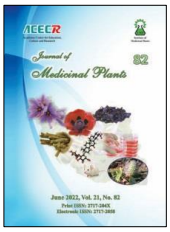
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Research Article

Design, reformulation, and standardization of a traditional-based memory enhancer herbal preparation originated from Persian medicine

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ABSTRACT

Background: Alzheimer's disease (AD) is the most common type of dementia that has been cited in traditional Persian medicine (TPM) manuscripts under the heading "*Farāmūshkārī*", "*Fesād-e-Zekr*" and "*Nesiān*". Since AD is a significant challenge for health professionals, searching for natural and effective compounds is crucial. **Objective:** Given the importance of AD, this study has provided a standardized plant-based tablet based on TPM. **Methods:** Related preparations were extracted from leading pharmaceutical manuscripts of TPM. Out of 127 gathered compound formulations, a preparation containing *Bunium persicum* (Boiss.) B.Fedtsch. (Persian cumin) and *Piper nigrum* L. (black pepper) was selected from the Canon of Medicine of Avicenna. After evaluating various excipients for tablet preparation, the proportions of Persian cumin (40 %), black pepper (40 %), hydroxypropyl methylcellulose (HPMC), and polyvinylpyrrolidone (PVP) as solution binder (20 %) were chosen. The final formulation was undergone through ethanol and dichloromethane and essential oil extractions to prepare for HPTLC fingerprints and GC assessments, respectively. The total alkaloid in the tablet's ethanol extract was also determined via spectrophotometry. **Results:** The final tablet was standardized via GC based on trans-caryophyllene (29.22 %) and γ-terpinene (17.72 %). Each tablet contains an average of 0.041 mg of trans-caryophyllene and 0.018 mg of γ-terpinene. The amount of the total alkaloid in each tablet was determined 0.06 mg, equivalent to atropine. **Conclusion:** This prepared simple natural formulation can be considered a traditional-based product to be evaluated clinically and presented as a memory enhancer supplement.

Abbreviations: AD, Alzheimer's disease; TPM, Traditional Persian medicine; HPMC, Hydroxypropyl Methylcellulose; PVP, Polyvinylpyrrolidone; GC, Gas chromatography; MS, Mass spectrometry; FID, Flame ionization detector; HPTLC, High-performance thin-layer chromatography

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1. Introduction

According to the International Working Group (IWG), Alzheimer's disease (AD) is now recognized *in vivo* as a dual clinico-biological entity that can be identified prior to the onset of the dementia syndrome, on the basis of: i) a distinct core clinical phenotype characterized by hippocampal amnestic syndrome, and ii) changing of biomarkers that reflect the location or nature of Alzheimer's-type [1]. AD, the most common form of dementia, begins gradually and progressively disrupts the brain's cognitive functions [2]. About 35.6 million people worldwide have dementia, which is expected to double every 20 years, with an estimated 115.4 million people worldwide by 2050 [3]. The disease, which mainly affects the elderly [4], is flagrantly becoming a widespread problem as the population ages increase worldwide. AD is currently imposing a vast economic and social burden on societies. Economically, it is estimated that dementia accounted for approximately 604 billion dollars worldwide in 2010, indicating that the disease can cause many economic problems for communities, especially low-income groups [5].

There are still many unknowns about the pathophysiology of AD. Several investigations have been carried out into influential factors - including acetylcholine deficiency, Tau protein hyperphosphorylation, and oxidative stress- used to develop new medicines to treat this disorder [6]. Loss of memory and severity of AD is closely linked to cholinergic activity decline in certain parts of the brain [7]. According to the cholinergic hypothesis, medicines with acetylcholinesterase enzyme inhibitory activity might effectively manage AD [8]. Recently, known medicines such as Donepezil, Rivastigmine, Ladostigil, and Galantamine have been used as acetylcholinesterase inhibitors [9]. Much evidence also implies the role of oxidative stress and free radicals in the pathogenesis and possible cause of this disease [10]. Correspondingly, the use of antioxidant therapies in the pre-clinical stages has been successful [11].

However, no drug that can completely prevent AD has been developed. As a result, many pharmaceutical companies are developing and producing novel medications to help slow the progression of AD [12, 13].

Since AD is a significant challenge for health professionals, searching for natural and effective compounds is crucial. Different traditional medical systems may offer good opportunities for treating AD patients [14]. Traditional Persian medicine (TPM) offers various therapies to manage such chronic diseases [15]. Persian practitioners were well aware of amnesia, dementia, and Alzheimer's disease (*Nesiān* and *Fesād-e-Zekr*) [16]. They provided a variety of medicinal plants to help with memory loss, including *Piper nigrum*, *Cinnamomum verum*, *Zingiber officinale*, *Boswellia* spp., *Terminalia chebula*, *Bunium persicum*, *Acorus calamus*, *Allium sativum* and etc [17]. Animal studies and clinical trials have confirmed the biological activity of some of these; for example, when an aqueous extract of *Boswellia serrata* was administered orally to AD-induced rats, there was an increase in Ach levels and a decrease in AchE activity in brain homogenates [18] or the combination of *Piper nigrum* and *Cinnamomum zeylanicum* significantly increased the recognition index in mice with scopolamine-induced memory impairment [19]. In another study the ginger was given orally to volunteers to evaluate cognitive task and working memory of middle-aged women, and its use significantly improved digit vigilance, word recognition, choice reaction, spatial working memory, and

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numeric working memory scores [20]. As a result, these medicinal plants can assist us in developing effective medications to prevent or slow the progression of AD; but, in order to improve patient compliance, modern medicine dosage forms should be used instead of traditional medicine dosage forms such as *habb*, *majoon*, *yaqooti*, *itrifal* and etc. Some of these are produced in new dosage forms, such as a biocompatible active film of nanochitosan containing free and nanoliposome caraway seed extract or gastroretentive matrix tablets of Boswellia Oleogum Resin [21, 22].

