
SECONDARY METABOLITES IN THE EXTRACTS OF *ANISOMELES INDICA*(L.)
KUNTZE

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Abstract

The *Lamiaceae* (*Labiatae*) is one of the most important angiospermic aromatic families with wide application in term of traditional knowledge. The genus *Anisomeles* (L). R. Br. belongs to the Family Lamiaceae and comprises over 20 species whose centre of distribution is located in the tropical Asia and Australia. These are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony and rocky regions. The members of this genus are characterized by the presence of glandular hairs on the floral parts. Local and tribal people of Maharashtra used this plant against fever and common cold. Preliminary phytochemical analysis was carried out by using various reagents, revealed that the plants parts are rich in active compounds such as alkaloids, essential oil, tannins, anthraquinones, glycosides, flavonoids, quinines, phenols, coumarines and terpenoids. The present study was conducted to investigate the presence of secondary phyto-constituents.

Key words: *Lamiaceae*, *Anisomelesindica*, Phytochemical Screening, Secondary metabolites.

Introduction

Secondary metabolites are the classes of compounds which are known to show curative activity against several ailments in man, and therefore could explain the traditional use of medicinal plants for the treatment of some illnesses. The *Lamiaceae* (*Labiatae*) is one of the most important angiospermic aromatic families with wide application in term of traditional knowledge. The diverse species of Lamiaceae are ethnomedicinally important for which it is continuously exploited by people and also used for pharmaceutical, nutraceutical and ingredient in nourishment supplements (Sharma *et al.*, 2010). Nearly 16 species are reported to be in Vidarbha region (Dhore and Joshi 1988). Traditionally Lamiaceae (mint family) is considered closely related to Verbenaceae (Stevenet *al.*, 1998 Cantino 1992). The genus *Anisomeles* (L). R. Br. belongs to the Family Lamiaceae and comprises over 20 species whose centre of distribution is located in the tropical Asia and Australia (Aluri 1992). *Anisomelesindica*(L.) Kuntze. is a camphor-scented large perennial woody shrub which can reach up to 2 m tall. The stem is erect, brown or pinkish black, acutely quadrangular (Ushiret *al.*, 2009). Local and tribal people of Maharashtra use this plant against fever and common cold (Bhattachrjeeet *al.*, 2001). In Thailand the volatile oils of *Anisomelesindica* are used in preparation of dermatological products (Okonogiet *al.*, 1994).

Methodology

Collection of plant material

***Anisomelesindica*. (L.) Kuntze.**

Plant material was collected from Pohara forest. Amravati district, Maharashtra. in the month of September in year 2019. Plant *Anisomelesindica* grows in arid soil, sandy loam and granitic soil and rocky regions (Sivarajanet *al.*, 1974). Pohara Forest in Amravati district, Maharashtra located

between 20.57'N 77.57'E was found to be the best location for collection. It is a reserve forest for birds wherein total 171 bird species from 56 families have been recorded in this forest (Wadatkar *et al.*, 2000).

Extraction Method

Preliminary phytochemical analysis

Air dried plant powder (5g) was extracted with petroleum ether, acetone, benzene and double distilled water by Soxhlet extraction. The extract was concentrated up to 100 ml. Preliminary phytochemical tests were performed following Harborne, (1998) and Raaman (2006). According to standard protocol 10 tests were selected for present investigation, these are Wagner's reagent test for detection of Alkaloids, Ferric chloride test for phenol, Braymer's reagent test for tannin, Salkowski test for terpenoids, Alkaline reagent test for Flavonoids, Keller Kelliani's test for Cardiac glycosides, Sudan red-III test for essential oil. All the reagents prepared according to standard method.

Test for Alkaloids

Mayer's Test - Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to 2ml of extract and observed for the formation of white or cream colour precipitate which indicate the presence of alkaloids.

Wagner's reagent - 2ml plant extract was treated with 3-5 drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

Test for Phenols

Gelatin Test - 1% gelatine solution containing 10% sodium chloride was added to 2ml of extract. Formation of precipitate indicated the presence of phenolic compounds.

Nitric acid Test - 2ml of extract was treated with dilute nitric acid. Formation of reddish to yellowish colour indicated the presence of phenolic compounds.

Test for essential oil

Sudan Red-III - 2 ml of plant extract was treated with Sudan Red-III and formation of red colour indicate the presence of essential oil.

Tincture alkane Test - 2 ml of plant extract was treated with 3-5 drop of alcoholic tincture solution, formation of red colour indicates presence of essential oil.

Test for Tannins

Braymer's Test - 2ml of an extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution indicating presence of tannin.

Iodine Test - 2 ml of extract was treated with dilute iodine solution. Appearance of transient red colour indicated the presence of tannins.

Test for Flavonoids

1) Lead acetate Test - 2 ml of extract treated with few drops of 5% solution of Lead acetate, milky colour precipitate formation indicated the presence of flavonoids.

