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Preliminary phytochemical screening and HPTLC fingerprinting of *Anisomeles indica* (L.) Kuntze: A traditional medicinal plant

Rajput Nutan and Satya Veena

Abstract
Anisomeles indica (L.) Kuntze plant, belonging to family Lamiaceae, is used as folk medicine in the treatment of diverse conditions such as piles, viral fever, skin inflammation, liver protection, intestinal infections, Abdominal pain and immune system deficiencies.
The present paper reflects the preliminary phytochemical screening and HPTLC fingerprinting analysis of the plant. The extract, prepared from stem, leaves & inflorescence in Ethyl acetate, Methanol and distilled water revealed the presence of alkaloids, glycosides, flavonoids, tannins, terpenoids, steroids, saponins and phenolics. Alkaloids were present at low quantity but phenolics were present in high quantity as compare to flavonoids.

Keywords: Folk medicine, extract, phytochemical screening, HPTLC, alkaloids, phenolics, flavonoids

Introduction
Green plants patches are Green laboratories, which have been a source of medicine and are one of the wonderful gifts of nature to human being. Plants and their parts are used as crude drugs in many medicinal preparations from centuries. The medicinal properties of plants can be attributed to the chemical compounds; synthesized during various metabolic pathways in the life. Most of the biochemical constituents are extractable and used as crude drugs. The rich botanical wealth needs to be identified, analyzed and utilized for drug industry. Knowledge about the chemical constituents present in plants is useful not only for the invention of remedial sources, but also determining new sources of economic materials, such as, tannins, oils, gums and precursors for the production of complex chemical substances.
About 65% of the world's population has incorporated the value of plants as a methodology of therapeutic treatments into their primary health care. (Farnsworth *et al.,* 1985) [8]. In addition, the information regarding the presence of various chemical constituents in plants becomes important in discovering the authentic value of folklore medicines (Mojab *et al.,* 2003) [23].
Lamiaceae or mint family is one of the largest family comprising 252 genera and 7000 species. (Plant- list 2013) [25]. In India the family is represented by 65 genera and 400 species. Nearly all the members of this family possess ethnomedicinal properties.
Anisomeles indica (L.) Kuntze plant is used in folk medicine in the treatment of diverse conditions. Aerial parts of the plant are valued as stimulant, expectorant, diaphoretic and insecticide. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites (Chopra *et al.,* 1956; Kirtikar & Basu, 1999; Batish *et al*., 2007; Alagesaboopathi, 2009; Kunwar *et al*., 2010; Sutha *et al*., 2010) [5, 14, 4, 1, 16, 34].
The world health organization (WHO) [40] recommended that now a day's, use of plant compounds in the pharmaceutical industries has gradually increased worldwide. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time (WHO, 1989) [39].
In the present study the preliminary phytochemical screening and HPTLC fingerprinting analysis has been done to identify the chemical compounds and performed which may be used as markers for quality evaluation and standardization of the drug.

About study area
Dhar dist. is situated in the south-western part of Madhya Pradesh. The dist. comprises Badnawar, Dhar, Dharampuri, Kukshi and Mandu tehsils. District is situated between latitude $22^{0}1^{\prime\prime}14^{\prime\prime}$ to $23^{0}08^{\prime\prime}49^{\prime\prime}$ North and longitude $74^{0}28^{\prime\prime}15^{\prime\prime}$ to $75^{o}42^{\prime\prime}43^{\prime\prime}$ East. It is bounded by the dist. of Ratlam to the north, Ujjain to the northeast, Indore to the east, Khargone (West Nimar)

to the southeast, Barwani to the south, and Jhabua to the west. It is part of the Indore Division of Madhya Pradesh. The total geographical area of the district is 8,153 km^2, of which forest area occupy 117000 hectares. The recorded forest area of the dist. is 137 km^2 moderately dense forest and 597 km^2 open forest.

Methodology

Plant material

Plants were collected from different localities of Dhar dist. (M.P.) India. The plant species were authenticated by Dr. C.M. Solanki (Retd. Prof. of Botany) and herbarium specimen were preserved at Govt. College, Manawar.

Extraction

The different parts (Stem, leaves & inflorescence) of the plant *Anisomeles indica* (L.) Kuntze were dried in shade, collected in powder form. The extraction procedure was adopted from Harborne's 1984 [10]. It was carried out by soxhlation techniques by using different solvents according to increasing order of polarity viz, Ethyl acetate, Methanol and distilled water.