The purpose of this study is to design and reformulate one of Aviccena's traditional compounding formulations (containing Persian cumin and black pepper) as a memory enhancer from powder to tablet dosage form in order to improve patient compliance and satisfaction, and to standardize the new dosage form based on the major component of essential oil and total alkaloid in order to improve the accuracy of the amount of medicine used.

2. Materials and Methods

2.1 Materials

The required materials used in this study are listed as follows: ethanol, methanol, dichloromethane chloroform, toluene, Bromocresol Green (BCG) and sodium hydroxide (NaOH) were purchased from Merck Co. (Germany); all these analytical grade compounds. The following materials manufactured by Sigma-Aldrich Co. (Germany) have also been used: HPMC, PVP, starch, acacia and avicel.

2.2. Manuscripts review

A line of the traditional pharmaceutical encyclopedias (*Qarābādins*) in TPM, including *Qarābādin-e-Kabir* [23], *Qarābādin-e-Salehi* [24], *Qarābādins-e-Ghāderi* [25], *Qarābādin-e-Azam* [26], *Tohfah al-Momenin* (Rarity of the Faithful) [27] and the third volume of Avicenna's Canon of Medicine [28], were studied with the exact keywords; "*Nesiān*", "*Farāmūshkārī*", "*Fesād-e-Zekr*". Memory enhancer formulations were collected and cited [29]. From the third volume of the Canon of Medicine, a two-ingredient formulation with repeated citations in those encyclopedias was chosen to enter the experimental phases. This preparation contained two main components: *Piper nigrum* L. (Black pepper) fruits and *Bunium persicum* (Boiss.) B.Fedtsch. (Persian Cumin) seeds. The daily dose to prevent memory loss was recommended at 1.7 g.

2.3. Plants authentication and microscopic characterization

The purchased samples of *Piper nigrum* L. and *Bunium persicum* (Boiss.) B.Fedtsch. were authenticated and deposited with a specific voucher number in the herbarium of the School of Pharmacy of Shiraz University of medical sciences. The vouchers were PM 1008 for Persian cumin and PM 862 for black pepper. Pepper and cumin samples were ground to determine botanical organs using an electron microscope and a digital camera.

2.4. Tablet preparation and Pharmaceutical tests

Using several different excipients, those herbal components (milled, and sieved previously) were mixed (1:1 ratio) to prepare the initial mixture of the tablet. Also, solution binders were used to improve the pharmaceutical properties of the dosage form. For this purpose, Avicel, Starch, Acacia, HPMC, and PVP were used as solution binders in a concentration of 24

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% W/V in different proportions. Various pharmaceutical tests, including the angle of repose [30] and Hausner ratio for assessment of final powder characteristics and also various tablet tests such as hardness, weight variation, friability, and disintegration time, were performed according to British Pharmacopoeia [31].

2.5. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) fingerprinting

TLC technique was employed to seek for the optimized solvent system for HPTLC. Two types of extracts including dichloromethane and ethanol, were prepared by using ultrasonic bath technique from each component and the final formulation to determine the TLC profile. In this method, 10.0 g of each powder was poured into dichloromethane and then subjected to an ultrasonic bath for 15 minutes three times; the plant residues were then used for ethanol extraction, resulting in the extraction of polar and non-polar compounds separately. The extracts were then dissolved in their respective solvents (1:10) before being subjected to the TLC test. To determine the best solvent system for the TLC assessment based on the ability to distinguish different components of extracts, various solvents such as chloroform, ethanol, methanol, toluene, dichloromethane, and water were investigated. Chromatographic plates were examined in visible light first, followed by UV light at 254 nm and 366 nm, respectively. Anisaldehyde reagent was then applied to the plates. The stains reappeared after drying, and the plate was examined under visible and UV light again. Also, HPTLC was used to determine the final profile as a fingerprint, using an appropriate solvent system for each extract.

2.6. Essential oil preparation and Gas chromatography

Approximately, 50.0 g of Persian cumin, black pepper, and the final tablet were ground into powder and soaked in 500.0 ml of distilled water (1:10 w/v) separately to extract the essential oil. Hydrodistillation was carried out for 4 hours using Clevenger-type apparatus, yielding essential oils that were stored in the dark at 4 ºC until analysis.

Essential oil components were analyzed using Agilent technologies model 7890A gas chromatograph (column; HP-5MS, 25 mm, 30 mm) attached to a US Agilent technology mass spectrometer (MS). Helium was selected as the carrier gas with a 1 ml/min flow rate with a split ratio of 1:100. The injector temperature was 250.0 °C, while column temperature was linearly programmed from 60.0 to 220.0 °C (rate of 5 °C/min) and then held for 10 min at 220.0 °C. The applied voltage was 70 EV, and its interface temperature was set at 280.0 °C. The mass range was set at 30-600 m/z. Then, each sample was injected into the device, and the final compound profile was analyzed.

