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COMPARATIVE PHARMACOGNOSTICAL STANDARDIZATION OF GENUS *ANISOMELES* LINN. R. BR. (LAMIACEAE) SPECIES IN INDIA

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ABSTRACT

Comparative Pharmacognostical studies of *Anisomeles* Linn. R. Br. species (Lamiaceae) in India are carried out. These species are *Anisomeles indica* L. Kuntze (Syn. A. Ovata R. Br.) and *Anisomeles malabarica* L. R. Br. Ex Sims, which are highly found in throughout south (Deccan plateau) and northeastern India. The study conducted with the aim of drawing the Pharmacognostical standards for differentiate the species. Macroscopical and microscopical characters, physio-chemical constants, quantitative microscopy parameters, extractive values, fluorescence analysis of extracts, its reaction after treatment with chemical reagents under visible light and UV light carried out. Preliminary phytochemical screening and TLC fingerprint on the various extracts from aerial part of *Anisomeles* species was also studied. According to the anatomical comparison between two species, the pith structure, sclerenchyma ring of the stems, the cuticle thickness of the leaves, and stomatal distribution on the leaves are the distinguishing features of the species. The determination of these characters will help future researchers in their Phytochemical as well as Pharmacological analysis of this species.

INTRODUCTION: *Anisomeles* Linn. R. Br. one of the largest genera of the Lamiaceae, is a genus of herbs or under-shrubs, distributed in tropical Asia and Australia. With 29 species, India is one of the richest countries in the world in *Anisomeles* diversity. Three species found in India yet, *Anisomeles indica, Anisomeles malabarica* and *Anisomeles heyneana* [1]. Out of these *A. indica* and *A. malabarica* were investigated for their biological activities (Anonymous, 1959). *Anisomeles indica* are used in folk medicine all over the India. It is popularly known as 'Jirnya' in northeastern part of India, where it receives widespread used as folk medicine, predominantly in the treatment of intestinal disorders and intermittent fever. *Anisomeles indica* have anti-microbial, astringent, carminative, ethanolic extract (50%) of the herb showed hypothermic activity and when burnt, acts as a mosquito repellant. The essential oil present in the herb is useful in uterine affections [2, 3], recently the valued plant investigated for its herbaceous activity [4].

Whereas, *Anisomeles malabarica* useful in halitosis, epilepsy, hysteria, amentia, anorexia, dyspepsia, colic, flatulence, intestinal worms, fever arising from teeting children, intermittent fever, gout, swelling and diarrhea [5]. There are many Pharmacognostical studies on the family Lamiaceae in India. However some of them are related with *Anisomeles indica* [6], there has been no investigation of comparison study related to *Anisomeles* species as yet. This study is intended to establish, macroscopical, microscopical, Histological, quantitative evaluation and aerial part of the plant to be used as diagnostic features in the identification, evaluation and monograph preparation of the individual plant [7, 8]. The aim of this paper is not only to present the Pharmacognostical features and to discuss their taxonomic value but also to determine the distinguishing parameter of these two species, so that future researcher whose area of interest is Genus *Anisomeles* Linn. can easily identify them.

MATERIALS AND METHODS:

Plant Collection and Identification: Plant samples of the 2 species were collected from their type localities in October 2009. The identity of the plant material was verified by Dr. (Prof.) D. A. Patil botanist, SSVP'S Science College, Dept. of Botany, Dhule (MS), and Dr. (Prof.) H.B Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India. Voucher specimen of the plant material has been deposited at Institute level (HNSIPER/Herb-05 & 06). The collecting localities of the species were Toranmal (MS) and Dindigul (TN), India.

Plant Material: The powder of aerial part (inflorescence, leaves, and stem) of *Anisomeles indica* (AIA) and *Anisomeles malabarica* (AMA) was prepared by passing through sieve No. 44, and kept in zip pack polytene bags for further use. The proper precautions were taken while storing the powder drug.

Chemicals and Instruments: Compound microscope, simple microscope, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Photomicroscope (OLYMPUS Pvt. Ltd., New Delhi; Model- CH 2OiBIMF) provided Magn US camera was used. Some crystals, starch grains and lignified cell slides were taken under projection microscope. Solvents viz. petroleum ether, chloroform, methanol, ethanol and reagents viz. phloroglucinol, HCl, sudan red III, glycerin, iodine and potassium hydroxide were procured from Loba Chemicals, Mumbai, India.

Macroscopical Examinations: For morphological observations, fresh young leaves (approx. 2-5 cm in length) and herbaceous stem were used. The

macro- morphological features of the plant parts were observed under magnifying lens and simple microscope [9].

