 0.05$ was considered as statistical significance in comparing with relevant controls.

Results and Discussion

The results of total phenolic contents of plants studied in this study are given in Table 1. Total phenolic content of the plants varied from 70 mg catechol per 100 g dry weight to 90 mg catechol per 100 g dry weight [17]. In assays of the leaf proteins of the crude extract, significant changes in absorbance at 650 nm were observed (0.7–0.9mg/g) with increasing concentrations of extract (100–500 µg/mL). When a large part of the dialyzed protein contents of plants (70 to 90%) consists of amino acids, the remaining portion and the part called as non-proteins are formed from ammonium and nitrate salts. Further, leaf protein content is an important parameter in the determination of food values of plant nutrients [18, 19]. Protein contents of plant types used in the study were determined spectrophotometrically. The obtained experimental data have been given in Fig 2. As seen from Fig 3, Using PAGE analysis, gel protein contents of plant species have approximately changed in the range of 70 to 90 mg per 100 g dry weight of plants. These results substantiate to indicate that the protein contents of plants are high. Again, from Fig. 2, it can be said that B. monnieri L. has the highest protein content. The total protein content of *Bacopa monnieri* Wettst plants treated with Acetone and Ammonium per sulfate is reduced after 2nd day in below 70 mg/g concentrations [20]. But Phenol chloroform extraction in plants resulted in significant turn down only 4th day onwards ($P \leq 0.05$). Reduced protein content might be due to inhibition of protein synthesis [21, 22], unavailability of essential elements [23] or inhibition of amino acid mobilization [24, 25]. However, protein content was gradually increased after 4 days. The molecular weight of the protein present on the day of 4^{th} and 8^{th} of the interval of assay shows low molecular weight protein as 15 kDa were determine followed by protein molecular marker [25, 5]. The exceeding outcome shows that the protein from *Bacopa monnieri* plant leaves has significant antioxidant activity and *Bacopa monnieri* plant leaves protein has possess the low molecular weight protein is phytochelatin and phytohormone response of antioxidants due to heavy metal stress of *Bacopa monnieri* L Wettest [19, 26]. Previous studies showed that by treating rats with dementia infusion towards bacopa monnierri leaves which decrease the free radicals damage and opposite pharmacognostic signs of cognitive/memory impairment [27, 28]. No significant variation was observed in the methanol, Ammonium salts and Ethyl acetate extraction of protein content in extracts of *Bacopa monnieri* L. Wettst. However, if the rationale is promoted to analytical use of MALDI-TOF- mass spectrometry such methods could be a suitable alternative for protein translational modification and structural characterization. To conclude that, the above result shows that, the proteins from *Bacopa monnieri* plant leaves has more significant antioxidant activity ($P \leq 0.05$). The medicinal and nutraceutical use of *Bacopa monnieri* Wettst are fast rising as the general population are obsessive over the use of traditional ancient or alternative medicines and food supplements containing *Bacopa monnieri* L.) Wettest, which are hypothetical to enhance the proficient of memory and improving the function of the brain; antidepressant antioxidant, antiulcerogenic agent, and calcium antagonist. As a consequence, it can be accomplished that these medicinal herbs, which are used customarily, are protected and effective drug for treatment of dementia type Alzheimer.

