ETHNOBOTANY, CHEMISTRY AND PHARMACOLOGY OF AN AROMATIC GENUS ANISOMELES LINN. IN INDIA

K. SRI RAMA MURTHY*1 M. CHANDRASEKHARA REDDY AND T. PULLAIAH2

1School of Conservation Biology and Plant Biotechnology, Department of Botany and Biotechnology Montessori Mahila Kalasala, Vijayawada-520 010, Andhra Pradesh, India
2Department of Botany, Sri Krishnadevaraya University, Anantapur 515 055, Andhra Pradesh, India

ABSTRACT

Anisomeles is an aromatic genus that belongs to the family Lamiaceae. The members of this genus are characterized by the presence of camphor-scented flowers. Members of this genus are widely used in folklore medicine in countries like India to cure various diseases like fever, anti-inflammatory, antiseptic, skin diseases, abdominal pain, psoriasis etc. In the present review traditional uses, phytochemistry and pharmacology of members belongings to this genus are presented. A wide range of chemical constituents were isolated from different species of Anisomeles. Among all the constituents that were isolated from these species anisomelic acid and ovatodiolids are the more abundant bioactive compounds, which show greater biological significance. They show a wide range antimicrobial, antipyretic, anti-inflammatory, antioxidant, cytoprotective, anticancer, insecticidal, and herbicidal properties. These species have strong cytotoxicity and anticancer properties which should be further evaluated. The literature presented in this review will provide a platform for further research on this genus to develop new safe plant based drugs to cure cancer.

KEYWORDS: Anisomeles indica, A. malabarica, A. heyneana, A. salviaefolia, ethnobotany, phytochemistry, pharmacology

INTRODUCTION

The genus Anisomeles is one of the old world Asian-Australian genus that belongs to the family Lamiaceae. The members of this genus are characterized by the presence of glandular hairs on the floral parts. The exact number of species that belong to this genus is not known, however according to the available literature very few species are found in this genus. The members that are found in this genus are Anisomeles heyneana, A. indica, A. malabarica and A. salviaefolia. Among these A. indica and A. malabarica are native to Asia where as A. heyneana and A. salviaefolia are native to the Australia. In A. indica another variety A. indica var. albiflora was reported from India 1. The taxonomic basis of this genus was developed based on the detailed investigation of Asian species. The information regarding this valuable genus Anisomeles that is found in India was presented in this paper.

DISTRIBUTION

Anisomeles indica and A. malabarica are of Asiatic origin and the remaining A. heyneana and A. salviaefolia are of Australian origin. A. indica was distributed in countries like Sri Lanka, China, Indonesia, India, Japan, Philippines and in Australia. Among all the places it occurs, widespread distribution was found in India particularly in Indian Himalayas. The other species of Asiatic origin A. malabarica was limited in its distribution and is found only in south India, Sri Lanka and Australia. Whereas A. heyneana whose origin is not exactly known, but its distributions was found to occur in both Australia and in south India 2.

HABIT AND HABITAT

Members of this genus are perennial shrubby herbs. They may be the tall or under shrubs. These plants occupy various weedy habitats and can grow on both moist and arid soils. A. indica grows in moist sandy loam, lateritic and granitic soils. In India
particularly in Andhra Pradesh, it is restricted to stream edges of irrigated rice fields. *A. malabarica* grows in relatively arid sandy loam soils in India. Where as in Ceylon this species grows particularly in lateritic soils. In Australia sandy flats or coastal dunes are more suitable for this species. In south India *A. heyneana* seems to grow on sandy loam soils and on sandy flats in Australia.\(^2\)