Microscopical Examinations: Fresh leaves and herbaceous stem of the 2 species were studied transversely and longitudinally, using surface preparations and sections. The different parts of leaf like lamina and midrib were studied according to the methods of Brain and Turner.[10] For the microscopical studies, cross sections were prepared and stained as per the procedure of K. R. Khandelwal [11]. The different lens of photomicroscope as, OLYMPUS iNEA 5X, 10X/0.2; India, and 100X/1.25 oil India were used for capturing the photographs.

Histochemical Studies: Histochemical analysis was carried out on the specimen, separately, dilute iodine solution, Dragendroffs reagent, dilute ferric chloride solution, Phloroglucinol + HCl (1:1) etc. The reagent treated hard section of the plant tissue was observed and microscope to detect the presence of histochemical components.

Quantitative Evaluations of the Crude Drug: Moisture content of the powdered determined based on the loss of drying method [12]. The ash values (Total ash, acid-insoluble ash and water-soluble ash) were determined, to find out about the physiological state and level of extraneous matter. Extractive values (ether, alcohol and water) were determined according to the official methods prescribed in Ayurvedic Pharmacopoeia [13]. The successive extractive values carry out as per the procedure cited by Dr. C. K. Kokate [14].

Preliminary Phytochemical Investigation: The chemical investigation was carried out by using standard procedures [15]. Total ash of the drug was subjected for testing different inorganic constituents [16, 17]. Fluorescence analysis of powdered leaf was done by standard method of Chase and Pratt [18]. Behavior of AIA and AMA drug powder with various chemicals was carried out as per Rathee *et al* [19].

TLC Finger Print Profile: Thin layer chromatography of the methanolic and ethanolic extract was studied and R_f values were determined [20].

RESULTS AND DISCUSSION:

Macroscopic Examination: The macro-morphological characteristics of the leaves of *Anisomeles indica* identified were acute apex, crenate margin, asymmetric base, reticulate venation and hairy to softly pubescent shape. Leaves surface thick, with dimension 3.8-10 x 5.5-6 cm. Color is green to yellowish green; taste is slightly astringent with characteristic odor. The plant shows covering trichomes more on the lower surface of the leaves. Stem is erect, brown to pinkish black, acutely quadrangular, softly pubescent; internodes 7 to 10 cm long; pith white, fracture powdery & fibrous (**Fig. 1a** and **1b**).

1a

1b

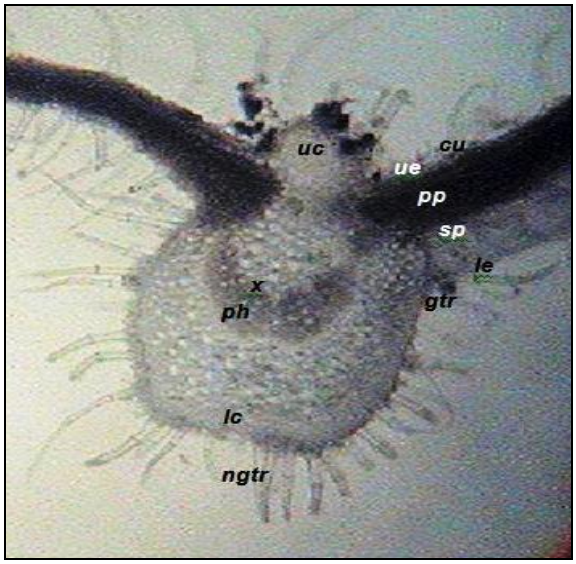
FIG. 1: Aerial parts of *Anisomeles* spp., [1a- *A. indica*; 1b- *A. malabarica*]

Whereas *A. malabarica* leaves simple, opposite, very thick, aromatic, oblong-lanceolate, acute, pale above, white below, crenate-serrate, softly woolly, venation reticulate and slightly winged. Stem is stout, erect, ash grey to blackish brown, densely tomentous, the hairs being woolly soft and white, obtusely quadrangular with a deep furrow on each side; internodes 5 to 10 cm long; pith white, soft, fracture fibrous.

