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Research Article

To Preparation and Evaluation of Bacopa and Cow Colostrum Powder Drug

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ABSTRACT

The present study focuses on the preparation and evaluation of a novel formulation combining Bacopa monnieri and cow colostrum, aimed at enhancing cognitive function and supporting overall brain health. Bacopa monnieri (L.) Wettest., traditionally known as Brahmi, is a valued medicinal plant in the Indian system of medicine, historically used as a brain tonic. Its primary active constituents, bacosides, are triterpenoid saponins known to enhance synaptic transmission, memory, and learning capacity. Cow colostrum, rich in immunoglobulins, growth factors, and nutrients, supports immune modulation and neuroprotection. In this study, a formulation was developed using 250 mg of Bacopa monnieri and 100 mg of cow colostrum per gram. The physicochemical evaluation of the formulation demonstrated acceptable organoleptic properties, pH, flowability, and stability. Results suggest a synergistic potential of Bacopa monnieri and cow colostrum for cognitive enhancement and neuro-immunological support, providing a promising basis for further pharmacological and clinical studies.

INTRODUCTION

Medicinal plant from the backbone of traditional system of medicine in india. Many plant Bacopa monneri (L.) Wettest. (Scrophulariaceae), is a well – known medicinal herb in Indian system of medicine as Brahami (Sanskrit) and Indian water hyssop. The plant is commonly found in wet, damp and marshy areas. Indian Materia Medica (1500 AD) cites the use of the plant as a brain tonic, which is responsible for the memory enhancing is a triterpenoid saponin called Bacosides'. Bacosides enhance the efficiency of transmission of nerve impulse there by strengthening memory and cognition. [1]

Why People Use Herbal Medicine:

The earliest evidence of human's use of plant for healing dates back to the Neanderthal period.

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Herbal medicinal is now being used by an increasing number of patients who typically do not report to their clinician concomitant use. There are multiple reasons for patients turning to herbal therapies. Often cited is a "sense of control, a mental comfort from taking action," which helps explain why many people taking herbs have diseases that are chronic or incurable viz. diabetes, cancer, arthritis or AIDS. In such situations, they often believe that conventional medicine has failed them. When patients use home remedies for acute, often self-limiting conditions, such as cold, sore throat, or bee sting, it is often because professional care is not immediately available, too inconvenient, costly or time-consuming. In rural areas, there are additional cultural factors that encourage the use of botanicals, such as the environment and culture, a "man earth relationship." People believe that where an area gives rise to a particular disease, it will also support plants that can be used to cure it.

Bioavailability of Herbal Drugs:

The bioavailability of the active constituents of the herb is another area of considerable importance. Before a compound can act systemically it must pass from the gastrointistinal tract into the blood stream. This is an area in which surprisingly little is known for herbal constituents. Compound, such as berberine and hydrastine in the popular botanical goldenseal are essentially not absorbed following oral consumption. Studies showing systemic effect in animal have all involved parenteral administration of these alkaloids. Yet goldenseal remains one of the best-selling herbs, is widely promoted, and is accepted by a misinformed public as a nonspecific immune stimulant. Cinnabar has been for a long time in traditional medicine.The toxic effects of inorganic mercury are well recognized, but because of its insolubility it has been assumed that this compound would not be significantly absorbed from the gastrointestinal tract. However, investigation of on the oral absorption of cinnabar in mice found a significant increase in mercury concentration in the liver and kidney. Concomitant use of cinnabar and drugs containing bromides, sulphates, sulphides, nitrates and iodine may enhance its toxicity by increasing the gastrointestinal absorption. [(2)]

Present Status of Herbal Medicine:

The wide spread use of herbal medicine is not restricted to developing countries, as it has been estimated that 70% of all medical doctors in France and German regularly prescribe herbal medicine. The number of patients seeking herbal approaches for therapy is also growing exponential. With the US Food & Drug Administration (FDA) relaxing guidelines for the sale of herbal supplement, the market is booming with herbal products. As per the available records, the herbal medicine market in 1991 in the countries of the European Union was about $ 6 billion (may be over $20 billion now), with Germany account for $3 billion, France $ 1.6 billion and Italy $ 0.6 billion. In 1996, the US herbal medicine market was about $ 4 billion, which have doubled by now. The Indian herbal drug market is about $ one billion and the export of herbal crude extract is about $80 million. In the last few decades, a curious thing has happened to botanical medicine. Instead of being killed by medical science and pharmaceutical chemistry, it has made come back. Herbal medicine has benefited from the objective analysis of the medical science, while fanciful and emotional claims for herbal cures have been thrown out, herbal treatments and plant medicine that works have been acknowledge. And herbal medicine has been found to have some impressive credentials. Developed empirically by trial and error, many

herbal treatments were nevertheless remarkably effective. In a recent survey estimated that 39% of all 520 new approved drugs in 1983-1994 were natural products or derived from natural products and 60-80% of antibacterial and anticancer drugs were derived from natural products. The penicillin that replaced mercury in the treatment of syphilis and put an end to so many of the deadly epidemics comes from plant mold. Belladona still provides the chemical used in opthalmological preparations and in antiseptics used to treat gastrointestinal disorders. Rauvolfia serpentina (The Indian snake root) which has active ingredient, reserpine, was the basic constituent of a variety of tranquilizer first used in the 1950's to treat certain types of emotional and mental problems. Though reserpine is seldom used today for this purpose, its discovery was a breakthrough in the treatment of mental illness. It is also the principal ingredient in a number of modern pharmaceutical preparations for treating hypertension. But reserpine can have a serious side effect-severe depression. [(2)]

What is Nootropic Activity:

Nootropics, popularly referred to as "smart drugs" are substances, which boost human cognitive abilities. Typically, these are alleged to work by increasing the brain's supply of neurochemicals, improving brain's oxygen supply or by stimulating nerve growth. Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory. Nootropic agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used for improving memory, mood and behavior, but the resulting side-effects associated with these agents have made their applicability limited. Indian system of medicine emphasizes use of herbs, nutraceuticals of life style changes for controlling age related neurodegenerative disorders. Alzheimer's disease (AD) is degenerative changes in the brain accompanied by loss of memory, especially for recent events. The learning and memory is closely associated with the functional status of the central cholinergic system. The basal forebrain provides the major source of cholinergic inputs to the neocortex and hippocampus are the projection from the medial septal nucleus and the nucleus of the vertical limb to the hippocampus via the fimbria-fornix and the projection from nucleus basalis cell ularis to the neocortex 3 Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, people are now motivated to explore the Indian traditional system to come up with a promising solution to manage this deadly disease (AD). [(3)]

Importance of Nutraceutical:

"A Nutraceutical is any substance that is food or part of food and provides medical or health benefits, Including prevention and treatment of disease". Neutraceutical are non- specific biological therapies used to promote wellness, prevent malignant process and control symptoms. These can be grouped into following three broad categories. Reagents derived from others sources (e.g. pyuruvate, chondroitin sulphate, steroid hormone precursor) serving specific functions such as sports nutrition, weight loss supplements. Neutraceutical needs have changed is we have evolved from a pre-agricultural industry dependant on mechanical processing for our food supply. [(7)]

Importance of cow colostrum:

Colostrum, a nutritient-rich fluid produced by female mammels immedietly after giving birth, is loaded with immune, growth and tissue repair

factor. It is a complex biological fluid, which helps in the development of immunity in the newborn. It contains significant quantities of complement componant that act as as natural anti-microbial agents to actively stimulate the maturation of infant's immune system. Bovine colostrum a raw material for immune milk preparation can be used to treat or prevent infection of the gastrointenstinal tract. It is possible that colostrum preparation aimed at specific consumers may play important role in healthcare in future. Colostrum has remarkable muscular-skeltal repair and growth capabilities. Study was shown that colostrum is the only natural, transforming growth factor were significant muscle and cartilage repair characteristics. They promote wound healing with surgical patients. Colostral growth factor have multiple regenerative effects that extend to all structural body cells. (7)

Scientific study of Neutraceutical with Herbs:

Nootropic are also called as cognitive enhancers. These are drugs, supplements that improve cognitive function like memory, creativity or motivation. These are also used for enhancing concentration and memory capabilities.

