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Qualitative and Quantitative Phytochemical Analysis of Medicinally Potential Plant of *Anisomeles malabarica* (L.) R.Br.sssss

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Abstract This investigation suggested that the qualitative phytochemical compounds based on leaves, flowers and seeds of *Anisomeles malabarica* with different solvents such as aqueous, benzene, chloroform and methanol were used. Aqueous extract of seeds including substances like alkaloids, carbohydrate, coumarin, flavonoid, phenol, protein, saponin, steroid, tannin and terpenoid had the highest concentration of qualitative phytochemicals compared to another solvents. Leaves and flowers were also identified, respectively. Obviously, the quantitative phytochemicals such as alkaloid, carbohydrate, coumarin, flavonoid, phenol, protein, saponin, steroid, tannin and terpenoid were profound of extracted in seeds of *A. malabarica* when compared to leaves and flowers with other solvents. However, each of the solvents also contained moderate phytochemicals, which were observed in the respective plant parts. *Anisomeles malabarica* is a tropical indigenous herbal plant. It is a member of the Lamiaceae family and has a strong scent (Labiatae). Both qualitative and quantitative research was performed on the main phytochemicals present in the seeds of the pharmaceutically important plant *Anisomeles malabarica*.

Keywords Medicinal Plant, *Anisomeles Malabarica*, Solvents, Qualitative and Quantitative Phytochemicals

1. Introduction

Medicinal plants have been used in traditional medicine and human diet since the time of the ancient Indians, Egyptians, Chinese, and many other cultures [1]. For the vast majority of people of earth, therapeutic plants and their parts are their primary source for strong health system. The liniments, herbal tea, infusion, syrup, ointment and powder were used by *Anisomeles malabarica* leaves [2].

Anisomeles malabarica is a fragrant perennial herb with a dense pubescence that grows to reach 1.2-2.0 metres tall. The plant has purple flowers in thick whorls with interrupted spikes, compressed and ellipsoid seeds [3]. The plant has leaves that are simple, opposite, thick, acute, oblong-lanceolate, aromatic, pale above, white below, crenate-serrate and leaves are woolly. *Anisomeles malabarica* vernacular names are peimiratti, malabar catmint and bhutan kusham.

Previous phytochemical investigations of *Anisomeles malabarica* [4] identified Anisomelin, Anisomelic acid, 2-Acetoxymalabaric acid, Anisomelolide, Betulinic acid, Gerainic acid, Anisomelyl acetate, Citral, sitosterol, Malabaric acid, Ovatodiolide and Triterpenebetulinic acid. As a medicinal herb, *Anisomeles malabarica* has been used as a folk medicine treatment for fevers, anorexia, rheumatism and swellings. *Anisomeles malabarica* has been used in traditional medicine to treat intermittent fever, colic dyspepsia, and wound healing. In rare cases, crude extracts of medicinal plants can be used as medications [5]. Theraputic plants provide bio-active molecules as well as lead structures for enhanced derivative development. Some naturally occurring botanical substances have a wide spectrum of chemical active components that can disrupt the mosquito's life cycle and dissemination, minimising the risk for humans and animals.

Several medical plants are performed as a pesticide and repellant properties as crude material and essential compounds and oils [6]. The various types of molecules found in plants, as well as secondary metabolites, aided in the development of their pharmacologically active molecules [7]. Phytochemical study of plant products has gained popularity in both organic chemistry and plant biochemistry in recent years. It not only deals with the various organic chemicals that plants acquire, but also assists in identifying the chemical structure, distribution, and biological function of plant substances [8].

As a result, advances in phytochemistry are inextricably linked to the effective use of existing techniques and the continuous development of new ways to address unsolved problems as they arise. In the truest sense, phytochemicals are compounds generated from the plants. Therefore, the term is most typically used to refer the plant derivate substances that may benefit organism health but are not necessary nutrients. Because plant-based diets are complex mixtures of bioactive molecules on the health impacts of individual phytochemical compounds were related to health care of foods containing those phytochemicals [9]. The vital role of bioactive compounds of plants contain alkaloids, flavonoids, phenols, phytochemical compounds, etc. Tribal tribes in India use thousands of plants, notably angiosperms, in a variety of ways. The seplantsist is most commonly used in medical plants, which contain flavonoids, which can be either conesor O or C glycosides. Flavonoids have recently gained popularity as a result of their pharmacological characteristics [10].