2) Alkaline reagent Test- 2 ml of extract treated with sodium hydroxide solution shows increase in the intensity of yellow colour which become colourless on addition of few drops of dilute hydrochloric acid, indicated the presence of flavonoids.

Test glycosides

Keller Kelliani's Test -2 ml of extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. A brown ring indicated the presence of Cardiac glycosides.

Borntagers Test – 2 ml of extract was treated with 2 ml of chloroform, followed by equal amount of concentrated ammonia, appearance of pink colour indicated presence of glycosides.

Test for Terpenoids

Salkowski Test- 2 ml of extract was treated with chloroform (2 ml) followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced indicated the presence of terpenoids.

Copper acetate Test- 2 ml of extract was dissolved in water and treated with 10 drops of copper acetate solution, formation of emerald green colour indicated presence of terpenes.

Anthraquinones.

H₂SO₄ Test -Five ml of the extract solution was treated with Conc. H₂SO₄ with 1ml benzene followed by addition of 1 ml of dilute ammonia. Rose pink coloration indicated positive response for anthraquinones.

Coumarin

Alcoholic NaOH Test -3 ml of 10% NaOH was added to 2 ml of extract, formation of yellow colour indicated presence of coumarins.

Test for Quinones.

HCL Test - 2 ml of all extract when treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration) indicated the presence of Quinones.

Table 1 : Phytochemical screening of the extracts of *Anisomelesindica*(L.) Kuntze

Sr.No.	Name of test	Ether	Benzene	Acetone	Water
1.	Alkaloids				
a.	Mayers reagent	++	++	-	++
b.	Wagner Reagent	+	+	++	+++
2.	Phenols				
a.	Gelatin test	-	+	++	-
b.	Nitric acid test	++	+	-	++
3.	Essential oil				
a.	Sudan red-III	+++	+++	+++	+++
b.	Tincture alkana test	++	+	++	-
4.	Tannins				
a.	Braymers test FeCl ₃ .	++	++	++	+++
b.	Iodine test	+	++	++	-
5.	Flavonoids				
a.	Alkaline Reagent Test	-	++	-	-

b.	Lead acetate test	-	++	++	++
6.	Glycosides				
a.	Borntragers test	+	++	-	+
b.	Keller kelliani test	-	-	++	++
7.	Terpenoids				
a.	Copper acetate test	+	++	-	++
b.	Salkowski test	-	++	++	++
8.	Anthraquinones				
a.	H ₂ SO ₄ test	++	++	++	++
9.	Coumarines				
a.	Alcoholic NaOH test	++	+	-	++
10	Quinones				
a.	HCL test	++	+	-	-

(+++ highly present, ++ moderately present, + presence, -absence of phytoconstituents.)

Table: Preliminary phytochemical analysis *Anisomeles indica* leaves

Colour observations

Anisomeles indica. (L.) Kuntze.

Sr.No.	Name of Plant	Plant Part	Solvent	Colour
1	<i>Anisomeles indica</i> .	Leaves	P. ether	Yellow
2	<i>Anisomeles indica</i> .	Leaves	Acetone	Black
3	<i>Anisomeles indica</i> .	Leaves	Benzene	Black Green
4	<i>Anisomeles indica</i> .	Leaves	Water	Brown

Table: Colour observations of extracts of *Anisomeles indica*.

Result and Discussion

Anisomeles indica leaf extracts exhibited color variation in different solvents; in petroleum ether, benzene, acetone and water it was show yellow, black, black green and brown respectively. Total 10 tests were selected for preliminary phytochemical analysis. The results presented in Table 1 revealed that the plant leaf extracts contained alkaloids, essential oil, tannins, anthraquinones, glycosides, flavonoids, quinines, phenols, coumarines and terpenoids. Alkaloids was confirmed through Mayer's reagent and Wagner's reagent that was moderately present in all leaf extracts. Phenols analyzed through Gelatin test and Nitric acid test, revealed moderately presence of phenolic compound. Occurrence of essential oil checked through Sudan red-III and Tincture alkana test. Sudan Red III reagent shows positive (+++) reaction in all four plant leaf extracts. The data indicated presence of abundant essential oil in the plant. Availability of tannins was validated by using Braymer's test (FeCl₃) and Iodine test that was abundantly present inside plant leaf. Confirmation of Glycosides carried out by using Borntrager's test and Keller kelliani test showed less amount in plant. Through H₂SO₄ test analyzed the presence of anthraquinones that was found to be less in all plant extracts. Coumarines and Quinones confirmed through Alcoholic NaOH test and HCL test revealed minimum concentration in all the plant extracts.

Comparatively maximum phytoconstituents were found in aqueous extract, while those were minimum in petroleum ether extract. Sudan Red III reagent shows positive (+++) reaction in all four plant leaf extracts. For preliminary test double distilled water is a good solvent as compared to other solvents used in extraction. More than 40,000 terpenoids exist in nature, maximum terpenoids originated from plants, these phytoconstituents used in aromatherapy against skin problem and mild fever, strengthens its importance in traditional practices.

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