- The cognitive mechanism such as,
- Increasing circulation to the brain,
- providing chemical messengers to neurotransmitters,
- providing useable energy to the brain,
- preventing free radical and oxidative damage to the brain cells and other cells.(8)

Colostrum: Nutraceutical products are used to endow with health and medical benefits for prevention and treatment of different diseases. Colostrum is a natural product which is rich in macro- and micronutrients, and because of this, it is measured as a best natural food supplement**.** Colostrum is the first milk secreted at the time of parturition, is also the sole source of passive immunization because the colostrums is an excellent source of immunoglobulins and highly biological value proteins, Growth Factor, lipids, carbohydrates, antioxidants, vitamins, minerals and viable cells. A viable cell like neutrophils, macrophages secretes cytokines and antimicrobial proteins and peptides, such as lactoferrin, defensins, and cathelicidins. In view of so many health factor through Colostrum, the use of Colostrum has been extended to so many health problems like treatment of autoimmune disorders, gastrointestinal conditions, including non-steroidal anti-inflammatory drug–induced gut injury, Hpylori infection, deficiency related diarrhea for all age group. This review explores the recent knowledge on the advantageous effect of immune factors containing Colostrum in the above conditions as well as the results of research aimed at realizing untouched significance in milk. (9)

➢ Plant Of Bacopa Monneri:

Figure 1: Plant of Bacopa monneri

Classification:

Table 2. Scientific classification of Bacopa monneri

Kingdom	Plantae
Class	Dicotyledonae
Sub Class	Gamopetalae
Order	Lamiales
Family	Scrophulariaceae
Genus	Bacopa
Species	Monneri

Habitat, Geographical Distribution, and Ethnomedical Description: Bacopa monnieri is a perennial, creeping herb whose habitat includes wetlands and muddy shores. The leaves are succulent, relatively thick, and are arranged oppositely on the stem. It is commonly found in wet marshy and damp places throughout India. It is also found in Nepal, Sri Lanka, China, Taiwan, Vietnam, and some southern states of USA. Bacopa monnieri is a major constituent of the traditional Medhya Rasayana, formulations, which are considered to facilitate learning and improve memory. In traditional medicine, the plant is used as a nerve tonic, diuretic, and to treat asthma, epilepsy, insanity, and hoarseness. [10]

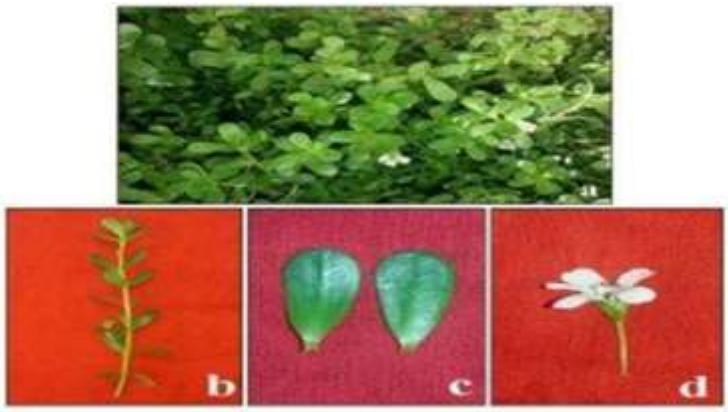


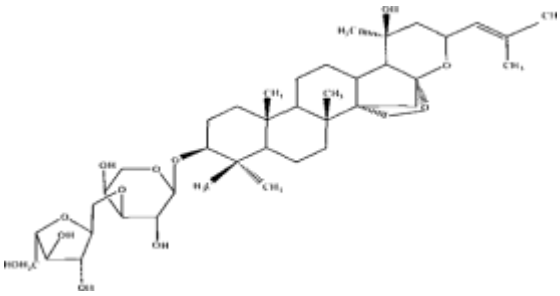
Fig. 2: Bacopa Monnieri (A) Plant In Natural Habitat, (B) Twig, (C) Leaf, (D) Flower