**ANISOMELES INDICA (LINN.) KUNTZE**

*Anisomeles indica* is locally known as in Hindi: Kala bhangra, Gobara, Manipuri: Thoiding angouba; Marathi: Gopali; Malayalam: Chedayan; Telugu: Adabeera; Kannada: Mangamari soppu; Bengali: Gobura, gopali, apang; Konkani: Gopali in India. Where as in other countries it is named as - in Malaysia: Babadotan; Indonesia: Javanese; Philippines: Kabling parang; Thailand: Komko huai; Laos: San nga. The synonyms of *A. indica* are *Nepeta indica*, *Anisomeles ovata* and *Phlomis indica*. *Anisomeles indica* is a camphor-scented large perennial woody shrubby herb, which can reach up to 2 m tall. The stems are quadrangular and sparsely hairy to densely hairy. The leaves are (broad) ovate, measuring 5-12 x 2-7 cm, hairy on both sides and with 5-celled hairs. The petiole is 1.5-4 cm long and covered with soft hairs. The inflorescence is a terminal spike, accompanied by more than 2 lateral spikes. The sepal measures 6 mm x 6.5 mm with the longest teeth 1.7-2 mm long. The fruit is 9-10 mm long where the upper part of the tube and teeth are hairy inside and the petal is up to 11 mm long while the lower lip measures about 8 mm x 3 mm, greenish to whitish, and with dark red lines inside but sometimes purple or blue. The filaments are didymous and 5-6 mm long with the style about 9 mm long. The nutlets are subglobular, measuring 1.2 mm x 1 mm and shiny black.

**ANISOMELES MALABARICA (L.) R.BR.**

*Anisomeles malabarica* are locally known as Malabar catmint in English, Kalpanath, Codhara, Gopali in Hindi, Kannu thumbai in Irula, Karitumbi, Gandubirana Gida in Kannada, Kaktumbo in Konkani, Karintumpa, Karithumba, Karimthumba, Perumtumpa in Malayalam, Gojibha in Marathi, Vaikuntha in Oriya, Mahadronah, Vaikunthah in Sanskrit, Aruvachadachi, peyimarutti in Tamil, where as in Telugu it is named as Mogabheri or Moga-biran. The synonyms of *A. malabarica* are *Anisomeles salviifolia*, *Nepeta malabarica*, *Stachys mauritiana*, *Craniotome mauritiana*, *Nepeta pallida* and *Ajuga fruticosa*. Perennial, semi-shrubby herb; stem to about 2 m high, much branched from base, subquadragular, thickened below to 1.8 cm in diam., densely lanate. Leaves ovate-lanceolate to oblong-lanceolate, 3.6-16 x 1.3-7 cm, narrowed and rounded at base, acute, crenate-serrate, slightly bullate and velvety lanate above, densely so beneath; petioles 0.7-3.5 cm long, stout, lanate. Racemes to 34.5 cm long; verticils close, dense; peduncles densely lanate. Floral leaves 8-10 mm long, densely lanate. Bracts linear, to 5 mm long. Calyx to 9 mm long; tube to 5 mm long, lanate without, glabrous within; lobes lanceolate, to 4 mm long, acuminate. Corolla 1.4-2 cm long; tube to 9 mm long, white, glabrous without; throat pilose towards base of lower lip; Upper lip oblong, 4-6 mm long, obtuse at apex, slightly arched, whitish; lower lip to 1 cm across, coral pink, with 2 white streaks towards base, pilulose without with gland-tipped hairs, the lateral lobes shallowly rounded, the median one larger, broadly orbicular, 2-fid. Style glabrous; branches linear, unequal. Nutlets ovoid, 2.5 x 1.5 mm, trigonous, glabrous, blackish-brown and shining.

**ANISOMELES HEYNEANA BENTH.**

This species is commonly known as western hill catmint, Chandhara in Hindi, Gopali in Marathi and in Sanskrit it is named as Osthaphala *Anisomeles heyneana* is a tall, erect herb, growing to 1-1.5 m high. Slender stems and branches are quadrangular. Oppositely arranged ovate lance-like leaves are 5-12 cm long. Flowers occur in cymes which are 10-30 cm long. Small 2 cm flowers are white, tinged with pink, and 2-lipped. Upper lip is 5 mm. The lower lip is 3-lobed. The flowers resemble cow's earlobes, which gives it its Marathi name. Flowering: October-November.

