FORMULATION AND IPQA EVALUATION OF HERBAL BILAYER TABLET OF ANTHOCYANIN AND GINKGO BILOBA POWDER FOR ALZHEIMER'S DISEASE.

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ABSTRACT:

Alzheimer's disease (AD) is associated with cognitive impairment and dementia, both of which are widespread in the elderly population due to neurodegeneration. It slowly deteriorates the patient's memory. The development of senile plaques and neurofibrillary tangles are important diagnostic findings (NFTs). The inability of the neurotransmitter acetylcholine (ACh) to function in the cerebral region is due to metabolism by an enzyme called acetylcholine esterase, and neuronal death is the primary cause of AD. There are numerous anti-medication Alzheimer's categories on the market for the management of Alzheimer's disease; however, satisfactory outcomes have not been found due to a lack of patient compliance. Herbal medicines are becoming more popular owing to their alleged efficacy, safety, and inexpensive cost. Due to the ageing population and increased frequency of Alzheimer's disease, it appears that new treatment drugs with the highest efficacy and fewest side effects are necessary. The aim of this research was to create bi-layer tablets of anthocyanin and ginkgo biloba so that the synergistic impact of this combination could be employed to treat Alzheimer's disease effectively. The wet granulation procedure was employed to create both layers, which were then tested for thickness, weight variation, hardness, friability, disintegration time, and dissolution studies. It was found that anthocyanin and ginkgo biloba bi-layer tablets can be effectively made since they meet all of the criteria for a bi-layered tablet and would be an alternative to the already available conventional tablets.

KEYWORDS: Bilayer tablet,Alzheimer's disease, Neurodegeneration, Anthocyanin, Ginkgo Biloba Powder.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia, characterized clinically by a progression from episodic memory problems to a slow general decline in cognitive function.[1]Dementia was estimated to affect 44 million people worldwide in 2013, with a steep rise to 136 million predicted by 2050.[2]There are currently no treatments with proven disease-modifying effectsand Alzheimer's disease remains the most unmet medical need in neurology. Changes in amyloid precursor protein metabolism, phosphorylation of the tau protein, oxidative stress, impaired energetics, mitochondrial dysfunction, inflammation, membrane lipid dysregulation, and neurotransmitter pathway disruption all play a role in AD pathology.[3]The majority of these pathological features are directly related to metabolic abnormalities, and it is now clear that metabolic dysfunction is an important factor in Alzheimer's disease.[4]Impaired cerebral glucose uptake, for example, occurs decades before the onset of cognitive dysfunction and is an unavoidable feature of Alzheimer's disease.[5] Aβ42 well-documented neurotoxicity is thought to contribute to impaired neuronal energetics by initiating a cascade of pathological events; interaction between Aβ42 and mitochondrial enzymes leads to increased release of reactive oxygen species (ROS), affecting glycolysis, the TCA cycle, and mitochondrial respiratory chain activity via the accumulation of deleterious intermediate metabolites in the mitochondria.[6-7]

Causes and Risk Factors of Alzheimer’s Disease

Alzheimer's disease is regarded to be a complex illness with several risk factors, including advancing age, hereditary factors, head traumas, vascular problems, infections, and environmental variables (heavy metals, trace metals, and others). The fundamental etiology of the pathological alterations associated with Alzheimer's disease (Aβ, NFTs, and synaptic loss) is yet unclear. Several hypotheses have been proposed as causes of Alzheimer's disease, but two are thought to be the most important: some believe that cholinergic dysfunction is a critical risk factor for AD, while others believe that an alteration in amyloid-protein production and processing is the primary initiating factor. There is presently no recognized explanation to explain the pathophysiology of Alzheimer's disease.

Problems with memory

Memory loss is a widespread and serious problem in dementia; thus, it is critical to first identify the extent to which it is a problem and for whom it is an impediment.[10] Memory is said to consist of four stages: registration, encoding, storage, and retrieval. To be kept in memory,

information must first be attended to or recorded. Encoding is the process through which this information is semantically or phonologically encoded, that is, encoded in terms of meaning or sound, respectively. [11-12] Storage is the process of retaining information in memory. It is commonly known that different forms of information appear to be stored differently, such that, for example, knowing what a person ate for lunch (episodic knowledge) appears to be retained differently.