Propagation: The plant Bacopa monnieri L. is not capable of producing seeds and well registered for their regeneration using their mature stem cuttings. Sandy soil/black soil is more suitable for this plant to regenerate in a wide range. Stem cuttings used for regeneration requires to present 4-6 nodes. In the initial stage of its cultivation, it requires a moderate irrigation. Each one mature nodular part of this plant is efficiently producing new root and shoot system in a favorable environmental condition which further support to develop its new individuals like their parental ones. After establishment, plant spread rapidly around the sites of its cultivation.

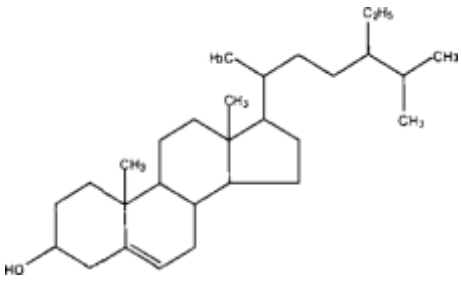
Botanical Features: B. monnieri is a small creeping, spreading, succulent herb with numerous branches and small fleshy, oblong leaves. Flowers and fruits appear in summer and the whole plant is medicinally important (Chopra et al., 1956). The salient botanical features are: Stem - prostrate, (sub) succulent, herbaceous; Leaves - decussate, simple, oblong, 1 × 0.4 cm, succulent, punctate, margin entire, apex obtuse, sessile; Flower(s) - axillary, solitary, linear, purple, pink or white in colour; Calyx - 5 lobes (unequal); outer 2 lobes larger, oval, 7 × 3.5 mm; inner 2 lobes linear, 5.5 × 0.7 mm; median 1 lobe oblong, 5.5 × 2 mm, imbricate, (sub) succulent, punctuate, obtuse, acute; Corolla - white with violet and green bands inside the throat, 0.8 cm across, 5 mm tube; 5 lobes, obscurely 2-lipped, obtuse or emarginated; Stamens - 4, 16 filament pairs 1 and 2.5 mm anthers oblong, contiguous, 1.5 mm; Ovary - oblong, 2 mm; style slightly deflexed, 5.5 mm; Stigma - flat capsule, oblong, 5×2.5 cm septicidal or locilicidal or 4 valved; Seed - oblong, testa striate; Fruit - small, capsule form, less than 0.5 inch in length [12]

Phytochemistry: The pharmacological properties of Bacopa monnieri were studied extensively, and the activities were attributed mainly due to the presence of characteristic saponins called

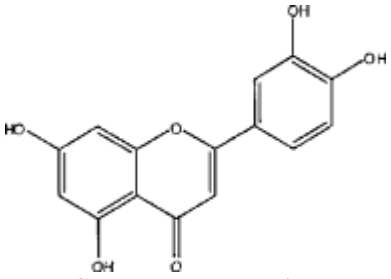
"bacosides." Bacosides are a complex mixture of structurally closely related compounds, glycosides of either jujubogenin or pseudojujubogenin. Bacosides comprise a family of 12 known analogs. Major bacopasaponins were bacosides A3, bacopaside II, bacopaside I, bacopaside X, bacopasaponin C, bacopaside N2 and the minor components were bacopasaponin F, bacopasaponin E, bacopaside N1 bacopaside III, bacopaside IV and bacon aside V. Four cucurbitacins, bacitracin A-D, a known cytotoxic, cucurbitacin E and three phenylethanoid glycosides, monnieraside I, III and plant inside B were isolated from the aerial part of Bacopa monnieri. Two common flavonoids, luteolin, and apigenin, have also been detected in B. monnieri. A simple reversed-phase HPLC method has been developed and successfully analyzed for the simultaneous determination of all 12 Bacopa saponins present in the extracts of B. monnieri. [11] The Phytochemical screening of the extracts showed the presence of glycosides, alkaloids and flavonoids. The antimicrobial investigation showed that ether extract showed antimicrobial activity against four bacteria and one fungus. From the literature review to was also found that the plant has direct therapeutic effects on piles, dysentery, dyspepsia, diarrhea, vomiting, giddiness, worms, burning of the skin and menorrhea. The literature survey reveals that the plant has antibacterial and antimutagenic activities. Thus an attempt was made to reinvestigate aforementioned antimicrobial as well as the cytotoxic properties for the bark constituents. [13]