2. Materials and Methods

Anisomeles malabarica seemed to be the most valuable source of medical benefit in many respects. Plant collection and identification: *Anisomeles malabarica* was collected in its whole from A.Veeriya Vandayar Memorial Sri Pushpam College (Autonomous) in Poondi – 613 503. The plant has been authenticated by Rapinat herbarium, St. Joseph's College (Autonomous), Trichy (Herbarium No. NT001) (Fig.1).

2.1. Processing of Plant Materials

The *Anisomeles malabarica* leaves, flower and seeds were washed under running water and air dried at room temperature. The dried plant parts were pulverised. The grinded plant parts were extracted for 24 hours in a Soxhlet apparatus with methanol (80℃) using a hot continuous percolation process. Whatmann number 1 filter paper and a Buchner funnel were used to filter the plant extracts. Excess solvents were recovered in a rotary evaporator at low pressure to thick oily natured crude, and the extract was dried by lyophilizer and dry powder were obtained.

2.2. Qualitative Phytochemical Analysis [11]

Phytochemical tests were performed on powdered specimens of leaves, floweres and seeds of *Anisomeles malabarica* with acetone, aqueous, benzene, chloroform, and methanol extracts using established techniques to identify components.

It was carried out to determine the qualitative phytochemical compounds from crude extracts which were used and its results were confirmed by reaction basis, such as Alkaloids, carbohydrate, Coumarin, flavonoids, Phenols, protein, saponin, steroid, tannin and terpenoid.

2.3. Test for Alkaloids (Mayer's Test)

One mL of HCl and mayer's reagent (2 mL of 5%) added 1 mL of *Anisomeles malabarica* plant leaf, flower and seed individually extract. The appearance of green colour precipitates indicated the alkaloid presence.

2.3.1. Aminoacids

0.5 mg of extract of leaf, flower and seed with 0.2% Ninhydrin reagent was boiled. The formation of purple or pink colour indicated the amino acids presence.

2.3.2. Carbohydrates

Two mL of leaves, flowers and seeds extract and two drop of alcoholic solution of a napthal are added and few droplets H_2SO_4. The appearance of red or violet ring indicated the carbohydrates presence.

2.3.3. Flavonoids

Sodium hydroxide solution in a few drops was used to treat the extracts. Flavonoids were detected by the formation of a bright yellow colour that turned colourless when diluted acid was added.

2.3.4. Phenols

Few drops of $FeCl_3$ and ethanol were added 1 mL leaves,

flowers and seeds extract of *Anisomeles malabarica*. Formation of violet colour indicated the formation of phenols.

2.3.5. Proteins

In a test tube, 2 mL of plant extract was combined with 2 mL of a 10% sodium hydroxide solution, and the mixture was then heated for 10 minutes. In the mixture mentioned above, a drop of a 7 percent copper sulphate solution was added. Purplish violet coloration is a sign that proteins are present.

2.3.6. Saponins

When two drops of coconut oil and a few drops of water were applied to a plant extract from *Anisomeles malabarica* leaf, flower and seeds a layer or foam showed the presence of saponins.

2.3.7. Steroids

2 mL of plant extract of *Anisomeles malabarica* leaves, flowers and seeds with acetic acid was added to sample heated and added sulphuric acid. Finally occurring the upper side of green color layers determined the presence of steroids.

2.3.8. Tannins

5 mL of the *Anisomeles malabarica* plant parts extract was mixed with 2 mL of 5% of $FeCl_3$. The formation of greenish-black precipitation showed presence of tannins.

2.3.9. Terpenoids

The plant extract was combined with two millilitres of chloroform, evaporated over a water bath, and then heated with two millilitres of concentrated H_2SO_4. Terpenoids generated a grey colour, which served as a sign of their presence.

2.4. Analysis of Quantitatively Phytochemicals

2.4.1. Alkaloids [11]

The *Anisomeles malabarica* leaf, flower, and fruit were weighed into a 250 mL beaker, to which 200 mL of acetic acid in ethanol at a 10% concentration was added. The beaker was then covered and let to stand for 4 hours. After filtering, the extract was concentrated to a quarter of its original volume on a water bath. The extract received drop wise additions of concentrated ammonium hydroxide until the precipitation was finished. After allowing the entire solution to settle, the precipitate was collected, cleaned with weak ammonium hydroxide, and then filtered. The alkaloids that were dried and weighed make up the residue.