Preliminary phytochemistry

In order to determine the presence of alkaloids, glycosides, flavonoids, tannins, terpenoids, steroids, saponins, phenolics, a preliminary phytochemical study (Color reactions) with various plant extracts were performed. (Harborne, 1973; Sofowora, 1993 and Krishnaiah *et al.*, 2009) [9, 32, 15].

Crude quantification of the major phytochemicals

The methods applied for crude quantification of major phytochemicals were adapted from Harborne, 1973 and Krishnaiah *et al*., 2009 [9, 15]. Only alkaloids, Phenolics, flavonoids and Saponins from the plants under study were quantified.

Thin layer chromatography (TLC) analysis

The TLC analysis was done as per standard protocols (Harborne, 1973) [9]. Synthetic TLC plates (Merk) were used for the analysis. The powdered material was extracted in aqueous solution, ethyl acetate and methanol and then loaded on the TLC plate about 1 cm from below.

High performance thin layer chromatography (HPTLC)

The HPTLC analysis of methanolic leaf extracts of sample was done using CAMAG LINOMATE TLC scanner 4. The solvent systems used were- (1) Petroleum ether: Acetone: Formic acid (35:10:05) and (2) Chloroform: Methanol (7.5:2.5) and the concentration of each sample was 5.00 ml. The interpretation of Chromatograms obtained was done in the light of recent standard references and using online library for *Rf* values of phenolic compounds considering the *Rf* values of sample bands obtained.

Observations

Preliminary phytochemical analysis (Qualitative analysis)

Preliminary phytochemical analysis of stem, leaf and inflorescence of *Anisomeles indica* (L.) Kuntze was done.

Table 1: Preliminary Phytochemical analysis of *Anisomeles indica* (L.) Kuntze-stem, leaf and inflorescence powder

Class of compound	Stem			Leaf			Inflorescence		
	Aqueous	Ethyl acetate	Methanol	Aqueous	Ethyl acetate	Methanol	Aqueous	Ethyl acetate	Methanol
Alkaloids	-	++	+	+	++	+	-	++	+
Phenolics	+	+++	++	-	+++	++	+	++	+
Flavonoids	--	+	+	-	++	+	-	-	+
Tannins	-	++	+	-	+++	++	-	+	+
Glycosides	-	+	+++	-	++	+++	-	-	+
Terpenoids	+	+++	++	+	+++	++	+	+	+
Steroids	+	++	+	+	++	+	+	+	+
Saponins	-	-	+	+	+	++	-	-	+

(+ indicates presence in sample, ++ or +++ indicates strong positive in sample & - indicates absence or not detected in sample)

Quantitative analysis of major phytochemicals

The quantitative analysis was done only in methanolic leaf extracts. It was observed that plant has significant level of tested phytochemicals. The average values of the content of respective phytochemical are presented in Table-2.

Table 2: Quantitative analysis of alkaloids, phenolics and flavonoids (mg/g sample)

Sr. No.	Phytochemicals	*Anisomeles indica* (L.) Kuntze
1	Alkaloids	0.83± 0.15
2	Phenolics	1.85± 0.25
3	Flavonoids	0. 90± 0.20

Thin layer chromatography (TLC) analysis

The powdered extract of leaves of *Anisomeles indica* (L.) Kuntze was used to identify the available phytochemicals using Thin Layer Chromatography (TLC). The analytic mode was designed to identify alkaloids, phenolics, flavonoids & saponins using suitable solvent systems and spraying reagents or visualizer.

Table 3: Thin layer chromatography analysis of leaf powder of *Anisomeles indica* (L.) Kuntze.