Identification of essential oil components was accomplished based on the comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (NIST Chemistry WebBook and the Adams' textbook [32]).

2.7. Gas chromatography coupled to flame ionization detector (GC/FID)

Following GC/MS analysis of the compounds in Persian cumin, black pepper, and final formulation, Agilent technology US 7890A GC/FID was used to standardize the product

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based on major components (*trans*-caryophyllene and γ-terpinene). For this purpose, nitrogen gas with 1 ml/min flow was selected as the carrier gas. Since the device had an FID detector, it needed two hydrogen and oxygen gases that gave it the best flame at the ratio of 1 to 10. We used split mode ($^1/_{50}$), and 1 µl of the sample was injected into the device. The injection chamber was set to 270 °C, and the column used was HP-5 (30 m × 320 mm). The stationary phase was phenylmethyl siloxane with a diameter of 0.25 µm. The column temperature was set at 60 °C. The sample was kept at this temperature for 2 minutes, then increased at a rate of 6 °C/min until it reached 250 °C, where it was held for 10 minutes. The temperature of the detector was also set at 300 °C.

As standards, *trans*-caryophyllene and γ-terpinene (Analytical Standard grade, Sigma Aldrich) were used. By sequential dilution with n-hexane, different concentrations of *trans*-caryophyllene (0.18, 0.36, 0.9, 1.8, and 3.6 mg/ml) and γ-terpinene (0.85, 1.7, 8.5, 17, and 42.5 mg/ml) were prepared. Three injections were performed for each standard to check the reproducibility, and the standard curve was plotted by the mean of three injections for each standard. The mean of three injections was used as the sample concentration for the essential oil in the final formulation. In addition, to validate the method, a concentration of 8.5 mg/ml was injected into the device three times daily for three different days to calculate intra- and inter-day differences as well as relative standard deviation (RSD). The limit of detection and the limit of quantification (LOQ) were measured separately for each marker.

2.8. Determination of total alkaloid content in the final tablet

Dried ethanol extract of the final formulation was dissolved in hydrochloric acid (2 N) and filtered subsequently. Afterward, 1 ml of the solution was transferred to a funnel and washed three times with chloroform (10 ml every time). Enough sodium hydroxide was added to the residual solution to neutralize the solution. Finally, 5 ml of BCG, 5 ml of phosphate buffer, and separately, 1, 2, 3 and 4 ml of chloroform were added to the neutralized solution, respectively. The chloroform phases were collected, and the volume was increased to 10 ml with chloroform [33]. This procedure was also used for atropine as a standard alkaloid, and absorption at various concentrations (40, 80, 100, and 120 mg/ml) was measured using a spectrophotometer at 470 nm, and a calibration curve was established. Finally, the absorbance of the resulting solution of the sample was measured at 470 nm, and the total alkaloid content of the formulation was calculated.

3. Result

3.1. Traditional literature review

In total, 127 prescriptions were extracted from the aforementioned traditional encyclopedias. During this search, 3442 pages of traditional texts were studied. Finally, a medicinal prescription mentioned in Canon of Medicine, consisting of Persian cumin and black pepper, was chosen for subsequent processes.

3.2. Microscopic Characterization

An electron microscope was used to examine the fruit of Persian cumin and black pepper. Specific characteristics of each fruit limb were determined in Fig. 1.