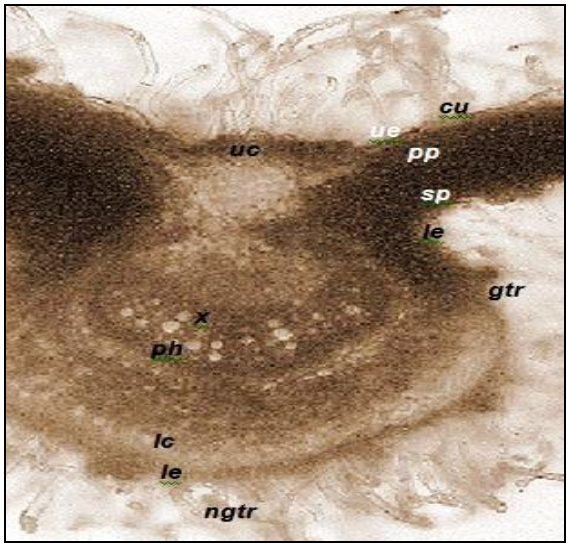
Microscopic Examination: (Fig. 2a & 2b)

Trans-sections of Leaves: They are a dorsiventral (bifacial) leaf. Following tissues are present in lamina and midrib;

Lamina: In TS, the upper and lower epidermises comprise uniseriate, spherical to polygonal cells. Both epidermises are cover with cuticle. The upper cuticle layer is thicker than the lower one in *A. malabarica*. But, the cuticle thickness is approximately the same on both epidermises in *A. indica*. There are covering and non covering trichomes on both epidermises. In *A. malabarica*, the trichomes are fairly dense on the both surfaces. In *A. indica* the density on the upper and lower surfaces is approximately same. Numerous caryophyllaceous or diacytic stomata present in epidermises, the stomatal distribution in upper epidermis of *A.indica* is dense comparatively *A. malabarica.* Mesophyll is traversed by large number of veins and is represented by groups of few spiral vessels.



2a

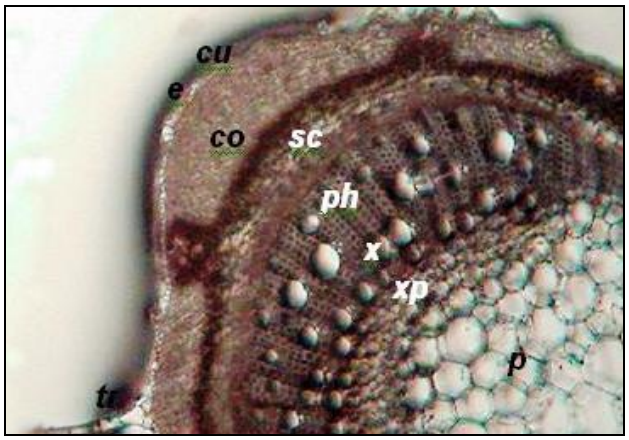


2b

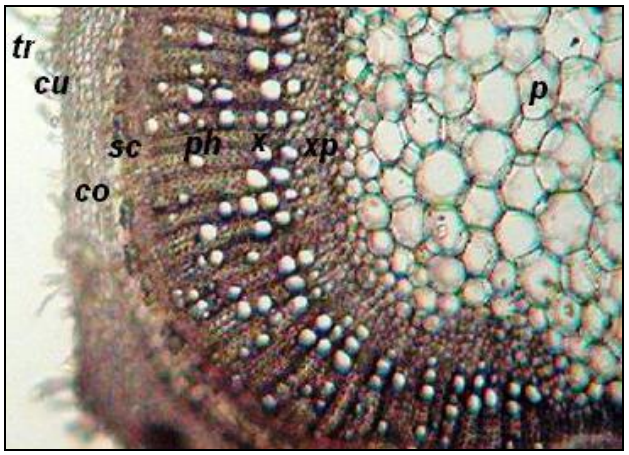
FIG. 2: Trans-Sections of *Anisomeles* SPP. LEAVES (2a- *A. indica*; 2b- *A. malabarica*); [cu: cuticle, ue: upper epidermis, uc: upper collenchyma, pp: palisade parenchyma, sp: spongy parenchyma, x: xylem, ph: phloem, lc: lower collenchyma, le: lower epidermis, gtr: glandular trichomes, ngtr: non-glandular trichomes]

Midrib: *A. indica* midrib shows concavo-convex outline in the basal and middle region which becomes more or less plano convex in the apical region. Where in *A. malabarica* midrib present both surface with different degree of concavity. 4-6 layered collenchymas located below both epidermises, vascular bundles are surrounded by a parenchymatic bundle sheath. Palisade parenchyma are triseriate in *A. indica* & biseriate in *A. malabarica* under the upper epidermis. Spongy parenchyma cells are 3-4 layered under the lower epidermis Phloem cells almost encircling the arc of xylem is embedded with dark brown content and occasionally shows isolated or group of few fibres in *A. malabarica*. Collateral vascular bundle is prominent, occupying the central portion of the midrib. Xylem vessels are covered by xylem fibres in *A. indica*.