2.4.2. Aminoacids [12]

Pipette out the corresponding labelled test tubes with 0.2, 0.4, 0.6, 0.8, and 1 mL of the standard amino acid solution. In each test tube with the label "Blank," pour distilled water. Now fill all of the test tubes, including the ones marked blank and unknown, with 1 mL of the ninhydrin reagent. By shaking or vortexing the tubes, combine the contents inside. Then immerse all of the test tubes for 15 minutes in a pot of boiling water. The test tubes should be chilled in cold water before adding the 5 mL of diluent solvent and thoroughly mixing. Using a colorimeter, now note each solution's absorbance at 570 nm.

2.4.3. Carbohydrates [13]

5 mL of 2.5 N hydrochloric acid, 100mg of the material was hydrolyzed over a 3-hour period. When the effervescence stopped, solid Na_2CO_3 was added after it had been chilled to room temperature. The liquid was centrifuged, and 100 mL of distilled water was added to the supernatant. From this, 0.2 mL of the sample was pipetted and 1 mL of distilled water was used to make up the volume. Then 1 mL of phenol reagent and five millilitres of H_2SO_4 were added. For 20 minutes, the tubes were maintained at 25 to 30 ℃. At 490nm, the absorbance was measured.

2.4.4. Flavonoids [14]

Anisomeles malabarica leaf, flower, and fruit samples weighing one gramme were repeatedly extracted at room temperature with 100 mL each of 80% acetone, water, ethanol, and methanol. A Whatmann No. 1 filter paper was used to filter the mixture into a pre-weighed 250 mL beaker. The filtrate was put into a water bath, allowed to dry completely, and then weighed.

2.4.5. Phenols [15]

The phenolic component was extracted from the fat-free samples by boiling them in 50 cc of ether for 15 minutes. A 50 mL flask was filled with 5 mL of the appropriate extract, followed by 10 mL of distilled water. Additionally, 5 mL of concentrated amyl alcohol and 2 mL of NH_4OH solution were added. Plant leaf, flower, and fruit samples were prepared according to specifications and allowed to respond for 30 minutes before being coloured. 550nm was used to read this.

2.4.6. Protein [16]

The Bradford's method was used to ascertain the total protein content. 3 mL of Bradford's reagent was added to 100 l of the appropriate sample extract, which was then incubated for 5 minutes in the dark. At 595nm, the absorbance was measured. Dilutions of bovine serum albumin (0.1 mg/mL and 0.5 mg/mL) are employed as reference solutions.

2.4.7. Saponins [17]

The leaf, flower, and fruit samples of *Anisomeles malabarica* were each ground separately. 200 mL of 20% ethanol were mixed with 20g of each plant sample in turn. The suspension was cooked at roughly 55℃ over a hot water bath for 4 hours while being constantly stirred. The mixture was filtered, and the leftover material was extracted once more using 200 cc of 20% ethanol. A water bath heated to roughly 90℃ was used to decrease the combined extracts to 40 mL. Transferring the concentrated samples into a 250 mL separator funnel, 20 mL of diethyl ether was added, and the mixture was violently agitated. While the ether layer was discarded, the aqueous layer was recovered. The cleansing procedure was repeated. N-butanol 60 mL was added. Two separate washes with 10 mL of 5 percent aqueous sodium chloride were performed on the combined n-butanol extracts. In a water bath, the residual solution was warmed. Following evaporation, the corresponding samples were baked to a consistent weight. The amount of saponins was calculated as a percentage.

2.4.8. Total steroids [18]

20 mL of ethanol were macerated with the extract (1 g) and then filtered. After adding two mL of chromagen solution, the mixture was allowed to stand for 30 minutes. At 550 nm, the absorbance was measured.