Class of Compounds	Solvent system	Powdered Extract	Spraying reagent/ Visualizer	Total Bands observed	R_f values
Alkaloids	Methanol: Conc. NH_4OH (200:3)	Aqueous	Dragondraffs reagent	00	--
		Ethyl acetate		00	--
		Methanol		01	0.17
Phenolics	Chloroform: Methanol (27:0.3)	Aqueous	Ferric chloride reagent	00	--
		Ethyl acetate		01	0.62
		Methanol		01	0.28
Flavonoids	Chloroform: Methanol (19:1)	Aqueous	UV-light	01	0.48
		Ethyl acetate		01	0.56
		Methanol		02	0.56, 0.58
Saponins	Chloroform: Glacial acetic acid: Methanol: Water (64:34:12:8)	Aqueous	Iodine vapour	01	0.79
		Ethyl acetate		01	0.95
		Methanol		02	0.63, 0.92

High performance thin layer chromatography (HPTLC)

The HPTLC analysis of methanolic leaf extracts of sample shows the presence of various phytochemicals in both the solvent systems. They are shown in the tables below-

(A) Analysis using solvent system- petroleum ether: Acetone: Formic acid (35:10:05)

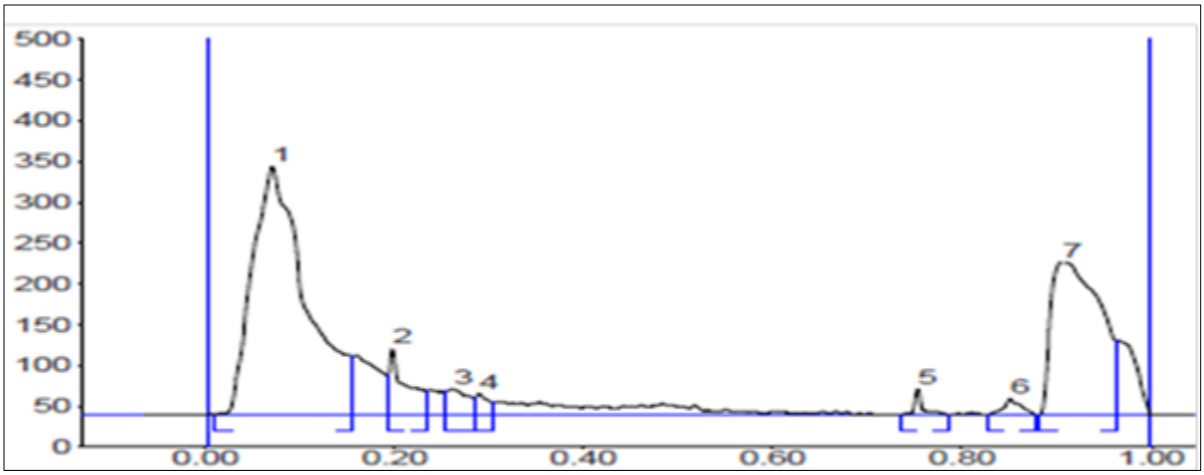

Fig 1: Chromatogram of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 366nm.

Table 4: HPTLC data of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 366nm.

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compound
1	0.01	0.3	0.07	304.2	44.60	0.16	71.4	15399.4	57.09	**1. Chlorogenic acid**
2	0.19	47.4	0.20	80.9	11.86	0.23	28.8	1352.4	5.01	2. Unidentified
3	0.25	28.1	0.26	31.5	4.62	0.28	20.0	689.6	2.56	3. Unidentified
4	0.29	20.8	0.29	26.2	3.84	0.30	14.9	301.5	1.12	**4. Gallic acid**
5	0.74	0.6	0.75	32.3	4.73	0.79	0.7	229.1	0.85	**5. Ferulic acid**
6	0.83	0.4	0.85	19.4	2.84	0.88	0.3	305.9	1.13	6. Unidentified
7	0.88	0.3	0.91	187.6	27.51	0.97	90.6	8694.9	32.24	**7. Apigenin**