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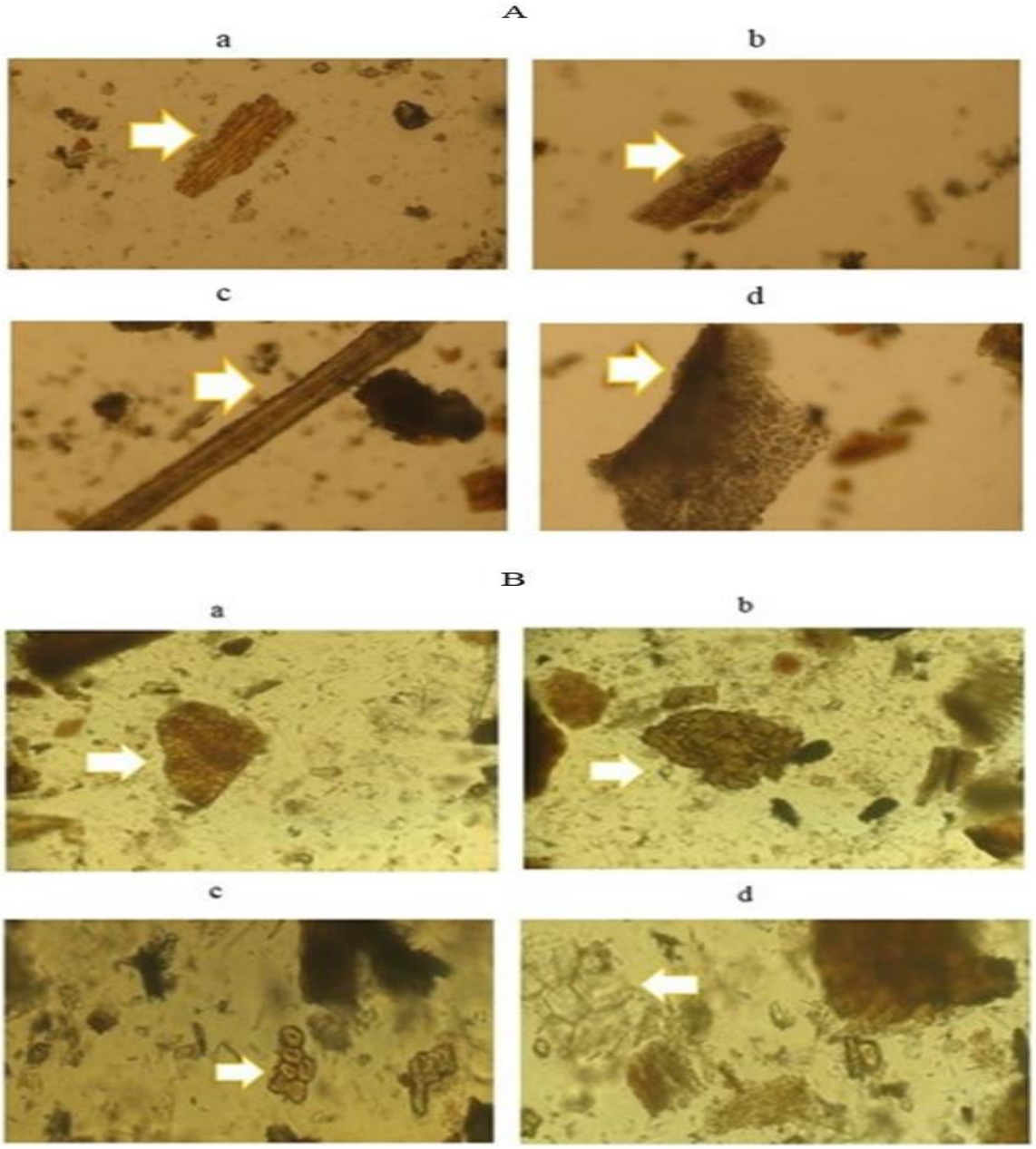


Fig. 1. A: Microscopic characterization of Persian cumin (in chloral hydrate): a) Endocarp b) Epicarp c) Sclereid of mesocarp d) Cells of inner fruit with duct. B: Microscopic characterization of black pepper (in chloral hydrate): a) Exocarp b) Sclereid of endocarp c) Stone cells d) Pitted cells of mesocarp

3.3. Assessment of different tablet formulations

Following the use of various excipients and methods to create the tablet from the selected prescription, each formulation was tested for hardness, weight, and disintegration time. Among 54 different formulations, a tablet containing 40 % Persian cumin, 40 % black pepper, 10 % HPMC, and 10 % PVP as solution binder had good characteristics and was selected for more specific pharmaceutical controls (Fig. 2). The evaluation of powder flow by measuring the angle of repose revealed that the

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flow improved after using HPMC and PVP solution binders from 42.56º to 33.55º.

The mean weight of the 20 tablets was 0.401 g, and the percentage of weight changes was calculated as 1.412 %. Furthermore, the measured thickness of 10 tablets was 3.02 ± 0.042 mm, the friability was less than 1 %, the hardness was 4.87 ± 0.24 kilogram-force and the final formulation tablets were disintegrated in 24 minutes. The final tablet contained an average of 0.041 mg *trans*-caryophyllene, 0.018 mg of γ-terpinene, and 0.06 mg total alkaloid (equivalent to atropine).