Trans-sections of Stems: Schematic TS of stem is quadrangular in shape exhibiting 4 equidistantly placed pubescent ridges, central wide parenchymatous 4 angled pith encircled by a ring of xylem, very narrow phloem and collenchymatous hypodermis. The detailed TS of the stem are quadrangular shaped. The epidermis consists of single layer rectangular cells, and is surrounded by a thin cuticle layer, traversed with few stomata and bearing simple covering multicellular (2-3 cell) and glandular (non-covering) trichomes. The sessile glandular (non-covering) trichomes observed in *A. indica.* Cortex is collenchymatous, 2 to 4 layered but many more; reaching up to 10 beneath the primary rides. Endodermis is distinct. There are lignified sclerenchyma fibers between the cortex and vascular tissue. Sclerenchyma fibers are seen as a continuous ring in *A. indica* and it was uninterrupted in *A. malabarica*. Cambium is indistinguishable. Central wide pith is parenchymatous; it shows presence of simple starch grains and calcium oxalate crystals **(Fig. 3a & 3b)**.



3a



3b

FIG. 3: Trans-Sections of *Anisomeles* SPP. STEMS (3a- *A. indica*; 3b- *A. malabarica*); [cu: cuticle, e: epidermis, tr: trichomes, co: cortex, sc: sclerenchyma, ph: phloem, x: xylem, xp: xylem parenchyma, p: pith.]

Histochemical Studies: The counter idea about presence of phytoconstituents is obtained through this study like phenolic compound in palisade as indicated by brownish black stain on ferric chloride solution treatment (**Table 1**).

Quantitative Evaluations of the Crude Drugs: The moisture content seems to be lower than necessary to support the growth of microbes to bring any change in the composition of the drugs. Physical constant as ash value of the drug gives an idea of the earthy matter or the inorganic composition and other impurities present along

with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs. The results of the quantitative evaluations of the drug powders are given in **Table 2**.

Preliminary Phytochemical Investigation: Revealed the presence of primary and secondary metabolites as carbohydrates, mucilage, Tannins, Terpenoids, Glycosides, Alkaloids and Phytosterols (**Table 3** & **4**). Various inorganic elements present in the plant are Na^+, K^+, Fe^{++}, So_4^-, Cl^- and No_2. The results of fluorescence analysis of the various extracts are presented in **table 5**.

TLC Finger Print Profile: Thin layer chromatography of the methanolic and ethanolic extracts was carried out using Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8) and Toluene: Ethyl acetate: Formic acid (7:3:1) as mobile phase respectively and the R_f were recorded (**Table 6**).

TABLE 1: HISTOCHEMICAL STUDIES OF AIA & AMA

Reagent	Phytoconstituents	Histological zone in leaves	Inference	
			AIA	AMA
Phloroglucinol + HCl (1:1)	Lignin	Vascular bundle	+	+
Aniline Sulphate + H_2SO_4	Lignin	Vascular bundle	+	+
Weak Iodine Solution	Starch	Vascular bundle, lamina	+	-
Sudan III Solution	Oil globules	Vascular bundle	+	+
Aqs. $FeCl_3$ Solution	Phenolics	Pallisade cells	+	+
Dragendroff's reagent	Alkaloid	Lamina	+	-
Libermann-Burchardlt reagent	Steroids	Lamina	-	+
Millon's Reagent	Proteins	Midrib region	-	-

+ Test is positive; - test is negative

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF AIA AND AMA

Parameter	% W/W *	
	AIA	AMA
Ash Values		
Total	07.70±0.351	11.65±0.545
Acid - insoluble	01.53±0.147	02.56±0.160
Water – soluble	06.39±0.284	04.46±0.155
Extractive Values		
Pet. Ether Soluble (40-60^o)	01.25±0.117	04.29±0.176
Ethanol Soluble (95%)	10.45±0.232	11.71±0.761
Water Soluble	16.12±0.675	13.33±1.106
Moisture content	08.67±1.335	09.23±0.656

*mean of three readings ± standard deviation

TABLE 3: SUCCESSIVE EXTRACTIVES (%), FLUORESCENCE ANALYSIS, AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF AIA AND AMA