Structure: Bacoside A:



Structure Sitosterol:



Structure Luteolin:

Traditional use:

Bacopa has traditionally been used as a brain tonic and is commonly recommended to improve memory and heighten learning capacity. It is one of the important drugs of Ayurveda. Classical therapeutic claims suggest that the entire plant is prescribed for large number of diseases like asthma, epilepsy, insanity and memory enhancement. It is also used as a nerve tonic to treat anxiety, nervous exhaustion or debility and is prescribed to enhance rehabilitation after any injury causing nervous deficit, such as stroke. Other traditional uses include promoting longevity, and treating diarrhoea. It is used as an anti- inflammatory, analgesic and anxiolytic agent. It is also found to have laxative effect and the

whole plant is used in treatment of constipation and stomach disorders. [14]

MATERIAL AND METHOD:

Procurement of sample: Plant material of bacopa monneri (L) obtained from Sunrise Agro Service, Pune. The powder obtained was then sieved and kept in air tight container. The herbarium of bacopa monneri (L) was prepared and authentification has been obtained from Sangamner Nagarpalika Arts, D. J. Malpani commerce, B.N. Sarda Science College, Sangamner, Ahmednagar from Botany Department.

Isolation and characterization of bacoside rich fraction [1]

The bacoside fraction was prepared by the standard method. The aerial part of the plat were washed in running tap water. Blotted dry between use tissue paper, shade dried and coarsely powdered. The powder sample (100gm) was packed into thimble of a soxhlet apparatus. To defeat the sample 25 ml of hexane was taken in a flask and continuously refluxed for 5 hours. The hexane extract was discarded and the powdered sample was dried to remove solvent. The dried powder repacked into thimble and re-extracted with 625 ml of acetone for 5 hours to remove unwanted constituents and colouring matter from the sample. The acetone extract was discarded and the powder sample air dried to remove acetone. The residue was then extracted with 625 ml of methanol for 6 hours to completely dissolve the bacosides. The methanolic extract was concentrated to 100mlm using a rotatory vaccum evaporator .the concentrated extract containing the bacosides was slowly added to 100 ml of acetone with constant stirring to precipitate bacosides along with acetone insoluble material. The solution was filtered through a vaccum filter. The precipitate was dissolved in 50 ml of water. This solution was then extracted with 5×15ml of n-butanol to transfer the bacosides to the solvent phase. The two phases were seaparate, butanol extracts were pooled and concentrated under high vacuum at 50-55ºC. The dried mass containing bacosides was dissolved in 100ml of water in a stirred vessel. To this, mannitol was added at 1% as a stabilizer. Stirring was continued for 1.5 hours. The water was evaporated to dryness and the resulting residue was weighed and dissolved in water. The presence of these components in the fraction was identified by HPTLC in comparison with standard bacoside A. [1]

Physical evaluation:

Table no: 3 Physical evaluation of Bacopa monneri

Sr. no	Test	Observation
1	Colour	Bright green
2	Odour	Characteristic
3	Taste	Bitter
4	Surface	Smooth

PRELIMINARY PHYTOCHEMICAL SCREENING OF THE EXTRACTS

Procedure :

The preliminary phytochemical screening of various extracts of leaf was carried out using standard procedures (Khandelwal, 2010)

- **Test for saponin glycosides.**

a. **Foam test:** Shake the extract vigorously with water Persistent foam is formed. This indicates the presence of saponin glycosides.

b. **Haemolytic test:** Extract + drop of blood placed on a glass slide. A haemolytic zone is appeared. This indicates the presence of saponin glycosides.

c. **Libermann burchard test:** Alcholic extract of drug was evaporated to dryness and extracted with trichloromethane, add few drop of Acetic chloride followed by conc. sulphuric acid from side wall of test tube to the dichloromethane formation of violet to blue ring at the junction of two liquid indicate the presence of steroidal moiety.

