

**PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY STUDY OF DIFFERENT SOLVENT EXTRACT OF DIFFERENT PARTS OF *ANISOMELES INDICA* (L.) KUNTZE. FROM DIBRUGARH, ASSAM**

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**Abstract:** *Anisomeles indica* (L.) Kuntze, a commonly used and easily available medicinal plant which have not studied in detail from Dibrugarh, Assam. The study revealed that tannin, flavonoid, terpenoid, steroid, glycoside, cardiac glycoside, saponin, reducing sugar and phenol are recorded in all the tested parts of the plant. Methanol and ethanol extracts recorded highest phenol and flavonoid content respectively. Water extract of mature leaves recorded highest antioxidant inhibition (86.84±3.17 percent) against DPPH. Acetone extract of all the parts recorded good inhibition against the tested microbial strains than other extracts of the plant. The use of different parts, instead of using whole plant will help in sustainable management of the medicinal plant.

**Key words:** Phytochemical, Antioxidant, Antimicrobial, Sustainable, Management

### **Introduction**

*Anisomeles indica* (L.) Kuntze is an erect aromatic perennial herb found throughout India as weed in waste places, roadsides and forests clearing (Wealth of India, 1985). The plant belongs to the family Lamiaceae having numerous traditional uses. The various parts like-leaves, stem, flowers and essential oils are used in various practices. The plant is used in various folk medicines and have various medicinal properties like-antioxidant, antimicrobial, anticancer, neuroprotective, antimalarial (Ramachandran and Nair, 1981; Sikarwar and Kaushik, 1993; Tiwari, 1995; Chatterjee and Pakrashi, 1997; Sudhakar and Chetty, 1998; Dharmasiri *et al.*, 2000; Wang and Huang, 2005; Batish *et al.*, 2007; Hsieh *et al.*, 2008; Shaik and Balakumar, 2014; Ulhe and Narkhede, 2014). Besides having various medicinal values, the plant is not studied well from the study area. As, the biochemical properties of plant always differ from place to place and habitat to habitat, their study is necessary from various places to know their differences in their activities. The main aim of the work is to perform qualitative and quantitative phytochemical analysis, antioxidant activity and antimicrobial assay of different extracts of different aerial parts of the plant. Use of different solvent extracts on the basis of their polarity level also reveal differences in their activities. The use of different aerial parts besides using whole plant will also help in sustainable management of these medicinal plants.

### **Analysis**

Samples were collected from Dibrugarh district of Assam at their full bloomed stage. The samples were brought to the laboratory of Department of Life Sciences, Dibrugarh University. Different parts were separated and cleaned properly and washed under running water to remove dust and other debris. The materials were air dried at room temperature. The materials were grounded to fine powder using electric grinder. The fine powder was kept in air tight bottles for further analysis. Extracts were prepared in five solvents viz-water, methanol, ethanol, acetone and petroleum ether by cold maceration methods and are known as cold extracts. Hot extract of petroleum ether was prepared in soxhlet apparatus. The dried extracts were dissolved in DMSO (Dimethyl Sulfoxide) to obtain

sample solution at 1mg/ml of concentration. Aqueous extracts were dissolved in distilled water at 1mg/ml of concentration. Qualitative analysis for detection of various phytochemicals were performed using standard laboratory methods. Total phenol content (TPC) and flavonoid content (TFC) of the sample extract was estimated following the method described by Malik and Singh (1980) and Mervat and Hanan (2009) respectively. DPPH and ABTS radical scavenging activity was determined by the method described by Stanojevic *et al.* (2009) and Re *et al.* (1999) respectively. Antimicrobial activity of the bacterial strains was carried out by agar well diffusion method described by Nair *et al.* (2005) using 6mm borer. All the experiments were done in triplicate and mean and SD was calculated and are presented in  $\pm$  form.