**ETHNOBOTANY**

*Anisomeles* Linn. Is an ethnomedicinally important aromatic genus and widely used for the treatment of various kinds of ailments in various parts of world. Among all the species of this genus *A. indica* has distinct role in traditional medicine in curing various diseases. \(^3\) *A. indica* possesses aromatic astringent, carminative and tonic properties and is employed as a cure in gastric catarrh and intermittent fevers. Decoction prepared from this species is used to cure convulsions. The essential oil obtained from this plant is useful in urine infection. \(^4\) The leaves of this species are used to cure diseases like inflammation and they have antiseptic and antibiotic properties. \(^5\) The fresh leaves as...
well as greenish parts containing volatile oil used to
cure ailments like stomachache, cough and cold. A. indica is commonly used in numerous
conditions of immune system deficiencies in
treating diseases such as gastrointestinal and liver
disorders. The juice of the leaves is administrated
to children for colic dyspepsia and fever caused by
teething. Inhaling the vapor of the hot infusion
induces copious perspiration. A decoction of this
plant is an excellent fomentation and used
externally as an embrocation in rheumatism
arthritis. Bantar tribes of Bhaudaha, Morang,
Nepal use leaf extract of A. indica to cure
stomachache. Leaves are considered useful to
cure skin diseases, abdominal pain and psoriasis.
Bruised leaves are applied in snake bites. According to Sri Lankan traditional medicine, a
decocion made from stems and leaves of A. indica
possesses analgesic activity. Leaf juice of A. indica is given to fever and dyspepsia. The leaf
paste is applied on snake bites and scorpion sting. It is reported that leaf extract of A. indica is used to
cure fever. Anisomeles malabarica is useful in
halitosis, epilepsy, hysteria, amentia, anorexia,
dyspepsia, colic, flatulence, intestinal worms, fever
arising from teething children, intermittent fever,
gout, swelling, diarrhea and rheumatism. This
species is reported to possess anticancer, allergenic,
antihelmintic, antibacterial, antimalarial and
antiperotic properties. The plant also shows other
properties like antiinflammatory, diuretic, emmenagogue etc. Ethnobotanically, the leaves of
A. malabarica are used against convulsions,
dyspepsia, intermittent fever, colic, boils, tetanus. The plant is also used in curing various
ailments like anticancer, allergenic, antihelmintic,
antiallergic, antianaphylactic, antibacterial,
anticarcinomic, antiedemic, antihistaminic, anti-
inflammatory, antileukemic, antinoiceptive,
antiplasmodial, antisptic and antiperotic properties. A. malabarica was investigated for its
pharmacogostical and various biological
activities. Recently the valued plant was
investigated for its herbicial activity. Mixture of
A malabarica and Alangium salvifolium leaves
made in to paste and applied externally to cure
chronic wounds. Stem paste of A. malabarica
mixed with coconut oil is applied topically over the
wound. Leaves of A. malabarica are used to treat
Eczema.

PHARMACOCOGNOSTIC STUDIES
The proper identification of this valuable species is
very important because they have great medicinal
importance. For this purpose it is very important to
carry out pharmacognostic studies. Pharmacognostic studies of A. malabarica were carried out by
Brindha. The pharmacognostical studies of A. malabarica were also conducted by studying
morphological and anatomical characteristics of the
leaf with the determination of physio chemical
constants. Pharmacognostic studies on leaves of other species A. indica were studied by Ushir et al.,
Comparative Pharmacognostical studies in
between the species of genus Anisomeles that are
found in India were carried out by Ushir. Further
histological and phytochemical characteristics of
leaves of A. indica was reported for proper
identification of this species.