Dementia

Dementia is a condition marked by disruptions in numerous brain processes, including memory, thinking, direction, understanding, computation, learning ability, language, and judgment. The consciousness is clear. Impaired cognitive performance is frequently accompanied by, and sometimes preceded by, decrease in emotional regulation, social conduct, or motivation. Dementia may affect a person in a variety of ways, and the disease's course is determined by the disease's impact as well as the individual's personality and state of health. Dementia is classified into three stages: early (first year or two), medium (second to fourth or fifth years), and late (fifth year and later). These times are presented as a rough guideline, and not all people with dementia may exhibit the same symptoms. [13-14] Common symptoms experienced by people with dementia syndrome have been illustrated by Table 1

TABLE 1: COMMON SYMPTOMS EXPERIENCED BY PEOPLE WITH DEMENTIA SYNDROME [15]

Early stage	Middle stage	Late stage
The early stage is often overlooked. Relatives and friends (and sometimes professionals as well) see it as "old age", just a normal part of ageing process. Because the onset of the disease is gradual, it is difficult to be sure exactly when it begins.	As the disease progresses, limitations become clearer and more restricting.	The last stage is one of nearly total dependence and inactivity. Memory disturbances are very serious and the physical side of the disease becomes more obvious.
Become forgetful, especially regarding things that just happened	Become very forgetful, especially of recent events and people's names	Usually unaware of time and place

May have some difficulty with communication, such as difficulty in finding words	Have difficulty comprehending time, date, place and events; may become lost at home as well as in the community	Have difficulty understanding what is happening around them
Become lost in familiar places	Have increasing difficulty with communication (speech and comprehension)	Unable to recognize relatives, friends and familiar objects
Lose track of the time, including time of day, month, year, season	Need help with personal care (i.e. toileting, washing, dressing)	Unable to eat without assistance, may have difficulty in swallowing
Have difficulty making decisions and handling personal finances	Unable to successfully prepare food, cook, clean or shop	Increasing need for assisted self-care (bathing and toileting)

Mood and behaviour: may become less active and motivated and lose interest inactivities and hobbies may	Unable to live alone safely without considerable support	May have bladder and bowel incontinence
show mood changes, including depression or anxiety may react unusually angrily or aggressively on occasion.		
	Behavior changes may include wandering, repeated questioning, calling out, clinging, disturbed sleeping, hallucinations (seeing or hearing things which are not there)	Change in mobility, may be unable to walk or be confined to a wheelchair or bed
	May display inappropriate behavior in the home or in the community (e.g.,disinhibition,	Behavior changes, may escalate and include aggression towards career,

	aggression).	nonverbal agitation (kicking, hitting, screaming or moaning)
		Unable to find his or her way around in the home.

Source: World Alzheimer's Report 2009. (16)

Stages Of Alzheimer Disease

AD is characterized by different stages:

1) Mild cognitive impairment (MCI);

2) Mild AD;

3) Moderate AD;

4) Severe AD; and

5) Very Severe AD.

Numerous studies show that the very early stage of MCI, which can last for years, is characterized by significant memory loss as well as cognitive abnormalities. However, there is no conclusive proof for this condition. MCI is fraught with uncertainty, with some determining that it is a forerunner to AD and others classifying it as a typical ageing occurrence. Notwithstanding the controversy, a few studies have found that cognitive impairment is the forerunner of dementia, which is 15 times more likely than normal ageing to evolve into Alzheimer's disease.Nowadays, imaging methods can detect both early metabolic and structural changes in the brain, but there is no one screening approach or test to identify patients with MCI. Those with cognitive decline can be successfully predicted in this condition using a combination of neuroimaging and neuropsychology testing.

The stable MCI phase is characterized by a deterioration in cognitive function that may last two to five years, with impairment of implicit memory (connected to experience) and semantic memory (of stored general knowledge and facts). Yet there is no evidence linking such memory loss to Alzheimer's disease. Yet, data shows that these risks can be decreased only if moderate cognitive impairment is detected and assessed early.[17]

Mild Alzheimer's disease is distinguished by short-term memory loss combined with forgetfulness, a lack of interest and hobbies, recurrent questioning, and changes in routine.Patients may also experience linguistic disorientation as a result of a reduced vocabulary and difficulty with fluency. As the condition progresses, individuals may become unable to do

various tasks alone, necessitating supervision or support for cognitively demanding activities.[18]

After the continual cognitive impairments and shifts in care, there are more severe alterations and impaired routines noticed, along with indicators of growing psychosocial behaviours in mild AD. At this point, long-term memory loss is detected, and 30% of patients may have illusionary misidentifications.