Fig. 2. Final prepared tablets

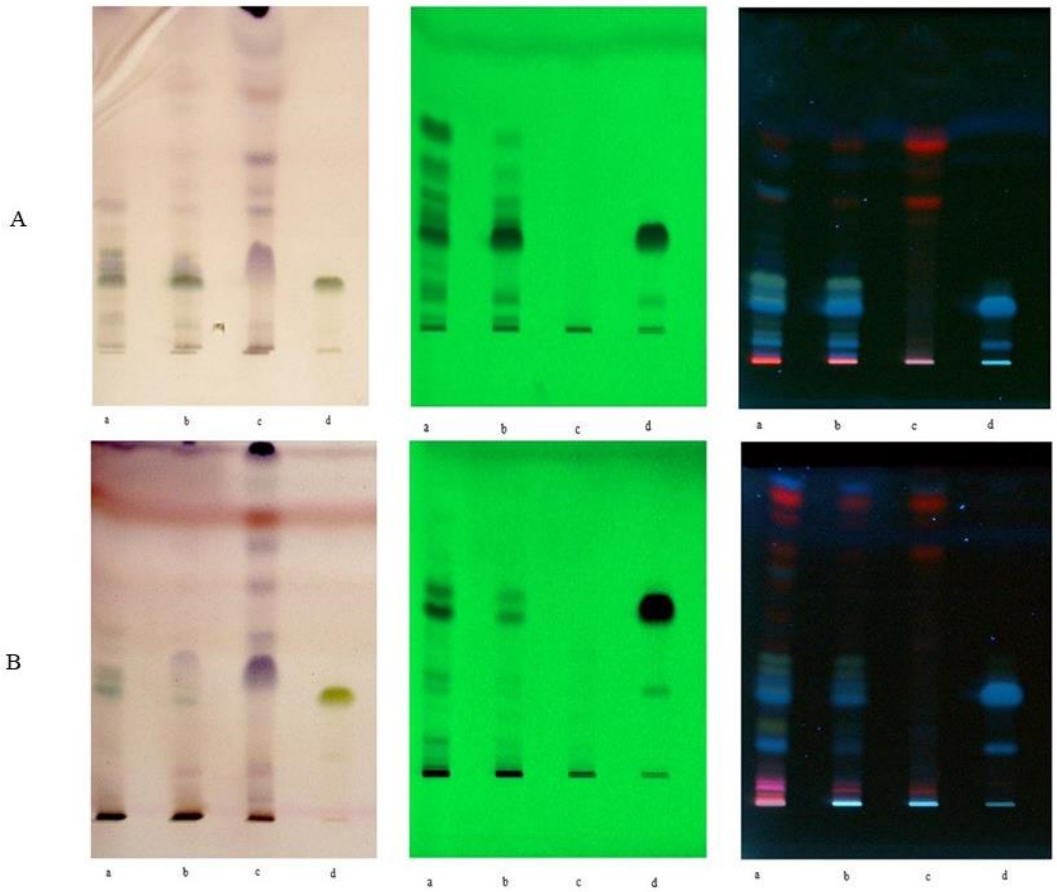


Fig. 3. High-performance thin-layer chromatogram (A: Dichloromethane extract, B: Ethanol extract) under UV light at 366 nm before adding reagent (right), at 254 nm before adding reagent (middle), under visible light after adding reagent (Left side). Sample arrangement from left: a: Black pepper, b: Final formulation, c: Persian cumin, d: Piperine

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3.4. Results of pharmacognostic evaluations

TLC, HPTLC, GC/MS, GC/FID, and total alkaloid content were all determined through various analyses.

3.4.1. HPTLC Analysis of Extract

As previously stated, a concentration of 3.0 mg/ml was prepared for various extracts of Persian cumin, black pepper, and the final tablets. Finally, toluene and ethyl acetate (80:20) was used for (70:15:5) was selected for ethanol extracts. HPTLC chromatogram for dichloromethane and ethanol extracts is shown in Fig. 3.

3.4.2. GC/MS analysis of the essential oil samples

The yields of the essential oils of Persian cumin, black pepper, and final formulations were 2.8 %, 1 %, and 3.4 %, respectively. *trans*-Caryophyllene and *γ*-terpinene were identified as major compounds after determining more than 91 % of the essential oil components of the finalformulation. The chemical constituents of the essential oil samples are shown in Table 1.

Table 1. Essential oil analysis of compounds in Persian cumin (PC), Black Pepper (BP) and Final formulation (F) by GC/MS

Components	PC	BP	F	KI_{CAL}*	KI_{ST}**
α-Pinene	0.63	0.7	2.01	935	939
Sabinene	0.66	-	1.55	975	975
β-Pinene	1.34	1.95	3.69	980	979
Myrcene	1.04	0.83	1.67	991	991
α-Phellandrene	-	1.68	2.11	1008	1003
δ-3-Carene	-	5.98	7.17	1015	1015
o-Cymene	6.93	-	-	1030	1026
Limonene	22.73	7.15	-	1033	1029
(*E*)-*β*-Ocimene	0.43	-	0.34	1048	1050
γ-Terpinene	**26.8**	-	**17.72**	**1064**	**1060**
Terpinolene	0.3	0.74	0.76	1091	1089
Linalool	-	0.75	0.72	1102	1097
Terpinen-4-ol	0.66	-	0.41	1180	1177
Pulegone	1.23	-	0.81	1197	1207
cis-Dihydro carvone	2.08	-	1.04	1201	1193
trans-Carveol	1.15	-	0.56	1208	1217
2-Methyl-3-phenylpropanal	2.65	-	1.04	1244	1244
Carvone	13.11	-	-	1258	1243
p-Menth-2-en-7-ol, trans-	0.39	-	-	1271	1268

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Table 1. Essential oil analysis of compounds in Persian cumin (PC), Black Pepper (BP) and Final formulation (F) by GC/MS (Continued)

Components	PC	BP	F	KI_{CAL}*	KI_{ST}**
Perilla aldehyde	0.43	-	-	1280	1272
Unknown	5.48	-	2.45	-	-
Unknown	-	-	5.9	-	-
Unknown	7.95	-	-	-	-
1,4-*p*-Menthadien-7-ol	0.65	-	-	1332	1332
δ-Elemene	-	7.48	4.68	1344	1338
α-Copaene	-	6.26	2.81	1383	1377
β-Elemene	-	1.86	0.39	1397	1391
Cuminyl acetate	0.88	-	-	1425	1432
(*E*)-Caryophyllene	-	**51**	**29.22**	**1439**	**1419**
α-Humulene	-	4.22	2.46	1464	1455
Germacrene D	0.29	0.54	0.63	1486	1485
β-Selinene	-	1.24	0.95	1494	1490
α-Selinene	-	1.21	0.91	1502	1498
β-Bisabolene	-	0.59	0.62	1512	1506
δ-Cadinene	-	2.86	1.52	1530	1523
Caryophyllene oxide	-	1.03	0.85	1591	1583
Dill apiole	1.89	-	2.65	1630	1621
(-)-Spathulenol	-	1.89	1.51	1637	1619
Palmitic acid	-	-	0.86	1966	1963
Identification	**86.63**	**99.96**	**91.66**	-	