## Results and Discussion

The qualitative phytochemical analysis of different parts of the plant are presented in Table 1. Tannins, flavonoids, terpenoids, steroids, glycosides, cardiac glycosides, saponins, reducing sugar and phenols are recorded and phlobatanin, anthraquinone, free-anthraquinone and alkaloids are absent. Yasmin *et al.* (2011); Ulhe and Narkhede (2013); Shaik and Balakumar (2014) also reported these phytochemicals in the plant. The difference in presence and absence of these phytochemicals might be due to the microclimate and soil condition of the area from where the plant is collected. The quantitative estimation for total phenol and flavonoids content are presented in Table 2. Methanol and ethanol extract showed better extraction of phenolic substances than water, acetone and petroleum ether extracts at 1mg/ml of concentration. Methanol extract of young leaves recorded highest ( $34.97 \pm 5.02$  mgCE/gm dry extract) phenol content. The flavonoid content was recorded highest ( $2.88 \pm 0.00$  mgQE/gm dry extract) in ethanol extract of inflorescence. Maqbool *et al.*, (2016) recorded a wide range of phenol and flavonoid content from  $1145.5 \pm 0.593$  to  $198.5 \pm 0.395$  mg/L of GAE and  $3123.7 \pm 0.395$  to  $1154.5 \pm 0.376$  mg/L of Quercetin Equivalent respectively in methanol extract of the leaves. This experiment suggested that the extraction efficiency of various solvents may vary with respect to the phytochemicals present in the plant samples.

Table 3 presents the antioxidant activity of the sample extracts of *A. indica*. Water extracts of young and mature leaves ( $84.03 \pm 0.00$  percent and  $86.84 \pm 3.17$  percent) and methanol extract of young leaves ( $81.95 \pm 0.00$  percent) recorded higher antioxidant activity than other extracts of other parts of the plant against DPPH at 500 $\mu$ l of sample at 1mg/ml of concentration. The results of the present study supported the previous reports from different parts of the country (Yasmin *et al.*, 2011; Baranwal *et al.*, 2012; Kundu *et al.*, 2013; Shaik and Balakumar, 2014; Dixit and Sharma, 2014). Maqbool *et al.*, (2016) studied the antioxidant activities of methanol extract of leaves and recorded significant antioxidant activities in TEAC (Trolox Equivalent Antioxidant Activity) and FRAP (Ferric Reducing Antioxidant Power). The more antioxidant activity recorded by leaves might be due to the more amounts of phytochemicals present in leaves. The results of antimicrobial activity study of the sample extracts of *A. indica* are presented in Table 4. The acetone extract recorded more antibacterial activity against various tested bacterial and fungal strains. Yogesh and Krishnakant, (2011); Naise and Bhadange, (2013); Dixit and Sharma (2014) also revealed antimicrobial activities of solvent extracts of the plant. The difference in antimicrobial activities in different solvent extracts of different parts might be due to the phytochemicals which are responsible for the antimicrobial activity of plant.

**Table 01: Qualitative phytochemical analysis of different parts of *Anisomeles indica* (L.) Kuntze**

Sample	Tannin	Phlobatannin	Flavonoid	Terpenoid	Steroid	Glycoside	Cardiac Glycoside	Saponin	Anthraquinone	Free Anthraquinone	Carotenoid	Alkaloid	Reducing Sugar	Phenol
Young Leaf	+	-	+	+	+	+	+	+	-	-	+	-	+	+
Mature Leaf	+	-	+	+	+	+	+	+	-	-	+	-	+	+
Inflorescence	+	-	+	+	+	+	+	+	-	-	+	-	+	+
Stem	+	-	+	+	+	+	+	+	-	-	-	-	+	+

'+' present, '-' absent

**Table 02: Quantitative estimation for total phenol and total flavonoid content of sample extracts of different parts of *Anisomeles indica* (L.) Kuntze**

Sample (mg/ml) ↓	Total phenol content (mg catechol equivalent/gm dry extract)					Total flavonoid content (mg quercetin equivalent/gm dry extract)				
	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract
Young leaf	2.22 ±0.12	34.97 ±5.02	6.53 ±0.96	5.80 ±0.14	1.30 ±0.01	1.06 ±0.02	1.61 ±0.00	2.62 ±0.13	1.65 ±0.02	0.95 ±0.00
Mature leaf	4.47 ±0.00	12.59 ±2.65	10.22 ±0.78	5.91 ±0.98	1.26 ±0.00	1.31 ±0.00	2.09 ±0.66	1.68 ±0.00	1.54 ±0.00	1.00 ±0.10
Inflorescence	1.57 ±0.00	2.98 ±0.00	13.08 ±0.94	4.09 ±0.00	1.26 ±0.00	0.98 ±0.02	2.00 ±0.01	2.88 ±0.00	2.87 ±0.00	0.89 ±0.00
Stem	1.79 ±0.00	3.77 ±0.52	1.01 ±0.00	1.10 ±0.10	1.05 ±0.00	0.91 ±0.01	1.08 ±0.00	1.05 ±0.03	1.73 ±0.04	0.84 ±0.01