The fourth stage, severe AD, is distinguished by changed and agitated sleep patterns, as well as growing indications of dementia-related psychosocial disorders, and may need assistance even to bathe, eat, or dress. As a result, patients are completely dependent on careers. The ultimate stage is known as extremely severe Alzheimer's disease, and it is characterized by decreased speaking or oration, such as the use of one word or short phrases, eventually progressing to no speech and bed rest.[19]

Considering the serious public health threat that dementia poses, just five pharmacological therapies for the condition have been licensed to far, including only two types of medications that operate to regulate symptoms rather than modify the disease's trajectory. Nevertheless, few clinical studies in Alzheimer's disease have been conducted in the recent decade, with a 99.6% failure rate.[20] As a result, the objective of disease-modifying treatment remains elusive, with currently available drugs merely functioning to reduce symptoms. This review summarizes current Alzheimer's disease therapy. Ayurveda is an ancient medical system that employs a variety of plants and herbs to effectively treat a wide range of sick conditions. Some herbs have also been utilized to treat neurocognitive illnesses such as Alzheimer's.[21]

Herbal Treatment to Cure Alzheimer Disease

The AD medication discovery process is crucial, risky, and expensive.[22] Herbal treatments are indicated to treat a variety of ailments because medicinal plants and herbs contain a complex blend of phytocompounds with varying pharmaco-biological relevance. Yet, plants have remained an important source of medications in a variety of therapeutic practices. A few plants are used in anti-ageing therapy to aid in the maintenance of homeostasis.[23] Herbal formulations have grown in commercial and scientific relevance in recent years. "Nervines" are therapeutic herbs and phytochemicals that have the ability to restore memory, stimulate neurons, and increase neuronal function. Tannins, sterols, flavonoids, alkaloids, lignins, triterpenes, and polyphenols are phytochemical components that are responsible for the plant's pharmacological actions, such as antioxidants, anti-cholinesterase, anti-inflammatory, and anti-amyloidogenic properties.[24] Because of the increased activity of cholinesterase in the brain, studies have

shown that using herbs against AD has a tremendous influence on the patient's memory skills.[25]

Benefits of combination therapy over monotherapy

In terms of safety and efficacy, monotherapy has numerous drawbacks when compared to combination therapy. At larger dosages, single medication therapy is typically successful, although it has a number of side effects. Such medications boost the drug's efficacy at the expense of the host's tolerance to it in a dose-dependent way. The many mechanisms of action examined in combination therapy may give more efficacy than monotherapy.[26] Herbal combinatorial treatment improves efficacy by delivering synergistic benefits, acceptability, and safety at lower dosages, in addition to additive effects aimed at slowing disease development. According to these results, a relatively novel herbal combination treatment strategy offers multiple mechanisms of action that may have additive effects over monotherapy for the successful management of Alzheimer's disease. As a result, it is an urgent but realistic technique that has to be researched further in order to be incorporated into AD therapy regimens.[27]

Bilayer tablets

The bilayer tablet represents a new era in the successful creation of controlled release formulations as well as different characteristics necessary to produce a successful drug delivery system. Bilayer tablets can be a key alternative for avoiding chemical incompatibilities between APIs through physical separation as well as enabling the development of diverse drug release profiles, such as rapid release with prolonged release. A bilayer pill is an anti-inflammatory and analgesic in a totally distinct way. A bi-layer tablet is appropriate for the sequential release of two medications in combination as well as for a sustained release tablet in which one layer is immediate release as the first dosage and the second layer is maintenance dose. A bilayer tablet is a more advantageous technology that addresses the shortcomings of a single-layered tablet.