PHYTO-CHEMISTRY
Two acylated flavone glucosides were isolated
from A. indica. The presence of 6-methoxy
flavones in A. indica was also reported by Rao et al., Later the same group reported the presence of
5,6-Dimethoxy-7,3',4 - trihydroxyflavone in A.
indica. A. indica is well documented to possess
phytoconstituents like glycosides, flavonoids,
terpenoids and steroids. The same group latter
reported the occurrence of flavonoid glycosides in
A. indica. Preliminary chemical examination of
A. indica revealed presence of triterpenoids in
entire plant. Whole plant is reported to contain
anisomelic acid (terpenoid) (Fig – 1A),
ovoatodiolide (terpenoid) (Fig – 1B), 4,7-
oxycloanisomelic acid (terpenoid), iso-
ovoatodiolide, β sitosterol stigmasterol, flavones and
apigenin and yields an essential oil. Constituents of the essential oils of A. indica are
found to be α- pinene (Fig – 1D), β- pinene (Fig – 1E), d- limonene, methyl chavicol, d- alpha-
thujene, citral, borneol, 1,8 cineole, α- terpineol
eugenol, azulene, and caryophyllene (Fig – 1C). Complete chemical syntheses of anisomelic acid
were reported. Presence of terpenoids in entire
plant, A. indica was reported. Five new cerebrane-
type diterpenoids with a trans-fused alpha-
methylene-gamma-lactone, a new flavonoid
glucoside, and with some other compounds were
isolated from a methanol extract of A. indica. 14
constituents were isolated from the methanolic
extract of the whole plant of A. indica among them
eone is cerebrane-type diterpenoid, two are
benzenoids, five are flavonoids, and six are phenyl
propanoids. They are 7- methoxy-3,4,5,6-
tetraydroxylavone (pedalitin), apigenin, ovoliodolide, methylgallate, 3,4- dihydroxybenzoic
acid, scutellarein 7-O-d-gluconurone methyl ester,
apigenin 7-O-gluconuride, desrhamnosylverbascoside (calceolarioside),

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cistanoside F, betonyoside A, campneoside II, acteoside, isocateoside and apigenin 7-O-d-(6-O-p-coumaroylglucopyranoside) (terniflorin) respectively. 27 components were resolved from essential oil obtained from aerial parts and roots of A. indica by GC-MS analysis. The 1H- and 13C-NMR spectral studies reported the presence of ovatodiolide, a diterpenoid in A. indica. The presence of stigmasterol, β-sitosterol tetra cosine, tetra coranel β-amyrin in seed along with macrocyclic diterpenes, ovato-diolide and anisomelic acid in the flowers was reported. The ovatodiolide compound showed cytotoxicity effects by causing apoptosis with producing reactive oxygen species and down-regulation of FLICE inhibitory protein leading to cell cycle arrest towards oral squamous cell carcinoma. The major components that present in essential oil of A. indica are eugenol, α-terpeneol (α-terpineol, ), β-pinene, bornyl acetate. In Anisomeles malabarica preliminary phytochemical analysis revealed that the presence of alkaloids, steroids, proteins, flavonoids, saponins, mucilage, carbohydrates, tannins, fats and oils. The ethanolic and diethyl ether extracts of A. malabarica revealed the presence of alkaloids, flavonoids, tannins, saponins, and glycosides. The hexane extract derived from the whole plant of A. malabarica reported to contain beta sitosterol (Fig – 1F). Anisomelic acid is one of the major compounds in A. malabarica which is a cembrane type diterpenoid. Ovatodiolide and Anisomelic acid are two diterpenoid lactones isolated from A. malabarica and A. indica. It has been shown to be cytotoxic to KB cells. A. malabarica has been shown to possess many other compounds viz., anisomelolide (Fig – 1G), malabaric acid (Fig – 1H), 2-acetoxy malabaric acid, anisomethyl acetate, anisomelol and some other flavones glucosides. New phyllocladane diterpene, phyllocladan-16α, 17-dihydroxy-19-oic acid, together with known phyllocladane diterpene, phyllocladan-16α, 19-diol, cembrane diterpene ovatodiolide, sitosteryl-3-O-β-D-glucoside and verbascoside, were isolated from aerial parts of A. heyneana. The structure of compound 1 was elucidated by 1D and 2D NMR analyses which included HSQC, HMBC, and nuclear overhauled effect spectroscopy (NOESY) experiments as well as X-ray crystallography. Compounds 1, 3, 4, and 5 significant biological properties like inhibition of Mycobacterium tuberculosis and 3 was found to exhibit antimycobacterial activity with IC₉₀ 6.53 μg/ml. Compounds 1, 3, and 5, at 100 μg/ml, were also evaluated for inhibition of Thp-1 cell lines, and compounds 1 and 3 showed 59.02% and 96.4% inhibitions respectively.

Figure 1
Chemical constituents of the aromatic Genus Anisomeles Linn.