*** KI_{CAL}**: Calculated kovats index.
**** KI_{ST}**: Standard kovats index.

3.4.3. GC/FID-based determination of trans-caryophyllene and γ-terpinene in the final formulation

According to GC/MS data analysis, the main volatile compound in the essential oil of the final formulation was *trans*-caryophyllene, followed by γ-terpinene. A stock solution and subsequent serial dilutions of *trans*-caryophyllene (0.18, 0.36, 0.9, 1.8, and 3.6 mg/ml) and *γ*-terpinene (0.85, 1.7, 5 8, 17, 42.5 mg/ml) were prepared to draw calibration curves with correlation coefficients of 0.9996 and 0.9962, respectively. All data related to the determination of markers are presented in tables 2 to 4. Also, respective calibration curves are shown in Fig. 4 and Fig. 5.

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3.4.4. Determination of total alkaloid

The final formulation was standardized based on the amount of total alkaloid equivalent to atropine in each tablet, in addition to the major compounds in the essential oil. The absorption of atropine at different concentrations (40.0, 80.0, 100.0 and 120.0 mg/ml) were 0.031, 0.048, 0.056, and 0.063, respectively. The calibration curve was drawn by this data and the total alkaloid equivalent to atropine for ethanol extract of final formulation was calculated.

The extract concentration of 12.5 mg/ml resulted in absorbances of 0.049, 0.049, and 0.048 in the first, second, and third replicates, respectively. The absorbances were entered into the following equation:

$$Abs = 0.0004 \times Concentration + 0.01512$$

The alkaloid content was calculated to be 664.906 ± 11.44 mg in 100.0 g of extract.

Table 2. *trans*-Caryophyllene and γ-terpinene calibration data

Compound	Concentration(mg/ml)	Sample number	Area	Mean± SD	RSD
trans-Caryophyllene	0.18	1 2 3	3190.5 2798.7 3473.5	3272.7 ± 174.8	5.3
	0.36	1 2 3	6512.7 6826.7 6446.6	6595.3 ± 203.7	3
	0.9	1 2 3	13046.8 13181.4 13219.8	13149.3 ± 90.84	0.69
	1.8	1 2 3	27561.1 23985 24762	25436.03 ± 1880.92	7.3
	3.6	1 2 3	53811.9 51453.2 46655.7	50460.2 ± 3646.7	7.2
γ-Terpinene	0.85	1 2 3	12564 10920 14345	12609 ± 1712.95	13.58
	1.7	1 2 3	34645 30241 30584	31823.3 ± 2449.64	7.7
	8.5	1 2 3	147534 145819 138750	144034 ± 4656.01	3.23
	17	1 2 3	280561 268687 237145	262131 ± 22438.2	8.6
	42.5	1 2 3	494772 532713 502671	510085 ± 20018.47	3.9

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Table 3. Intra- and inter-day differences of *trans*-caryophyllene and γ-terpinene

Compound	Days	Area			Mean ± SD (intra-day)	RSD % (intra-day)	RSD % (inter-day)
		A_1	A_2	A_3			
trans-Caryophyllene	1	3154.2	3297.66	2969.06	3140.3 ± 164.7	5.2	2.29
	2	3195.6	3181.7	2865.72	3081 ± 186.5	6.02	
	3	3203.3	3198	3273.04	3224.7 ± 41.8	1.29	
γ-Terpinene	1	154413	154880	147534	152275.7 ± 4113.04	2.7	5.6
	2	159115.5	162315	145819	155749.83 ± 8747.9	5.62	
	3	144034	140119	138750	140967.7 ± 2742.32	1.94	

Table 4. *trans*-Caryophyllene and γ-terpinene content in the essential oil of the final formulation

Compound	Injection No.	Area	Concentration (mg/ml)	Mean ± SD	RSD %	LOD (mg/ml)	LOQ (mg/ml)
trans-Caryophyllene	1	41442.1	2.94	3.04±0.12	3	0.06	0.18
	2	42692.4	3.03				
	3	44615.8	3.17				
γ-Terpinene	1	23439.6	1.429	1.359±0.06	4.57	$2.6* 10^{-4}$	$8*10^{-4}$
	2	22937.3	1.339				
	3	24955	1.309				