Ginkgo Biloba

Ginkgo biloba (Gb) has received a lot of attention recently, especially because of its possible significance in the treatment of Alzheimer's disease. Gb also looks to be potential as a treatment for a variety of other chronic and acute disorders. Flavonoids and terpenoids are the most pharmacologically active classes of chemicals. Virtually all clinical investigations employ Gb extract, which includes flavonoid glycosides, terpene lactones, and ginkgolic acids.[28] Gb extract has been proved to be effective in treating Alzheimer's disease, cardiovascular disease, cancer, tinnitus, and other age-related disorders. The antioxidant impact of the Gb extract, anti-platelet activating factor activity for vascular disorders, prevention of β-amyloid peptide aggregation in AD, and lowered expression of peripheral benzodiazepine receptor for stress

relief are hypothesizedexplanations. [29-30] Gb is a prominent therapy for early-stage Alzheimer's disease.Gb extract prevents -amyloid and NO-induced toxicity in vitro and decreases apoptosis both in vitro and in vivo.[31-33] Gb extract increased memory retention in both young and elderly rats, as well as short-term memory in mice.[34] Many studies show that ginkgo can postpone the course of Alzheimer's disease and is equally effective as cholinesterase inhibitors in treating it. Many randomized, double-blind, placebo-controlled trials in Alzheimer's disease patients shown a moderate improvement in cognitive performance. [35-37] Gb extract also improves ADLs in AD patients and is chosen over other AD drugs because of its few side effects.[38]

Anthocyanin

The capacity of anthocyanins to mitigate CNS illnesses is now being investigated, while evidence on their effects on neurodegeneration are primarily taken from anthocyanin-rich plant extracts. Although data on the impact of pure anthocyanins is sparse, a few promising studies using specific molecules have been undertaken. Yet, multiple data demonstrate that anthocyanins mitigate many of the detrimental consequences of neurodegenerative processes such as oxidative and nitrosative stress, excitotoxicity, glial inflammation, protein aggregation, and activation of apoptotic signaling proteins. Additionally, research suggests that anthocyanins can pass the blood-brain barrier (BBB), implying that these substances may mediate these effects directly in the CNS, where neuronal death occurs.[39]

Preparation Of Bilayer Tablets

Bilayer tablets are created with one layer of medicine for immediate release and the second layer for later drug release, either as a second dosage or in a prolonged release form. Bilayer tablets containing two incompatible medications can also be made by compressing distinct layers of each drug to reduce the area of contact between two layers. An extra intermediate layer of inert material may be inserted as well. To make a suitable tablet formulation, several requirements must be satisfied, such as sufficient mechanical strength and the correct drug release profile.Because of poor flow and compatibility characteristics of the medication, which will result in capping and/or lamination, it may be difficult for the formulator to attain these conditions at times, especially in bilayer tablet formulation where twofold compression method is used. A material's compaction requires both compressibility and consolidation. Compression is described as a reduction in bulk volume achieved by reducing voids and bringing particles closer together. Consolidation is a material feature characterized by enhanced mechanical strength as a result of interparticle contact (bonding). The compression force on layer 1 was discovered to be a significant element determining tablet delamination.[40]

Steps for compression cycle of bilayer tablet

- Filling of first layer.
- Compression of first layer.
- Ejection of upper punch. Filling of second layer.
- Compression of both the layers together.
- Ejection of bilayer tablet.

PREPARATION OF BILAYER TABLET

MATERIALS AND METHODS

Equipment/ Apparatus

The following equipments were used: Vernier caliper, Monsanto hardness tester, electronic weighing machine (Erweka), tablet compression machine (CADMACH), Disintegration Tester machine (Erweka), Friability tester machine (Roche Friabilator).

Materials

The plant materials Anthocyanin powder was obtained from Herbo Nutra Extract Pvt, Ltd, Utter Pradesh and Ginkgo Biloba powder was obtained from Vijaya Herbal Products, New Delhi. The other materials (excipients) were obtained from the Ordain healthcare global Pvt ltd.

Determination Of Average Moisture Loss on Drying

The method described in BP 2007 was adopted with slight modification. Anthocyanin powder and Ginkgo Biloba powder (1 g) were weighed in a tared petri dish. The petri dish with its content was placed in an oven and dried at 105 °C for 3 hr. Thereafter, the petri dish with its content was cooled in a desiccator over anhydrous silica gel and reweighed. The moisture content was then determined as the ratio of weight of moisture loss to weight of sample expressed as a percentage. Triplicate determinations were made and the means of the values reported.