➢ **Chromatographic evaluation:**

Thin layer chromatography: Thin layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.

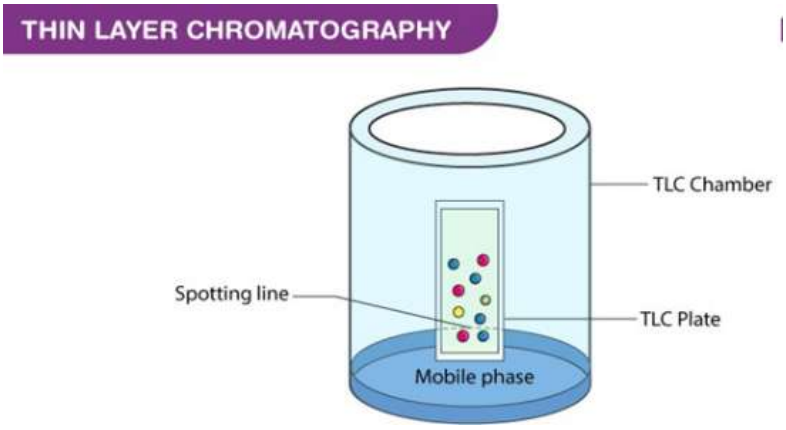


Figure- 3 Thin layer chromatography

Need Of TLC:

1) To characterize extract with respect to number of separable phytoconstituents.
2) To develop method for chemical constituent separation of phytoconstituents.

Preparation of sample:

About 25 mg of extract was weighed Bacopa monneri in to 10 ml test tube and volume up to the 5 ml add methanol solvent. Then after the test tube place in bath sonicator in 5 minutes for sonication purpose. Then sample throughout sonicator and filter its watman filter paper.

Table 4: Details about the TLC of Bacopa monnieri

Stationary phase	Silica gel G, aluminum silica plate
Mobile phase	1. Chloroform: methanol: Water (6.5: 3.5: 1) 2. Toluene: Ethyl acetate: Methanol: Glacial acetic acid (3:4:3:1) 3. Ethyl acetate: Methanol: Water (4: 1: 1)
Sample applicator	Capillary tube
Saturation time	10 minutes
Chamber	Twin through chamber
Spraying reagent	Vanillin (1%) - Sulphuric acid (5%)
Observation	2-3 band are observed

- **High performance Thin Layer Chromatography:**

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time "on-column". Hence, different constituents of a sample are eluted at different times. The signals are converted and recorded by a data management system (computer software) and then shown in a chromatogram. After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit or to the waste. In general, a HPLC system contains the following modules: a solvent reservoir, a pump, an injection valve, a column, a detector unit and a data processing unit (Fig. 1). The solvent (eluent) is delivered by the pump at high pressure and constant speed through the system. To keep the drift and noise of the detector signal as low as possible, a constant and pulseless flow from the pump is crucial. The analyte (sample) is provided to the eluent by the injection valve.

- **Chromatographic parameters:**

The separated analyte which are transported by the mobile phase are recorded as signal peaks by the detector unit. The total amount of all peaks is called chromatogram. Each individual peak provides qualitative and quantitative information of the analyte. Qualitative information is given by the peak itself (e.g.: shape, intensity of the signal, time of appearance in the chromatogram). In addition, the area of a peak is proportional to the concentration of the substance. Hence, the chromatography data management software can calculate the concentration of the sample by integration. This provides quantitative information. Ideally the peaks are recorded as a Gaussian bell-shaped curve. A schematic example is illustrated in Fig.6.2. The basic parameters of a chromatographic separation are discussed below.