2.4.9. Tannins [19]

In a 50 mL plastic bottle, 500 mg of *Anisomeles malabarica* were measured out. Aqueous solvent and 50 mL of methanol were added, and the mixture was agitated for an hour in a mechanical shaker. This was built up to specification and filtered into a 50 mL volumetric flask. Then, 2 mL of 0.1M ferric chloride in 0.1N hydrochloric acid and 0.008M $C_6N_6FeK_3$ were added to 5 mL of the filtrate in a test tube. Within 10 mm, the absorbance at 120nm was measured.

2.4.10. Terpenoids [20]

100 mg of a dried *Anisomeles malabarica* leaf, flower, and fruit extract were taken, and they were steeped for 24 hours in 9 mL of methanol and water. Following filtering, the extract was extracted using a separating funnel and 10 mL of petroleum ether. The plant ether extract was divided into pre-weighed glass vials, let to dry completely, and then the yield (percent) of the total terpenoids contents was calculated using the formula below.

$$(wi\text{-}wf/wi \times 100)$$

3. Results

Anisomeles malabarica seed sample had the highest concentration of sample, followed by flower sample, whereas leaves sample had the lowest concentration of sample in the compounds. Protein and carbohydrate are also found in all samples and solvents. Similarly, in the plant, leaf methanol extract is recorded as a maximum amount present, benzene extract is recorded as a minimum amount present, flower benzene extract is recorded as a maximum amount present, and aqueous extract is recorded as a minimum amount present. However, the largest concentration of the plant seed Chloroform extract is found, whereas the lowest concentration of methanol is found. In this study, the largest amount of phytochemical components was discovered in the methanolic extracts of both plant samples (Table 1).

The findings of quantitative analysis of the plant extracts in aqueous, benzene, chloroform, and methanol extracts from their three components leaves, flowers and seeds are reported. The methanol extract was present in the highest concentration of all samples, followed by the methanolic extract of the plant leaf, which had the highest quantity of protein (1.53±0.26) and the lowest amount of tannins (1.24±0.12). Following that, methanolic flower extract had the highest amount of carbohydrate (3.78±0.00) and the lowest amount of flavonoids (3.07±0.05), while seed extract had the highest amount of protein (5.06±0.05) and the lowest amount of coumarins (2.11±0.41). Many researchers have looked at the phytochemicals found in medicinal plants as well as their therapeutic properties (Table 2).

The highest value of aqueous extract (2.99±0.00) and benzene (2.98±0.00) were found in the investigation of phenol content, which was predominantly found in seed samples. Similarly, only one extract has been supplied for the flower sample of benzene (3.64±0.08) and the leaf sample of chloroform (1.39±0.19). A flower sample of the plant contains terpenoids, saponins, and coumarins in abundance in all solvents. Then the highest values are found in benzene solvent terpenoids (3.49±0.05), saponins (3.61±0.06), and coumarins (3.71±0.02). Chloroform solvent was entirely present in all tests for seed sample, with carbohydrate (4.16±0.01) and protein (5.53±0.65) being the most prominent components. Aqueous solvent (3.28±0.06) is the most effective at presenting flavonoids in flower samples. Protein content was found to be high in seed samples of the plant according to the researchers (Table 2).

Figure 1. Taxonomical identification of *Anisomeles malabarica*

Table 1. Qualitative phytochemical analysis of *Anisomeles malabarica* with different parts of plant

Phytoconstituents	Leaves				Flowers				Seeds			
	Aqueous	Benzene	Chloroform	Methanol	Aqueous	Benzene	Chloroform	Methanol	Aqueous	Benzene	Chloroform	Methanol
Alkaloids	+	-	-	+	+	+	+	-	+	+	+	-
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins	+	-	-	-	+	+	+	+	+	-	+	+
Flavanoids	+	+	-	+	+	-	+	+	+	-	+	+
Phenol	-	-	+	-	-	+	-	-	+	+	+	-
Protein	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	-	+	+	+	+	+	+	+	+	+	-	+
Steroid	-	+	_	+	-	+	-	+	+	+	+	-
Tannins	+	-	+	+	-	-	-	+	+	-	+	+
Terpenoids	-	-	-	-	+	+	+	+	-	+	+	+

Table 2. Quantitative phytochemical analysis of *Anisomeles malabarica* with different parts of plant