Particle Size Analysis

Anthocyanin powder and Ginkgo Biloba powder Particle size analysis were one according to a method described by previous researchers. Each sieve was tared to the nearest 0.001 g. Thereafter, 10 g of Anthocyanin powder and Ginkgo Biloba powder were carefully loaded on the coarsest sieve of the assembled stack (1000 µm to 150 µm) and the lid was replaced. The nest was subjected to mechanical vibration using a Shaker for 25 min at 5 min 8 interval per shaking session. Thereafter, the sieves were carefully separated and each sieve was carefully reweighed

with its content. The weights of powder retained on each sieve and the collecting pan was determined by difference. The values were used to calculate the percent of sample retained on each sieve and the average diameter of the particles (Dav) using the formula

Dav = ∑(% retained ×mean aperture size)/ 100 → (Equation 1)

Determination Of Bulk and Tapped Densities

The bulk and tapped densities of Anthocyanin powder and Ginkgo Biloba powder were determined by a method proposed by previous researchers.

The bulk density was determined by pouring 10 g (Anthocyanin powder and Ginkgo Biloba powder) of the powder into a 50 ml glass measuring cylinder Separately and the bulk volume (Vo) determined. The bulk density (Db) was then calculated from the following relationship:

Db = M / Vo → (Equation 2)

Triplicate determinations were made and the mean values reported.

The tapped density of the powder was determined using a Stamp Volumeter. The 10 grams (Anthocyanin powder and Ginkgo Biloba powder) of the powder sample after the bulk density determination was subjected to 250 taps mechanically and the volume V250 of the powder column determined and appliedto evaluate tapped density (Dt) using the following relationship:

Dt = M /V250 → (Equation 3)

Triplicate determinations were made and the mean values reported.

Determination of Angle of Repose

The Angle of repose was determined by a method proposed by previous researchers. The static angle of repose, θ, was measured according to the fixed funnel and free-standing cone method. A glass funnel was clamped with its tip of diameter 1 cm at a given height (h = 1.5 cm) above a graph paper placed on a flat surface. The powder/granules sample (10 g) was carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. 10 The diameter (d) of the base of the cone was measured. This procedure was repeated three times for each powder/granule sample and the mean was used to calculate the angle of repose for the powder/granules using the formula:

tan ɵ = 2h / d → (Equation 4)

Determination of Hausner's Ratio and Compressibility Index Hausner's Ratio (Hr)

Hausner's Ratio (HR)

This was calculated using the formula:

HR = Dt / Db (Equation 5)

Where Dt is Tapped density; Db is Bulk density

Compressibility Index (CI)

This was calculated using the formula:

C I=(Dt-Db/Dt)× (100/1) (Equation 6)

PREPARATION OF TABLETS

Three (3) batches of basic formulations of Anthocyanin powder and Ginkgo Biloba tablets were prepared.

Layer 1 - Anthocyanin and a HPMC dry – mixed for 10 minutes in a Mortar, moistened with the appropriate amount of binder solution PVP prepared, Filler, MCC, was used to standardize the weights of the different formulations. Wet massing of the ingredients was carried out in a mortar using a pestle for 10 min. The homogeneous wet mass was then offloaded and screened through a 1700 µm sieve and dried in a hot air oven at 50℃ for 2 hours. Thereafter, the dried granules were screened through a 600µm sieve in order to generate uniformly sized granules and transferred into a mortar. Talc was added as glidant mixed for 5 minutes. Magnesium stearate was then added as a lubricant and mixed for 1 minute. Mixing for 1 min after the addition of Magnesium stearate was done. The granulated material was then offloaded into well labeled clean containers ready for compression into tablets using a 10 mm round punch. Samples from the different batches were individually weighed and placed in the compression chamber. For each formulation, the compressive force was adjusted according to the properties of the material. The compression force of the machine was manually adjusted, i.e., for each material the behavior at a particular applied force was observed. To obtain the tablets, the machine engine was not engaged, so each form of the solid dosage was obtained individually by manual rotation of the punch. After compression, the formulations were collected and stored away from light and rehydration (in desiccation chamber) at room temperature until further analysis.