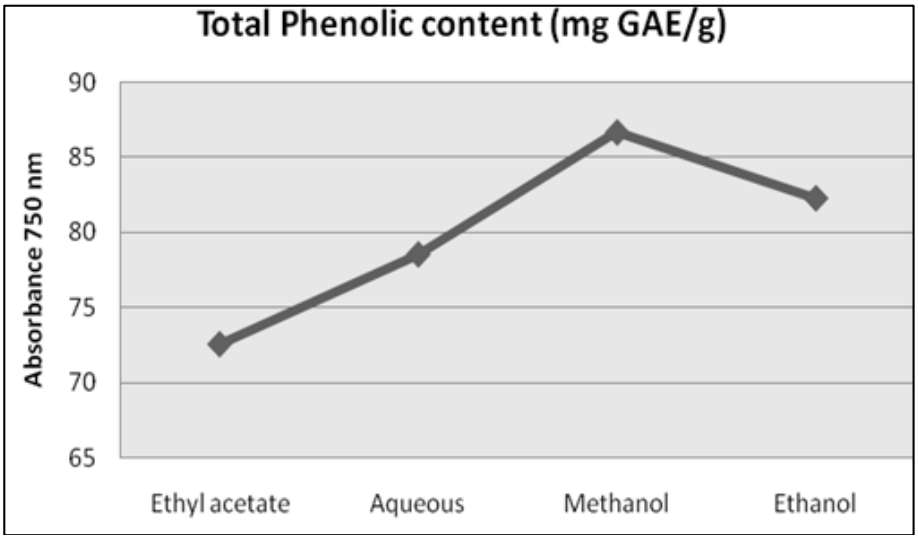

Fig 1: Total Phenolic content in Leaves extract of *Bacopa monnieri* Wettest

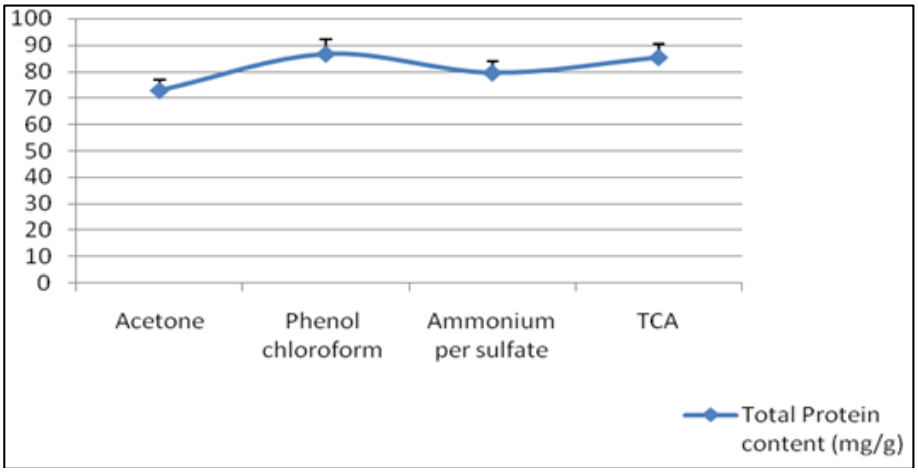

Fig 2: Total Protein content (mg/g) in leaves extract of *Bacopa monnieri* L.

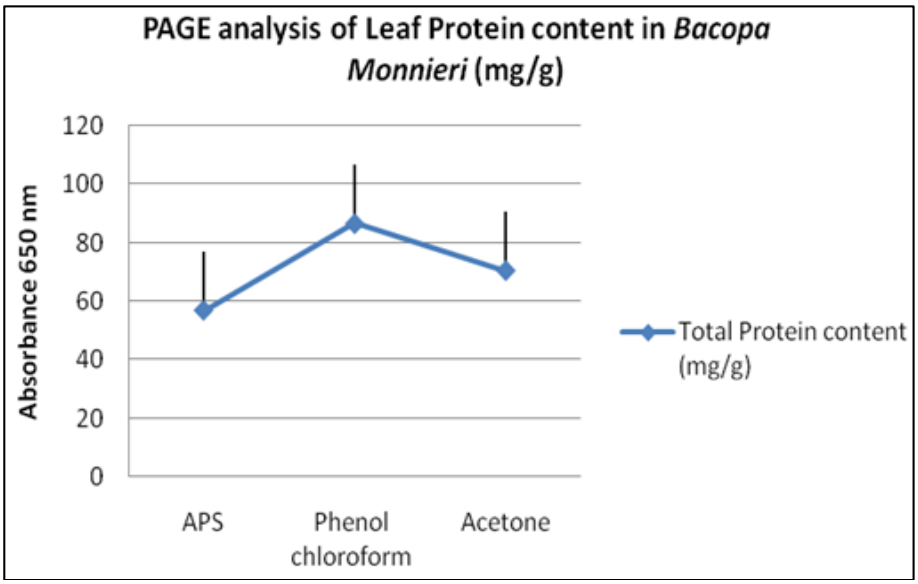

Fig 3: Isolation of gel Protein using Protein Block Expert method (PBE) method in *Bacopa monnieri* L.

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