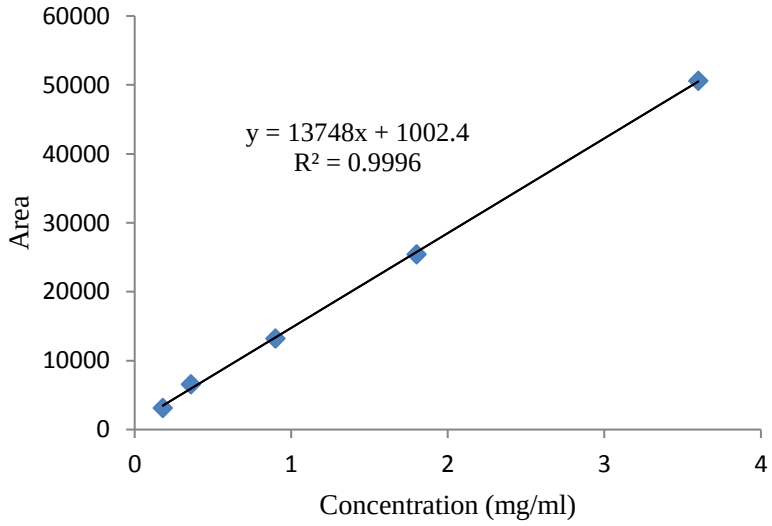


Fig. 4. Standard curve of *trans*-caryophyllene

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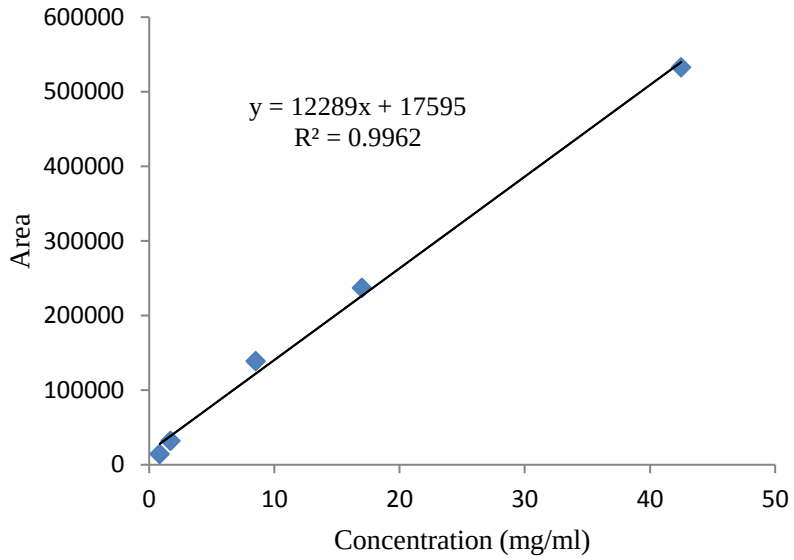


Fig. 5. Standard curve of γ-terpinene

4. Discussion

The ancient sages are thought to have established the foundation for modern medicine, and their instructions can be used to discover and design new pharmaceutical dosage forms and preparations. This valuable information could be used to either treat diseases directly or to provide good sources of various natural medicines [34, 35].

Natural and traditional medicines are now used to prevent and treat a wide range of diseases, including diabetes [36], cancers [37], gastrointestinal and neurological disorders [38], inflammatory issues [39], and bacterial and viral infections [40, 41]. Furthermore, the potential of traditional medicine in treating diseases like Alzheimer's, for which there is no complete cure, can be significant. However, due to a lack of standardization or quality controls, there are numerous concerns and pitfalls associated with the use of traditional medicines [42, 43]. As a result, these medicines must be designed and developed based on new dosage forms with standardized doses in order to improve drug quality and patient satisfaction.

The present study selected a simple traditional formulation reported as a memory enhancer from the list of memory enhancer preparations cited in TPM manuscripts. This formulation includes Persian cumin and black pepper.

Persian cumin has been used in folk medicine to treat digestive issues such as bloating and stomach pain [44]. There have also been reports of anticonvulsant [45], anti-anxiety, anti-depressant [46], and anti-inflammatory effects [47]. Furthermore, this herbal remedy may inhibit the acetylcholinesterase enzyme, potentially aiding in the treatment of AD [48]. The effects of caraway extract on nootropic activities in normal and stress-induced rats were studied, and it was discovered that it has significant anti-stress activity and reduces memory loss due to its central cholinomimetic effect as well as its free radical scavenging mechanism [49].

In addition, black pepper is used as an anti-flatulent, appetizer, and anti-inflammatory in medicine. It has nutritional value in addition to being a spicy condiment [50]. Various studies on the pharmacological effects of black pepper

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show that it can control and manage Alzheimer's disease through a variety of underlying mechanisms [51, 52]. Piperine, one of the bioactive compounds found in black pepper, has been shown in adult male Wistar rats at all doses to reduce memory impairment and nerve damage to the hippocampus. This role could be explained by a decrease in lipid peroxidation and an inhibitory effect on the acetylcholinesterase enzyme [53]. Furthermore, in numerous studies, this bioactive compound has been shown to act as an antioxidant and to eliminate the effects of acetyridine mustard acetylcholine ion brain destruction in an animal model of AD [54, 55]. Moreover, the use of methanol extract of black pepper in rat Alzheimer's disease models was shown to improve memory function and performance via antioxidant activities [56]. As a result, black pepper may play an important role in reducing brain neuron damage in the treatment of Alzheimer's disease [57].

Another study showed that oral consumption of β-caryophyllene reduces neuroinflammation by activating cannabinoid two receptors as well as the peroxisome proliferator-activated receptor-γ (PPAR γ) pathway in mice with Alzheimer's disease [58]. Also, this compound can play a role as one of the effective compounds in the treatment of Alzheimer's patients by reducing the load of β-amyloid, Nitric Oxide (NO), and Prostaglandin E2 (PGE2), Cyclooxygenase-2 (COX-2) and by acting on pro-inflammatory factors [59].

Standardization of herbal products is a critical step after formulation. In Traditional Chinese Medicine (TCM), for example, standardization is carried out in accordance with the principles of "unification, simplification, coordination, and optimization" to ensure TCM quality and safety, to promote TCM modernization and international communication in order to achieve good economic and social outcomes [60]. Standardization of herbal products ensures their quality, purity, safety, and efficacy [61].