Solvent Extract	% W/W	Fluorescence (365nm)	Alkaloid	Carbohydrate	Phytosterols	Triterpenoids	Saponins	Glycosides	Phenolics	Proteins	Gums
Petroleum ether (40-60 ^{0}C)			-	-	+	+	-	-	-	-	-
AIA	01.13±0.071	Blackish green	-	-	+	+	-	-	-	-	-
AMA	04.13±0.048	Pale yellow									
Benzene											
AIA	00.93±0.035	Grey	-	-	-	-	-	-	-	-	-
AMA	02.10±0.077	Black	-	-	-	-	-	-	-	-	-
Chloroform											
AIA	00.96±0.057	Grey	+	-	-	-	-	-	-	-	-
AMA	01.53±0.082	Golden yellow	+	-	-	-	-	-	-	-	-
Propanone											
AIA	01.55±0.037	Blackish green	-	-	+	-	-	-	+	-	-
AMA	01.05±0.067	Black	-	-	+	-	-	-	+	-	-
Ethanol (95%)											
AIA	06.84±0.041	Black	-	+	-	-	+	+	+	-	-
AMA	04.95±0.055	Dark red	+	+	+	-	+	+	+	-	-
Water											
AIA	16.59±0.065	Violet	-	+	-	-	+	+	+	-	+
AMA	13.94±0.068	Violet	+	+	-	-	+	+	+	+	+

Results are presented as mean ± standard deviation; + test is positive; - test is negative

TABLE 4: BEHAVIOR OF AIA AND AMA DRUG POWDER WITH VARIOUS CHEMICALS

Reagents	Color/ppt.	Phytoconstituents	Crude Drug	
			AIA	AMA
Conc. H_2SO_4	Red	Steroid	+	+
Aqs. $FeCl_3$ solution	Black	Tannins	+	+
weak Iodine solution	Blue	Starch	+	+
Picric acid solution	Yellow	Alkaloid	+	-
Aqs. $HgCl_3$ solution	Brown	Alkaloid	+	-
Mg-HCl Acid	Pink	Flavonoid	+	+
Aqs. $AgNO_3$ solution	Ppt.	Proteins	+	-
Ammonia Solution	Ppt.	Anthraquinone Glycoside	-	-
5% Aqs. KOH solution	Ppt.	Anthraquinone Glycoside	-	-

+ Test is positive; - test is negative

TABLE 5: FLUORESCENCE ANALYSIS OF AIA & AMA DRUG POWDER

Treatment	Day light		UV (254nm)		UV (365nm)	
	AIA	AMA	AIA	AMA	AIA	AMA
Powder as such	Faint Green	Dull Green	Green	Brown	Black	Black
Powder + 1N NaOH (Aqs.)	Red	Blood Red	Green	Green	Brown	Purple
Powder + 1 N NaOH (Alc.)	Yellow	Green	Green	Green	Purple	Blue
Powder + 1 N HCl	Faint Pink	Pink	Pink	Green	Blue	Blue
Powder + 1 N HNO_3	Green Yellow	Orange	Dull Green	Green*	Dark Blue	Purple
Powder + Ammonia	Reddish Brown	Yellow	Green*	Dull Green	Violet	Purple
Powder + Iodine	Blood Red	Red	Green	Green	Purple	Purple
Powder + $FeCl_3$	Blue	Brown	Green*	Dull Green	Black	Black
Powder + acetic acid	Yellowish Green	Green	Green	Green	Blue	Black
Powder + 50% KOH	Brownish Red	Reddish Brown	Green*	Green*	Purple*	Purple*

* Fluorescent

TABLE 6: TLC FINGERPRINT FOR AIA & AMA

Mobile phase	Extract	Number of spot and their R_f value
1. Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8) Detection- Alc. KOH	**Methanolic** AIA	Four spots; 0.04, 0.05, 0.87 and 0.91
	AMA	Five spots; 0.04, 0.05, 0.53, 0.82 and 0.88
2. Toluene: Ethyl acetate: Formic acid (7:3:1) Detection- 365nm	**Ethanolic** AIA	Six spots; 0.03, 0.07, 0.58, 0.72, 0.86 and 0.94
	AMA	Five spot; 0.04, 0.20, 0.57, 0.71 and 0.91

The pharmacogno-anatomical, physicochemical and TLC study of the aerial part of a plants may be useful in identifying and differentiate *Anisomeles* Linn. R. Br. species either in whole or powder form. These comparative data and parameters have been investigated for *Anisomeles* species to lay down standards which could be useful to find the authenticity of this traditional medicinal plant. These investigations may be useful to supplement existing information with regard to distinguish from substitutes and adulterants. In other words, the pharmacognostic features examined in the present study may serve as tool for differentiating the species for validation of the raw material and for standardization of its formulations at Herbal industrial level in the coming days.

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