**Table 03: Antioxidant activity study of sample extracts of different parts of *Anisomeles indica* (L.) Kuntze**

Sample (500µl) ↓	DPPH radical scavenging activity (% inhibition in mg/ml)					ABTS radical scavenging activity (% inhibition in mg/ml)				
	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract
Young Leaf	84.03 ±0.00	81.95 ±0.00	75.93 ±0.36	61.52 ±5.48	62.91 ±0.87	74.99 ±0.36	74.27 ±0.00	77.86 ±0.11	78.55 ±0.00	65.41 ±0.49
Mature Leaf	86.84 ±3.17	79.57 ±4.78	79.7 ±1.06	60.32 ±0.00	55.56 ±0.02	68.16 ±2.77	87.56 ±2.01	76.67 ±0.44	78.07 ±1.55	55.98 ±0.11
Inflorescence	72.65 ±0.53	73.78 ±0.00	74.68 ±2.48	72.33 ±0.00	65.47 ±1.30	63.79 ±1.73	63.44 ±0.44	58.07 ±0.36	55.87 ±0.56	51.64 ±0.51
Stem	55.22 ±0.14	39.34 ±1.77	30.07 ±0.37	44.58 ±0.33	63.22 ±1.01	81.93 ±1.24	44.88 ±0.00	45.00 ±0.00	48.99 ±0.23	50.29 ±0.64
Ascorbic acid	90.28 ±0.02					89.00 ±0.00				

**Table 04: Antimicrobial activity study of sample extracts of different parts of *Anisomeles indica* (L.) Kuntze**

Sample	Extracts (mg/ml)	Diameter of Zone of Inhibition (mm)								
		Bacterial strains							Fungal strains	
		<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>S. epidermis</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>P. vulgaris</i>	<i>C. albicans</i>	<i>P. chrysogenum</i>
Young Leaf	Water Extracts	-	-	-	-	-	-	-	10±0	-
	Methanol Extract	8±1	9±1	10.2±2.4	12±1	10±1	-	-	-	-
	Ethanol Extract	8±0	14.5±1.5	-	14±1	12±1	-	-	-	12±2
	Acetone Extract	15.25±1.05	8±1	14±2	-	16±3	12±2	10±0	-	-
	Petroleum Ether Extract	-	8±1	-	-	-	-	8±0	-	8±0
	Hot Petroleum Ether extract	8±1	12±2	8±1	8±1	8±1	10±1	12.6±0	-	-
Mature Leaf	Water Extracts	-	-	-	-	-	-	8±0	-	-
	Methanol Extract	-	8±0	9±1	-	8±1	-	-	-	10±2
	Ethanol Extract	8±1	10±2	10±2	-	12±0	-	-	-	-
	Acetone Extract	8±1	14±1	14±2	10±2	14±2	14±2	-	-	-
	Petroleum Ether Extract	10±2	8±1	-	10±2	8±0	12±1	10±0	10±1	-
	Hot Petroleum Ether extract	-	-	-	-	-	-	-	-	-
Inflorescence	Water Extracts	-	-	-	-	-	-	-	-	-
	Methanol Extract	-	10±0	8±1	8±1	11±1	-	-	-	14±2
	Ethanol Extract	-	8±0	8±1	8±0	8±0	-	-	-	-
	Acetone Extract	8±0	12±0	10±2	-	10.8±2.8	-	-	10±2	26.7±4.3
	Petroleum Ether Extract	-	12±2	-	12±2	14±2	-	12±0	-	-
	Hot Petroleum Ether extract	-	12±1	8±0	10±0	-	-	8±0	-	-
Stem	Water Extracts	-	-	-	8±0	-	-	-	-	-
	Methanol Extract	-	-	-	10±0	-	-	-	-	-
	Ethanol Extract	-	10.4±1.6	-	14±1	-	-	-	-	-
	Acetone Extract	10±0	8±0	8±0	8±0	9±1	-	-	16.4±2.8	-
	Petroleum Ether Extract	-	-	-	-	-	8±0	-	-	8±0
	Hot Petroleum Ether extract	10±2	8±0	8±0	8±0	10±2	10±0	8±0	-	-

Diameter of the cork borer=6mm, '-' indicates no inhibition

### Conclusion

The methanol and ethanol extracts recorded more phenol and flavonoid content respectively. The acetone extract of different parts of the plant recorded good antimicrobial inhibition than other extracts of the plant. The more polar solvent can extract more phytochemicals the non-polar solvent extracts. As all the solvent extracts recorded more or less antioxidant and antimicrobial activity, different parts of a plant can be used instead of using whole plant, which will also help in sustainable management of these traditionally used medicinal plant.

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