A. Anisomelic acid
B. Ovatodiolides
C. Caryophyllene
D. Alfa pinene
E. Beta pinene
F. Beta sitosterol
G. Anisomelolide
H. Malabaric Acid

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PHARMACOLOGY
The members of Anisomeles have been prescribed for their antipyretic, carminative, antiarthritic, and analgesic activities. Scientific evidence revealed that a decoction from pre-flowering leaves and stems of A. indica has anti-histaminergic, free-radical scavenging, membrane stabilizing, and cyclooxygenase-I inhibitory activities. Moreover, its aqueous extract was also shown to have analgesic and anti-hyperalgesic activities. The ethanol extract of the whole A. indica exhibited strong anti-Helicobacter pylori activity and it possessed significant anti-inflammatory activity by inhibiting the enhanced production of nitric oxide (NO) radicals, and pro-inflammatory cytokines (TNF- and IL-12) induced by LPS/IFN- on murine peritoneal macrophages.

ANTIMICROBIAL
The potent antimicrobial activity of essential oils obtained from the flowers of A. indica was reported by Yadava and Barsainya. The essential oil from aerial parts and roots of A. indica showed potent microbiological activity on E. coli, P. aeruginosa, B. pumilus and S. aureus with a range of minimum inhibitory concentration values extended from 31.25 to 250 µg/ml. The microbiological activity of the isolated essential oils from A. indica was investigated; it was found that the essential oil show strong activity against pathogenic strains of S. aureus, B. subtilis and K. pneumoniae. In polar studies the maximum zone of inhibition were found against S. aureus and K. pneumoniae. The ethanol, methanol, petroleum ether and aqueous extract from the leaf and boiled leaf of A. malabarica were investigated for antibacterial property. In polar studies the maximum zone of inhibition were found against S. aureus. Whereas in non-polar studies the maximum zone of inhibition were found against p. aeruginosa. The aqueous, petroleumether, acetone and methanolic extracts of A. indica showed potent antibacterial activity against S. citreus, P. vulgaris and K. pneumoniae. In vitro antibacterial activity of leaf extracts of A. malabarica was tested against E. coli, S. aureus, P. mirabilis, P. aeruginosa, K. pneumoniae by Rajarajan et al. They reported that the ethanolic extract exhibited a potent antibacterial activity at 200µg/ml and produced 25mm zone of inhibition against S. aureus whereas Diethyl ether extract produced 30 mm zone of inhibition in the same concentration.

WEED MANAGEMENT
A. indica was assessed for its potential inhibitory activity against Phalaris minor and other weeds of

potent antimicrobial activity. Ovatodioline demonstrated bactericide activity against H. pylori reference, as well as multidrug-resistant strains. On the other side, in vitro H. pylori infection model revealed that ovatodioline inhibited the H. pylori bacteria adhesion and invasion to human gastric epithelial cells. The antibacterial effect of leaf extract against five drug resistant urinary tract pathogenetic isolates viz., S. aureus, Enterococcus faecalis, E. coli, P. aeruginosa and K. pneumoniae was evaluated. They found that gram positive bacteria have shown higher susceptibility to leaf extract than gram negative bacteria. S. aureus and E. coli were inhibited to high extent among gram positive and gram negative bacteria respectively. K. pneumoniae was inhibited to least extent. The Antifungal activity of essential oil obtained from A. indica was evaluated by poisoned food technique and maximum activity against Pithium aphanidermatum (ED 50 51.58 µg/ml) followed by Rhizoctonia bataticola (ED50 72.80 µg/ml) was noticed. The synthesized silver nanoparticles from leaf and boiled leaf extracts of A. malabarica showed prominent growth inhibition on Pseudomonas species. The in vitro antimicrobial activity of A. malabarica was performed by agar well diffusion method against the clinically important multi drug resistant bacterial strains S. aureus, B. subtilis and K. pneumoniae with the concentration of extracts ranging from 25 to 75µL; it was found that the activity was varied with depending upon the concentration. The ethanol, methanol, petroleum ether and aqueous extract from the leaf and boiled leaf of A. malabarica were investigated for antibacterial property. In polar studies the maximum zone of inhibition were found against S. aureus. Whereas in non-polar studies the maximum zone of inhibition were found against p. aeruginosa. The aqueous, petroleumether, acetone and methanolic extracts of A. indica showed potent antibacterial activity against S. citreus, P. vulgaris and K. pneumoniae. In vitro antibacterial activity of leaf extracts of A. malabarica was tested against E. coli, S. aureus, P. mirabilis, P. aeruginosa, K. pneumoniae by Rajarajan et al. They reported that the ethanolic extract exhibited a potent antibacterial activity at 200µg/ml and produced 25mm zone of inhibition against S. aureus whereas Diethyl ether extract produced 30 mm zone of inhibition in the same concentration.
the wheat crop leaf and root powder applied as mulch at 1 and 2 t ha-1 significantly reduced the emergence and growth of *P. minor* and other weeds of wheat crop similar to herbicide, without any negative effect on the wheat growth and yield. At 2 t ha-1 dose of root powder, there was an enhancement in the grain yield of wheat and suppression of weeds under natural field conditions.