Layer 2 – Ginkgo Biloba and a SSG dry – mixed for 10 minutes in a Mortar, moistened with the appropriate amount of binder solution PVP prepared, Filler of MCC, DCP were used to standardize the weights of the different formulations. Wet massing of the ingredients was carried out in a mortar using a pestle for 10 min. The homogeneous wet mass was then offloaded and screened through a 1700 µm sieve and dried in a hot air oven at 50℃ for 2 hours. Thereafter, the dried granules were screened through a 600µm sieve in order to generate uniformly sized granules and transferred into a mortar. Talc was added as glidant mixed for 5 minutes. Magnesium stearate was then added as a lubricant and mixed for 1 minute. Mixing for 1 min

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TABLE 2: THE DIFFERENT FORMULATIONS OF (ANTHOCYANIN POWDER AND GINKGO BILOBA POWDER) ARE SHOWN ABOVE.

No	**Material**	**F1**	**F2**	**F3**
Layer 1				
1	Anthocyanin Extract powder	500 mg	500 mg	500 mg
2	HPMC	8 mg	10 mg	12 mg
3	PVP (K-30)	2 mg	5 mg	8mg
4	MCC	17 mg	12 mg	7 mg
5	IPA	q.s	q.s	q.s
6	Talc	1 mg	1 mg	1 mg
7	Magnesium stearate	2 mg	2 mg	2 mg
Average Weight of the tablet		530 mg	530 mg	530 mg
Layer 2				
1	Ginkgo Biloba powder	200 mg	200 mg	200 mg
2	Sodium starch Glycolate (Super disintegrant)	3 mg	7 mg	8 mg
3	PVP (K-30)	2 mg	3 mg	5 mg

4	DCP	8 mg	4 mg	2 mg
5	MCC	5 mg	4 mg	3 mg
6	Talc	1 mg	1 mg	1 mg
7	Magnesium stearate	1 mg	1 mg	1 mg
Average Weight of the tablet		220 mg	220 mg	220 mg

EVALUATION OF TABLETS

Appearance

The macroscopic characteristics of tablets of each formulation including the geometric shape, appearance, color and presence of foreign material or particles was observed.

Thickness:

The tablets thickness measured by using vernier calipers

Weight Variation Test (The Uniformity of Weight)

The uniformity of weight of each batch of tablets was determined using the methodology described in the British Pharmacopoeia (2007), using a range of tolerance of 5% for tablets with a mean weight above 250.0 mg. Twenty tablets were weighed individually using an electronic weighing balance, Shimadzu model TX3202L made in Philippines and the mean weight was determined.

Hardness

The hardness of the tablets was determined using a Monsanto Hardness tester machine, made in Switzerland. Ten tablets of each formulation were evaluated (BP, 2007).

Friability

According to the procedure recommended in the British Pharmacopoeia (2007), twenty tablets were weighed and submitted to a friability tester machine (Roche Friabilator made in Germany). After 25 rpm for 4 minutes, the tablets were de-dusted and weighed again. The difference between the initial and final weights representing the friability (FR), as estimated by the percentage of powder lost was calculated and the results reported.

Disintegration Test

According to the method described in the British Pharmacopoeia (2007), 6 tablets of each batch were placed each in a tube (with a mesh at the bottom) immersed in a water bath at 37℃ and the disintegration test machine (Erweka ZT3, GmbH Heusenstamm, Germany) was set to run. The time it took for all the tablets to disintegrate was observed and recorded for each formulation.

RESULT AND DISCUSSION

Pre formulation studies, the Anthocyanin and Ginkgo Biloba Powder were subjected to pre formulation studies which included determination of moisture loss on drying, average particle diameter (D av), bulk (Db) and tapped density (Dt), angle of repose (ɵ), Hausner's ratio (HR) and compressibility index (CI). The average moisture loss on drying Anthocyanin and Ginkgo Biloba 7.88 %. & 12.63%.