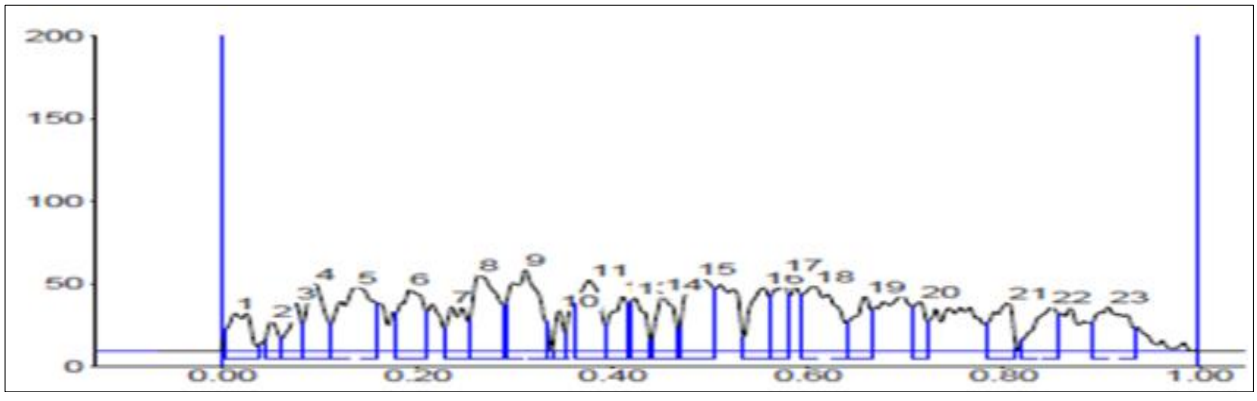

Fig 2: Chromatogram of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 540 nm.

Table 5: HPTLC data of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 540 nm.

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compound
1	0.00	13.0	0.01	22.9	2.93	0.04	2.5	417.8	2.87	1. Unidentified
2	0.04	4.8	0.05	17.6	2.25	0.06	7.2	169.3	1.16	2. Unidentified
3	0.06	7.2	0.08	28.6	3.66	0.08	16.3	291.0	2.00	**3.Chlorogenic acid**
4	0.08	17.7	0.10	40.0	5.14	0.11	15.9	636.3	4.37	4. Unidentified
5	0.11	16.7	0.14	38.3	4.91	0.16	28.4	1149.5	7.90	5. Unidentified
6	0.18	22.7	0.19	36.8	4.73	0.21	23.5	766.6	5.27	6. Unidentified
7	0.23	13.4	0.24	26.3	3.38	0.25	17.6	426.6	2.93	7. Unidentified
8	0.25	18.7	0.26	45.4	5.82	0.29	27.7	1016.2	6.98	8. Unidentified
9	0.29	27.9	0.31	49.1	6.30	0.33	17.9	1211.4	8.32	**9. Gallic acid**
10	0.34	0.0	0.35	23.9	3.06	0.35	10.9	165.0	1.13	10. Unidentified
11	0.36	28.1	0.38	42.4	5.44	0.39	14.9	827.4	5.68	**11. Catechin**
12	0.39	16.1	0.41	32.9	4.22	0.42	28.1	463.4	3.18	12. Unidentified
13	0.42	28.7	0.42	31.9	4.09	0.44	5.7	408.2	2.80	13. Unidentified
14	0.44	6.3	0.45	34.3	4.40	0.47	12.6	520.3	3.57	14. Unidentified
15	0.47	15.0	0.49	43.6	5.59	0.51	37.4	1098.4	7.55	15. Unidentified
16	0.53	8.3	0.56	37.9	4.86	0.56	32.4	640.0	4.40	16. Unidentified
17	0.56	33.4	0.58	45.7	5.86	0.58	33.4	572.9	3.94	17. Unidentified
18	0.59	33.6	0.61	38.7	4.97	0.64	17.0	1156.1	7.94	18. Unidentified
19	0.64	17.4	0.66	32.6	4.19	0.67	24.5	485.8	3.34	**19. Quercetin**
20	0.71	26.5	0.72	29.3	3.76	0.72	17.6	329.5	2.26	**20.Caffeic acid**
21	0.78	16.9	0.81	28.9	3.70	0.81	1.3	516.2	3.55	21. Unidentified
22	0.82	6.5	0.85	26.1	3.35	0.86	21.5	550.0	3.78	22. Unidentified
23	0.89	16.4	0.91	26.5	3.39	0.94	13.4	736.7	5.06	**23. Apigenin**

(B) Analysis using Solvent system- Chloroform: Methanol (7.5: 2.5).

(1) HPTLC analysis of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 366 nm.