Phytoconstituents	Quantity (mg/g)											
	Leaves				Flowers				Seeds			
	Aqueous	Benzene	Chloroform	Methanol	Aqueous	Benzene	Chloroform	Methanol	Aqueous	Benzene	Chloroform	Methanol
Alkaloids	1.17±0.18	-	-	1.28±0.14	3.72±0.00	3.33±0.00	3.11±0.00	-	2.16±0.01	2.72±0.03	2.45±0.01	-
Carbohydrates	1.19±0.15	1.43±0.218	1.41±0.18	1.45±0.12	3.09±0.01	3.18±0.06	3.84±0.08	3.78±0.00	4.16±0.08	4.48±0.03	4.16±0.01	4.93±0.01
Coumarins	1.25±0.05	-	-	-	3.56±0.22	3.12±0.58	3.44±0.11	3.71±0.02	2.10±0.46	-	2.74±0.08	2.11±0.41
Flavanoids	1.26±0.18	1.32±0.16	-	1.41±0.20	3.28±0.06	-	3.10±0.06	3.07±0.05	2.59±0.01	-	2.82±0.00	2.69±0.01
Phenol	-	-	1.39±0.19	-		3.64±0.08		-	2.99±0.00	2.98±0.00	2.47±0.32	-
Protein	1.22±0.41	1.51±0.25	1.40±0.20	1.53±0.26	3.77±0.00	3.82±0.02	3.28±0.52	3.54±0.02	5.36±0.07	5.09±0.01	5.53±0.65	5.06±0.05
Saponin	-	1.04±0.02	1.26±0.13	1.43±0.18	3.43±0.05	3.17±0.00	3.44±0.06	3.61±0.06	2.66±0.02	2.45±0.00	2.38±0.05	2.13±0.04
Steroid	-	1.48±0.24	-	1.31±0.15	-	3.56±0.05	-	3.09±0.04	2.82±0.06	2.85±0.00	2.79±0.06	-
Tannins	1.27±0.14	-	1.36±0.18	1.24±0.12	-	-	-	3.45±0.00	2.67±0.05	-	2.42±0.03	3.21±0.09
Terpenoids	-	-	-	-	3.36±0.00	3.49±0.05	3.25±0.08	3.32±0.04	-	2.45±0.81	2.21±0.04	2.16±0.00

4. Discussion

Phytochemical components of *Anisomeles malabarica* were investigated in dried leaf, flower and seed samples in this study. Aqueous, benzene, chloroform, and methanol were used to dissolve the dried leaf, flower, and fruit samples. To get the accessible bioactive ingredient in the sample, a variety of solvents were utilised for phytochemical extraction and quantification. In a solvent, the soluble phytochemical components were easily dissolved [21].

The qualitative analysis of ethanol extract of leaves of the plant was expressed in positive results of alkaloids, phenols, tannins, flavonoids, glycosides, steroids, terpenoids and saponins [22].

In this present investigation suggested that the seeds found to be remarkable quantity of phytochemicals than that of leaves and flowers recognized by respective plant part. Normally seed part was maximum nutrient sources.

The importance of many primary metabolites in medicinal substances lies in their role as precursors or pharmacologically active metabolites were determined [23].

The quantitative determination of phytochemical constituents from the plant showed alkaloids, flavonoids, tannin and poly phenol compounds. Among the phytochemicals showing maximum quantity was found in alkaloids [24].

Many researchers have looked at the phytochemicals found in medicinal plants as well as their therapeutic properties [25]. Plant growth, development, and disease resistance are all influenced by phenolic content. To guard against cancer, cardiovascular disease resistance, diabetes, osteoporosis, and neurological disease, on the other hand was reported [26].

5. Conclusions

The use of *Anisomeles malabarica* leaves, flowers and seed extracts on the plant resulted in greater phytochemical compounds. The plant has a wide range of medicinal effects in folk medicine. According to ethnobotany, numerous phytochemicals are found in commonly consumed plant foods. *A.malabarica* seeds posses maximum phytochemicals when compared to flower and leaves. The phytochemical molecules contain the features responsible for its physical, chemical and biological properties of the active chemicals for their therapeutic and nutraceutical importance. The plant examined for phytochemical elements appeared to have the potential to serve as a source of useful pharmaceuticals as well as to improve the health care of consumers due to the presence of several biochemicals that are required for good health.

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