**ANTIPLASMODIAL ACTIVITY**

Among 10 experimental plant extracts tested, the leaf methanol extracts of *A. malabarica*, and *Ricinus communis* showed good antileishmanial activity (IC (50)=126±19.70 and 184±39.33 μg/mL), respectively, against promastigotes of *Leishmania donovani*.

**ANTIINFLAMMATORY ACTIVITY**

The members of *Anisomeles* have potent antiinflammatory properties. The anti-inflammatory activity of decoctions of leaves and stems of *A. indica* at pre-flowering and flowering stages and possible toxic effects of the decoctions were evaluated. Decoctions of pre-flowering stage demonstrated a significant and dose-dependent anti-inflammatory effect in all three models, while decoctions during flowering stages did not demonstrate significant anti-inflammatory activity. Decoctions of pre-flowering stage demonstrated a significant dose-dependent antihistamine activity and free radical scavenging activities in addition to the membrane stabilizing and cyclooxygenase-I inhibitory activities. However, decoctions of pre-flowering stage failed to impair significantly the in vitro activity of lipoxygenase. A 30-day treatment with 500 mg/kg of decoction of pre-flowering stage was not liver toxic or renotoxic, and it did not have a significant effect on body weights. It was noticed that the anti-inflammatory activity of decoction of pre-flowering stage is contributed by cyclooxygenase-I inhibition, plasma membrane stabilization, antihistamine and free radical scavenging activities, but not by the inhibition of lipoxygenase. Among different compounds examined for their inhibitory effects on the inflammatory mediator's enhanced production from LPS/IFN-gamma-stimulated macrophages, ovatodiolide exhibited potent inhibition on NO, TNF-alpha and IL-12 enhanced production at a concentration of 5 micro M, followed by pedalitin, scutellarein 7-O-beta-d-glucuronide methyl ester and acteoside at 40 micro M. Furthermore, 2 micro M of ovatodiolide, and 20 micro M of 7-methoxy-3,4,5,6-tetrahydroxyflavone and scutellarein 7-O-B-d-glucuronide methyl ester significantly arrested the cell cycle of Con A-stimulated spleen cells at the G0/G1 stage. Different extracts of *A. indica* significantly inhibited the enhanced production of NO radicals, and pro-inflammatory cytokines (TNF-α, and IL-12) induced by LPS/IFN-γ in a dose-dependent manner. Furthermore, methanolic extracts of leaves and flowers significantly and dose-dependently arrest nitrogen-stimulated spleen cells in G0/G1 stage, in addition to their cell proliferation inhibition against Colon 205, MCF 7 and PC 3 by 94, 82; 98, 71; 82, 98%, respectively, at 200 μg/mL concentration. An *in vitro* *H. pylori* infection model revealed that 95% ethanol extract attenuated *H. pylori*-induced nuclear factor kappa B (NF-κB) activity and interleukin (IL)-8 secretions of gastric epithelial cells. The antiinflammatory activity of the various extracts of *A. malabarica* was evaluated based on their effects on carrageenan-induced paw oedema and cotton pellet granuloma in rats, among the extracts tested ethanol and aqueous extracts of leaves of *A. malabarica* produced significant anti-inflammatory activities in a dose-dependent manner. Presence of *In-vitro* anti-Inflammatory, anti-platelet and anti-arthritic activities to the leaves of *A. malabarica* was reported. Ovatodiolid isolated from *A. indica* inhibited the *H. pylori*-induced inflammatory response by the reduced nuclear factor (NF)-κB activation and interleukin (IL)-8 expressions in *H. pylori*-infected AGS cells. Furthermore, Ovatodiolide attenuated the cytotoxin-associated gene A (CagA) functions by reduced CagA translocation, phosphorylation, and caused hummingbird phenotype of AGS cells.