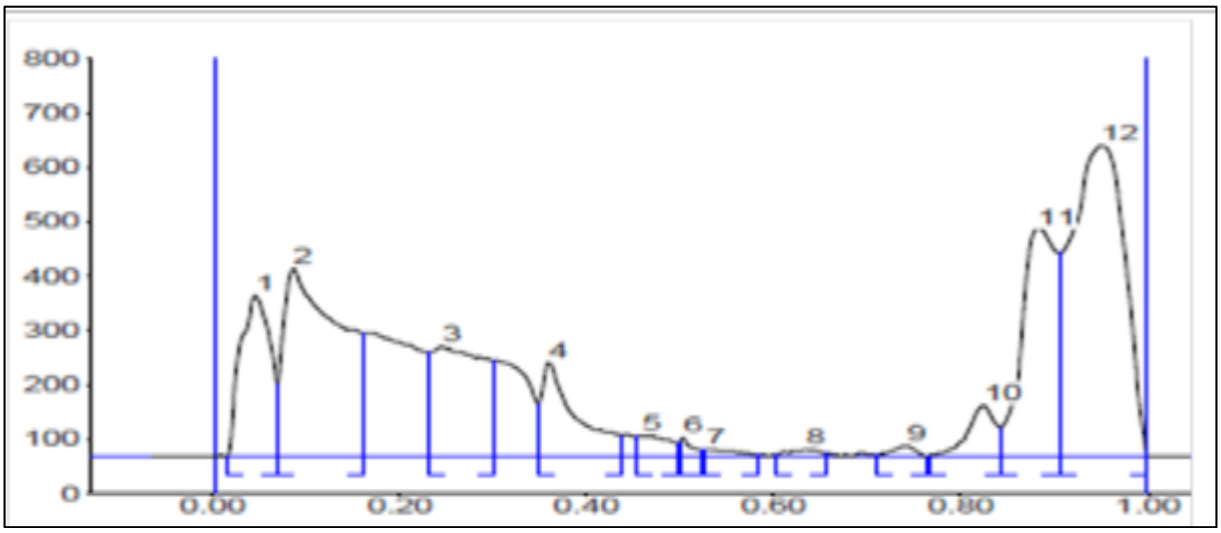

Fig 3: Chromatogram of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 366nm.

Table 6: HPTLC data of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 366 nm.

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compound
1	0.02	0.3	0.05	294.1	13.34	0.07	129.5	8379.1	9.28	1. Unidentified
2	0.07	133.9	0.09	344.6	15.63	0.16	224.8	18643.8	20.64	**2. Chlorogenic acid**
3	0.23	189.7	0.25	200.9	9.11	0.30	175.2	10152.2	11.24	3. Unidentified
4	0.35	96.1	0.36	171.9	7.80	0.44	37.6	5632.6	6.24	**4. Catechin**
5	0.45	35.1	0.46	37.0	1.68	0.50	23.3	1188.3	1.32	5. Unidentified
6	0.50	24.9	0.50	33.6	1.52	0.52	11.2	351.4	0.39	6. Unidentified
7	0.53	11.9	0.53	12.0	0.55	0.59	1.9	336.0	0.37	**7. Myricetin**
8	0.71	1.9	0.64	12.5	0.57	0.66	4.1	320.9	0.36	**8. Quercetin**
9	0.77	1.9	0.74	18.0	0.82	0.76	0.2	379.1	0.42	**9. Ferulic acid**
10	0.77	0.0	0.83	92.7	4.21	0.84	52.6	2387.0	2.64	10. Unidentified
11	0.85	53.1	0.88	417.4	18.93	0.91	373.0	13522.0	14.97	**11. Kaempferol**
12	0.91	373.3	0.95	569.7	25.84	1.00	13.1	29031.7	32.14	**12. Cinnamic acid**