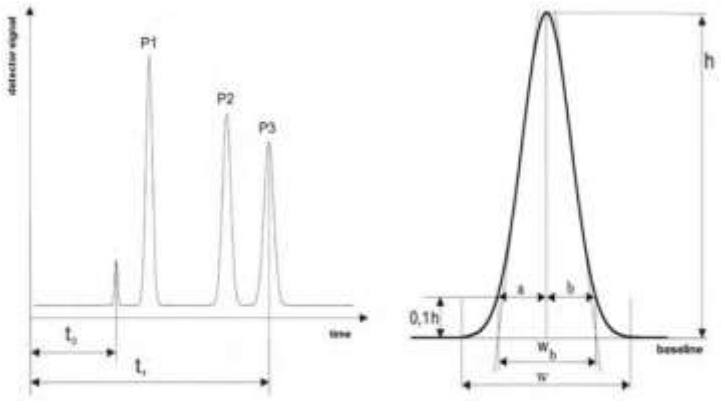


Fig. 4 Schematic illustration of chromatogram

Delay time (t0): The delay time refers to the time which is required for a non-retarded compound to be transported from the injection site to the detector unit (where the compound is recorded). During this time, all sample molecules are exclusively located in the mobile phase. In general, all sample molecules share the same delay time. The separation is caused by differing adherence of the substances with the stationary phase.

Retention time (tR): The Retention time refers to the time which is required for a compound from the moment of injection until the moment of detection. Accordingly, it represents the time the analyte is in the mobile and stationary phase.

Peak width (w): The peak width covers the period from the beginning of the signal slope until reaching the baseline after repeated drop in the detector signal.

Procedure:

The bacoside fraction and the standard bacoside A were dissolved in HPLC- grade methanol at a concentrated of 1mg/ml. The bacoside fraction and the standard bacoside A were dissolved in HPLC-grade methanol at a concentration of 1mg/ml. The sample (25µl) was injected into reverse phase C18 column of the HPLC system (Shimadzu, Japan) equipped with PDA. detector. The sample analysis was performed at room temperature, in the wavelength range of 200-320 nm at 1000. [11]

Table 5: HPLC instrument information

Parts of Instrument	Information
System	HPLC Agilent gradient system
Model no.	HPLC 1100 series
Company	Analytical technologies limited
Pump	P-1100 -M reciprocating
Column	Id 4.6 x 10 mm length
Detector	UV-1100 – M
Software	Chem-station

Table 6: Details about HPTLC of Bacopa monniri

Mobile phase	Acetonitrile: Sodium sulphate: Ortho phosphoric acid
Stationary phase	C18 (COSMOSIL)
Particle size	2.5m
Sample size	20
Flow rate	1.07 ml/min
Column	4.6 x 100mm
Detector	UV
Wavelength	205nm
Temperature	Ambient

RESULT AND DISCUSSION:

Description: The sample of Bacopa monneri extract was found to be bright green coloured powder with bitter taste

Table 7: physicochemical parameter of Bacopa monneri extract

Sr. No	Parameter	Results
1	water-soluble extractive values	10.1%
2	Alcohol- soluble extractive value	80.00%
3	Total Ash value	13.5
4	Acid insoluble Ash	13.5
5	Loss on Drying	1.5%

Solubility: Bacopa monneri extract was found to be less soluble in water, freely soluble in ethanol, methanol and chloroform.

TLC Thin layer of chromatography:

Figure 7.1: Thin layer of chromatography Bacopa monneri.

TLC pattern of Bacopa monneri extract in different mobile phase		
Chloroform: methanol: Water (6.5: 3.5: 1)	Toluene: Ethyl acetate: Methanol: Glacial acetic acid (3:4:3:1)	Ethyl acetate: Methanol: Water (4: 1: 1)
		

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