TABLE 3: Results of Pre formulation studies done on Anthocyanin and Ginkgo Biloba Powder

Parameter	Anthocyanin Powder	Ginkgo Biloba Powder
D av	269 (µm)	255 (µm)
Db	0.38 (g/ml)	0.27 (g/ml)
Dt	0.51 (g/ml)	0.26 (g/ml)
ɵ (◦)	20.3	19.3
HR	1.32	1.22
CI	25	22

Results of Pre formulation studies done on Anthocyanin Powder

Micromeritic property	Anthocyanin Powder	Formulated Granules		
		F1	F2	F3

Average particle diameter, Dav (µm)	269	368	366	363
Bulk density, Db (g/ml)	0.38	0.55	0.49	0.62
Tapped density, Dt (g/ml)	0.51	0.48	0.27	0.58
Angle of repose, ɵ (º)	20.3	25.2	19.5	30.3
Hausner's Ratio (HR)	1.32	1.26	1.10	1.39
Compressibility Index, CI (%)	25	16.27	09.02	18.22

TABLE 4.: MICROMERITIC PROPERTIES OF ANTHOCYANIN POWDER AND GRANULES OF THREE DIFFERENT FORMULATIONS

Results of Pre formulation studies done on Ginkgo Biloba Powder

Micromeritic property	Ginkgo Biloba Powder	Formulated Granules		
		F1	F2	F3
Average particle diameter, Dav (µm)	255	226	328	462
Bulk density, Db (g/ml)	0.27	0.30	0.51	0.86
Tapped density, Dt (g/ml)	0.26	0.29	0.28	0.80
Angle of repose, ɵ (o)	19.3	28.4	18.2	40.5
Hausner's Ratio (HR)	1.22	1.35	1.11	1.68
Compressibility Index, C I (%)	22.00	16.26	09.12	19.21

TABLE 5: MICROMERITIC PROPERTIES OF GINKGO BILOBA POWDER AND GRANULES OF THREE DIFFERENT FORMULATIONS

CI is a measure of powder bridge strength and stability, and the Hausner's ratio (HR) is a measure of the interparticle friction. Flow character is rated based on compressibility index and Hausner's ratio. Lower CI or lower Hausner's ratios of a material indicate better flow properties than higher ones. A Carr's CI of < 10 or HR of < 1.11 is considered "excellent'' flow whereas CI > 38 or HR > 1.60 is considered "very very poor" flow. There are intermediate scales for CI between 11 – 15 or HR between 1.12 – 1.18 is considered "good'' flow, CI between 16 – 20 or HR between 1.19 – 1.25 is considered "fair" flow, CI between 21 – 25 or HR between 1.26 – 1.34 is considered passable flow, CI between 26 – 31 or HR between 1.35 – 1.45 is considered "poor" flow, and CI between 32 – 37 or HR between 1.46 – 1.59 is considered "very poor" flow (Shah et al., 2008) Based on the results obtained, flow of Anthocyanin and Ginkgo Biloba powder was rated as "poor" , that of all formulated granules was rated as good. The angle of repose, a traditional characterization method for pharmaceutical powder flow, was also used to characterize the granules. Based on the angle of repose, a value of < 30 ° indicates "excellent" flow whereas > 56° indicates "very poor" flow. The intermediate scale indicates "good" (θ between 31 – 35°), "fair" (θ between 36 – 40 °), "passable" (θ between 41 – 45 °), and "poor" (θ between 46 – 55 °) (Shah et al., 2008). Based on this, the flow of both the powder and the granules of all formulations were rated as Good. Formulated granules had good flowability compared to the powder based on CI and HR with F2 having the best flow and F3 having least flow among the granules. The powder flow was rated poor with HR. However, based on the angle of repose results, both granules (F1,F3) and the powder flow were rated as fair. This discrepancy might be due to very qualitative nature of the scale of measurements and ratings for flow properties based on these compendial methods.

BILAYER TABLETING[40]

Three batches (F1 – F3) ranging from 60 to 120 tablets were made at a uniform compression force of about 6 kg/cm^2 (Monsanto tablet hardness tester machine) and uniform fill weight of 750mg.

TABLET TESTS

Appearance

Tablets were circular, smooth, shiny and Dark Brown and Pink in colour with some spots visible on the surface.

Thickness

The tablets had thickness ranging from 4.85 ± 2 mm measured using vernier calipers.

Uniformity Of Weight

Twenty tablets of each formulation were individually weighed and Table no 6 shows results of the uniformity of weight test.