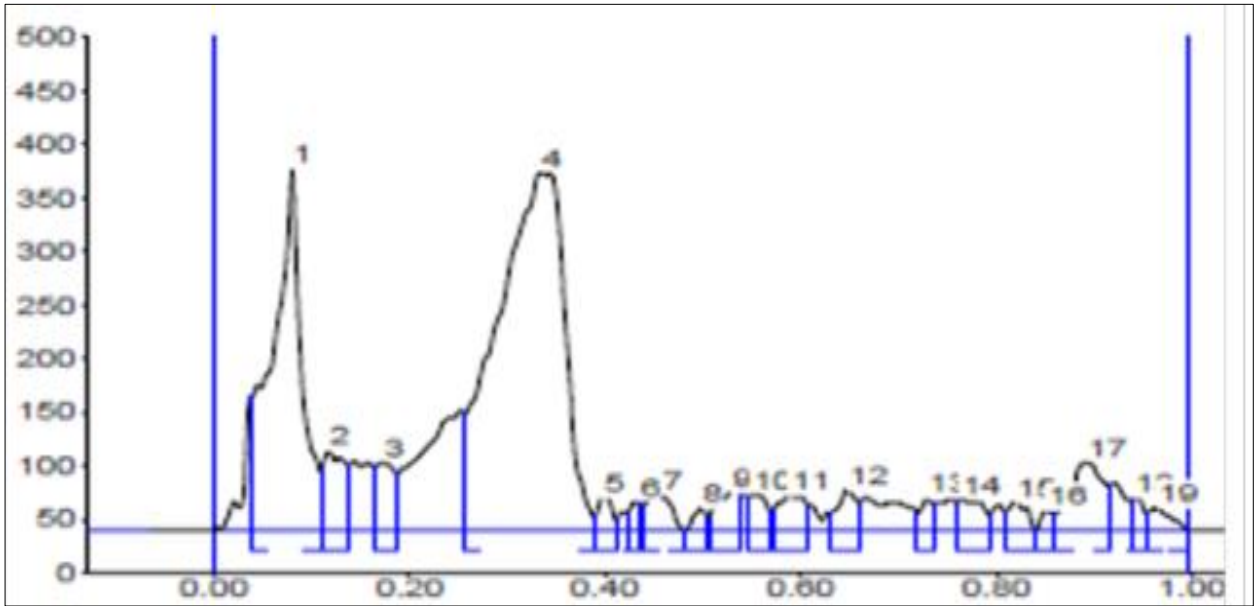

Fig 4: Chromatogram of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 540 nm.

Table 7: Analysis of HPTLC Chromatogram of *Anisomeles indica* (L.) Kuntze methanolic leaf extract at 540 nm

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compound
1	0.04	125.1	0.08	336.9	26.66	0.11	55.4	8883.8	22.09	**1.Chlorogenic acid**
2	0.11	58.3	0.12	73.3	5.80	0.14	60.3	1393.0	3.46	2. Unidentified
3	0.17	60.8	0.17	63.6	5.03	0.19	52.5	1073.8	2.67	3. Unidentified
4	0.26	108.2	0.34	333.8	26.41	0.39	12.1	20360.1	50.64	**4. Gallic acid**
5	0.39	12.6	0.40	30.1	2.38	0.41	8.3	384.2	0.96	5. Unidentified
6	0.42	15.3	0.44	25.7	2.03	0.44	25.1	267.0	0.66	6. Unidentified
7	0.44	23.6	0.46	31.3	2.48	0.48	0.2	706.9	1.76	7. Unidentified
8	0.48	0.1	0.50	20.8	1.65	0.51	11.6	222.3	0.55	8. Unidentified
9	0.51	11.9	0.53	34.8	2.75	0.54	32.1	760.1	1.89	**9. Myricetin**
10	0.55	30.7	0.55	32.6	2.58	0.57	17.8	539.8	1.34	10. Unidentified
11	0.57	18.3	0.59	31.6	2.50	0.61	22.1	813.7	2.02	11. Unidentified
12	0.63	16.1	0.65	36.6	2.90	0.66	25.8	640.9	1.59	**12. Quercetin**
13	0.72	15.8	0.73	29.1	2.30	0.74	25.8	355.8	0.88	**13. Vanillic acid**
14	0.76	26.4	0.77	28.5	2.26	0.79	14.1	663.4	1.65	14. Unidentified
15	0.81	16.8	0.82	25.2	1.99	0.84	1.3	429.3	1.07	15. Unidentified
16	0.84	0.3	0.85	17.7	1.40	0.86	16.5	197.1	0.49	16. Unidentified
17	0.86	16.3	0.90	63.2	5.00	0.92	41.8	1814.6	4.51	**17. Apigenin**
18	0.94	28.1	0.94	29.1	2.30	0.96	15.5	301.1	0.75	**18. Cinnamic acid**
19	0.96	16.1	0.97	19.9	1.58	1.00	1.8	402.0	1.00	19. Unidentified