According to the traditional use of Persian cumin and black pepper, as well as the mentioned related activities in modern medicine, these two components may have a synergistic effect, and act as a good supplement to prevent AD. Following formulation design, control, and standardization of the traditional prescription, a new tablet formulation containing 40 % Persian cumin, 40 % black pepper, 10 % HPMC, and 10 % PVP was established.

To investigate the phytochemical information of herbal medicines, the chromatographic fingerprint is used as a comprehensive identification method [62]. HPTLC was used to determine the presence of piperine in the final formulation. Because the components in each plant can vary depending on external conditions, reproducing the results without conforming to the analytical data is impossible. As a result, quantitative measurements on the final formulation were performed. Overall, the current study has created a new simple tablet formulation based on traditional Persian pharmaceutical manuscripts with memory enhancing effects. Analytical outcomes of this investigation include constituent and final product fingerprints, including GC/MS and HPTLC data, content determination of the final formulation via GC/FID, and spectrophotometry. In this study, each tablet contained an average of 0.041 mg trans-caryophyllene, 0.018 mg of γ-terpinene, and 0.06 mg of the total alkaloid (equivalent to atropine). The produced medicine can be clinically evaluated and considered a simple memory enhancer natural formulation.

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5. Conclusion

This work has designed, developed, and standardized a herbal formulation containing Persian cumin and black pepper based on traditional Persian medicine reports. This research can be used not only for the production and development of a new supplement, but also as a process for the preparation of herbal products in the form of solid dosage form while controlling and standardizing methods are taken into account. This new tablet has been standardized based on trans-caryophyllene, γ-terpinene and total alkaloid content. This new dosage form can ensure the quality and safety of this herbal product while also increasing patient compliance. The final formulation has introduced a memory enhancer preparation that can be evaluated clinically in various related groups of participants. With an adjusted low dose of administration and safe side for unwanted effects, this formulation may be applied for an extended period, mainly for preliminary steps of dementia or preferably as prevention.

Author contribution

F.D: experimental parts, writing the manuscript, A.A and MM.Z: Supervision, Experimental validation, and developing the draft of the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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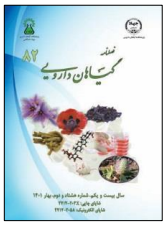
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مقاله تحقیقاتی

طراحی، ساخت و استانداردسازی داروی گیاهی تقویت‌کننده حافظه مبتنی بر طب سنتی ایران

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طب سنتی

چکیده

مقدمه: آلزایمر، شایع‌ترین نوع دمانس می‌باشد که در طب سنتی تحت عناوینی همچون "فراموشکاری"، "فسادِ ذکر" و "نِسیان" ذکر شده است. با توجه به چالش‌های این بیماری، تحقیقات جهت دستیابی به ترکیبات طبیعی موثر در آن حائز اهمیت می‌باشد. **هدف:** هدف، ارائه‌ی قرص گیاهی استاندارد موثر در پیشگیری و درمان آلزایمر مبتنی بر طب سنتی ایرانی می‌باشد. **روش بررسی:** فرآورده‌های مرکبه مرتبط از کتب مرجع طب سنتی ایران گردآوری شد. از میان ۱۲۷ فرمولاسیون، فرآورده حاوی زیره ایرانی و فلفل سیاه از کتاب قانون ابن سینا انتخاب و پس از ارزیابی اکسیپیان‌های مختلف، فرمولاسیون حاوی ۴۰ درصد زیره ایرانی، ۴۰ درصد فلفل سیاه و ۲۰ درصد HPMC و PVP به عنوان محلول چسباننده، تهیه گردید. فرمولاسیون نهایی و هریک از اجزای آن جهت ارزیابی GC و استانداردسازی فرآورده، اسانس‌گیری شدند؛ همچنین عصاره‌گیری با استفاده از اتانول و دی‌کلرومتان به منظور تهیه اثر انگشت HPTLC و تعیین میزان آلکالوئید تام موجود در قرص با استفاده از روش طیف‌سنجی انجام شد. **نتایج:** آنالیز اسانس قرص موید حضور دو ترکیب ترانس کاریوفیلن (۲۹/۲۲ درصد) و گاماترپینن (۱۷/۷۲ درصد) بود و استانداردسازی قرص براساس این دو ترکیب انجام شد؛ به گونه‌ای که بصورت میانگین در هر قرص ۰/۰۴۱ میلی‌گرم ترانس کاریوفیلن و ۰/۰۱۸ میلی‌گرم گاماترپینن وجود داشت. میزان آلکالوئید تام موجود در هر قرص، ۰/۰۶ میلی‌گرم معادل آتروپین محاسبه گردید. **نتیجه‌گیری:** این فرمولاسیون طبیعی می‌تواند به عنوان یک محصول طب سنتی ایرانی به صورت بالینی مورد ارزیابی قرار گیرد و به عنوان یک مکمل تقویت‌کننده‌ی حافظه ارائه شود.

مخففها: AD، بیماری آلزایمر؛ TPM، طب سنتی ایران؛ HPMC، هیدروکسی پروپیل متیل سلولز؛ PVP، پلی وینیل پیرولیدون، GC، کروماتوگرافی گازی؛ MS، طیف‌سنجی جرمی؛ FID، آشکارساز یونش شعله‌ای؛ HPTLC، کروماتوگرافی لایه نازک با کارایی بالا

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