Formulations	**Noof tablets weighed**	**Mean weight (g)**	**Deviation as Per Monograph (IP)**	**Noof tablets within range**	**Noof tablets outside range**
F1	20	0.740	5%	20	Nil
F2	20	0.749	5 %	20	Nil
F3	20	0.760	5 %	20	Nil

TABLE 6: RESULTS OF UNIFORMITY OF WEIGHT OF

UNCOATED TABLETS

For all the formulations there was no single tablet that deviated from the mean weight of the weighed tablets (20) by more than 5%. Based on compendial standards (IP & BP, 2007), all the formulated uncoated tablets passed the uniformity of mean weight test. Results of quality tests done on the tablets are shown in the Table.7

TABLE 7: RESULTS OF TESTS OF ANTHOCYANIN AND GINKGO BILOBA UNCOATED TABLETS

Quality Tests	**Formulations**		
	F1	**F2**	**F3**
Mean weight (g)	0.740 ± 5 %	0.749 ± 5%	0.760 ± 5 %
Hardness (kg/cm^2)	4 ± 2	6 ± 2	8 ± 2
Friability (%)	0.98	0.26	1.22
Disintegration time (min)	16	6	18

based on compendial standardstablet hardness ranged from 4 to 8 kg/cm^2, uncoated tablets of all the formulations passed the uniformity of weight and the Friability tests which are compendia tests. Only formulation F2 tablets passed the disintegration time test. All the six tablets disintegrated within 15 minutes while for the other formulation tablets (F1 and F3), complete disintegration of all the six tablets used in the test occurred beyond the stipulated 15 minutes (India Pharmacopoeia, 2010).

There was a direct relationship between mean weight and hardness. Formulation F2 tablets had the highest mean weight and they were the hardest. This is because formulation F2 contained 2mg PVP K-30 as a binder as compared to F1 and F3. It should also be noted that F2 granules had the least CI and the least HR value and therefore had the best flow properties.There was direct relationship between hardness and disintegration time and these had an inverse relationship with friability. This was especially true with formulations F2 disintegrated faster than the rest of the formulations and at the same time it was the most acceptable friability. However, its friability was within the acceptable range (less than 1 %) (Indian Pharmacopoeia, 2010). This makes polyvinyl pyrrolidine (PVP) total tablet weight a binder good enough to make strong tablets of Anthocyanin and Ginkgo Biloba Bi Layered Tablet and not too strong to interfere with disintegration.

CONCLUSION

From the above results it can be concluded that Anthocyanin and Ginkgo Biloba powder has acceptable Pre formulation characteristics which is also retained upon being granulated and compressed into Bi Layered tablets. It can also be concluded that granules of Anthocyanin and Ginkgo Biloba powder formulated with PVP K-30 as binder, MCC and SSG as disintegrant possess values of CI and HR that are rated as having good flow and resultant Bi Layered tablets that are strong enough to pass friability test and at the same time pass disintegration test. All in all, this study indicated that it is possible to make un coated Bi Layered tablets of Anthocyanin and Ginkgo Biloba powder especially formulated and granulated with PVP as a binder and MCC and SSG as disintegrant. The formulated Bi Layered Anthocyanin and Ginkgo Biloba tablets may produce Synergistics Activity to treatment of Alzheimer's Disease.

The future plan - formulated Bi Layered Anthocyanin and Ginkgo Biloba tablets will check the in vivo study of anti-Alzheimer's Activity.

ACKNOWLEDGMENT

The author would like to express their heartfelt appreciation to The School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies(VISTAS), for providing this opportunity to conduct research and for their contribution to the completion of this project titled "Formulation and IPQA Evaluation of a Bilayer Tablet of Anthocyanin and Ginkgo Biloba for Alzheimer's Disease."

The author would also like to thank their guide, Mr.Velmurugan Thiagarajan, Assistant Professor, Department of Pharmaceutics, Vels Institute of Science, Technology and Advanced Studies (VISTAS), for his guidance and constant supervision throughout the year. His helpful advice and suggestions were extremely beneficial throughout the project's completion. The author is grateful for his valuable insights and expertise, which have significantly contributed to the quality of this work. Without his support, this project would not have been possible.

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