Results

Preliminary Phytochemical studies of *Anisomeles indica* (L.) Kuntze revealed the presence of secondary metabolites as terpenoids, glycosides, alkaloids & phyto steroids. The presence of these chemical constituents accounts for their usefulness as medicinal plants. Aqueous stem extracts of stem show presence of phenolics, terpenoids, steroids and saponins while rest are absent. Ethyl acetate and methanol extract shows presence of all analyzed phytochemicals. Its aqueous extract of leaf and inflorescence indicates presence of alkaloids, terpenoids, steroids and saponins and phenolics, terpenoids and steroids (Table-1).However, ethyl acetate extract of inflorescence does not show presence of flavonoids, glycosides and saponins. The overall qualitative analysis suggests that the ethyl acetate might be useful in extracting available phytochemicals but the diversity analysis, methanol is most suitable solvent. The quantitative analysis was done only in methanolic leaf extracts. It was observed that plant have significant level of tested phytochemicals. The average values of the content of respective phytochemical are presented in (Table 2). The alkaloid content of the leaves in methanolic extract was found to be (0.83± 0.15) but less than phenolics content was found to be (1.85± 0.25) and flavonoids content was found to be (0. 90± 0.20).

The HPTLC fingerprinting analysis of *Anisomeles indica* (L.) Kuntze is also helpful in identification of drug. The HPTLC analysis showed presence of various phenolic compounds like, gallic acid, quercetin, catechin, ferulic acid, apigenin, vanillic acid etc. Thus, it could be stated that, the antioxidant potential of this plant is due to availability of these phenolic compounds. Earlier reports also showed similar results in different plants of Lamiaceae (Anandan *et al*., 2012; Karhikeyan *et al*., 2013; Annapandian & Rajagopal, 2017) [2, 12, 3].

Discussion

The Ethno- medicinal properties of plant can be attributed to the chemical compounds present in them. The study reveals that 11 phytochemical constituents were isolated from the methanolic leaf extract of the plant. The phytochemicals acting as Gallic acid is used as antioxidants against oxidative stress-mediated disorders (Soobrattee *et al*., 2005) [33]; has cardioprotective (Priscilla *et al*., 2009) [26]; anticancer (Locatelli *et al*., 2013) [19]; anti-inflammatory (Hsiang *et al.* 2013) [11]; and anti-allergic, anti-inflammatory effect (Kim *et al*., 2006) [13]. Ferulic acid is used as insulin-releasing compound (Sirichai *et al.*, 2008) [31]; used on cardiovascular, diabetes mellitus and in skin disease treatment (Mancuso & Santangelo, 2014) [20]; as antioxidant and anti-inflammatory agent (Yan *et al*., 2001) [41].Apigenin used as anti-inflammatory (Wang *et al*., 2014) [37]; Chlorogenic acid used as antibacterial, antioxidant (Meng *et al.*, 2013) [22]; anti-inflammatory & antidiabetic agents (Shimoyama *et al.*, 2013) [30]. Caffeic acid is used as anticancer and antimutagenic agent (Eduardo *et al*., 2007) [7]; has antioxidant & anti-inflammatory effects (Ozturk *et al.*, 2012) [24]. Catechin has hepatoprotective (Tsuchiya, 1999) [36]; and antidiabetic effect (Lee *et al*., 2009) [18]. Quercetin has gastric myorelaxant (Rotondo *et al.*, 2009) [29]; and antioxidant, inhibitory effect (Wattel *et al.*, 2003) [38].Cinnamic acid is used in treatment of chronic venous insufficiency & has anti-inflammatory activity (Rohdewald, 2002) [28]; increasing insulin secretion (Sirichai *et al*., 2005) [31]. Vanillic acid is used as anticoagulant agents (Dhananjaya *et al.*, 2006) [6]. Kaempferol has inhibitory effect on in vitro bone resorption (Wattel *et al*., 2003) [38]; has anti-inflammatory activity (Melo *et al*., 2009) [21]. Myricetin has antioxidant and prooxidant actions (Laughton *et al*., 1989) [17]; antioxidant (Robak *et al*., 1988) [27]; analgesic effect (Tong *et al*., 2009) [35]

Plant based traditional medicine system play an essential role in health care. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folklore remedies. *Anisomeles indica* (L.) Kuntze is a source of medicine in traditional system and also has medicinally active compounds and have various pharmacological effects. Hence this drug holds a good promise for managing variety of ailments.

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