**ANTI-PYRETIC ACTIVITY**

Anti-pyretic activity of various extracts of *A. malabarica* was evaluated using the brewer's yeast-induced pyrexia in rats. The extracts in dose levels of 50,100 and 200 mg/kg orally were used for anti-pyretic studies. The three extracts have shown a good anti-pyretic effect with all the doses used.

**ANALGESIC AND ANTIHYPERALGESIC ACTIVITY**

Water extracts were made from leaves and stems of both pre flowering (E1) and flowering plants (E2). E1 showed a dose-dependent analgesic effect up to 6 h in rats. Further, the analgesic effect of E1 was not accompanied by toxic effects. This effect was neither gender dependent nor dependent on the stage of the estrous cycle. E1 also showed a dose-dependent antihyperalgesic activity in rats. In contrast, E2 did not show any analgesic effect event
at higher concentration 500 mg/kg. E1 dose-dependently retarded the amplitude of the spontaneous contractions of isolated dioestrous rat uterus. Further, E1 induced a dose dependent plasma membrane stabilization effect on rat erythrocytes. The analgesic and antihyperalgesic effects of E1 are mediated from inhibition of COX-1, thus impairing the synthesis of prostaglandins. A change in chemical contents that accompanies during flowering may be reason for the inability of E2 to demonstrate analgesic effect  

**ANTI CANCER ACTIVITY**

Among all the extracts of *A. indica* tested 95% ethanol extract significantly inhibited lipopolysaccharide (LPS)-induced expression of inducible nitric oxide synthase (iNOS), as well as production of nitric oxide (NO) and tumor necrosis factor α (TNF-α) by macrophages  

The ethanolic extract of *A. malabarica* at a oral dose of 100 mg/kg body weight exhibited a significant protective effect by reduce in liver and serum levels of total protein glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, acid phosphatase, and alkaline phosphatase  

Treatment of Ca9-22 cells with ovatodiolide led to cell cycle arrest at G2/M phase. Ovatodiolide treatment also induced apoptosis, as indicated by caspase activation, DNA fragmentation, and poly (ADP-ribose) polymerase (PARP) cleavage. By using specific inhibitors of caspase-9 and -8, they demonstrated that the ovatodiolide-induced apoptosis is dependent on both intrinsic and extrinsic pathways. The action of ovatodiolide was correlated with a rapid and sustained increase in ROS production and down-regulation of FLICE inhibitory protein (FLIP), which is an endogenous caspase-8 inhibitor and is sensitive to intracellular redox status. Pretreatment of Ca9-22 cells with N-acetylcysteine, a thiol antioxidant, abolished all of ovatodiolide-induced effects, including ROS generation, down-regulation of FLIP, caspase activation, apoptosis as well as cell cycle arrest  

*n*-hexane and chloroform extracts of *A. malabarica* were cytotoxic to the cervical cancer cells in dose- and duration-dependent manner. The cells that responded to the treatments revealed typical apoptotic features. Early features of apoptosis, phosphatidyl serine translocation and loss of mitochondrial transmembrane potential, were noticed in the treated cells and comet assay revealed DNA damage. In the FACS analysis, the cells accumulated in the sub-G0/G1 phase of the cell cycle, except in *n*-hexane and chloroform extract–treated SiHa cells at 24 h, which showed arrest in S- and G2/M phases  

Anisomelic acid (AA) was tested for its cytotoxicity and apoptosis-inducing potential in breast and cervical cancer cells. The MTT assay for cell viability indicated that AA is cytotoxic to all types of cell lines tested in a dose- and duration-dependent manner. Acridine Orange and Ethidium Bromide (AO & EB) and Hoechst 33258 staining of AA-treated cells revealed typical apoptotic morphology such as condensed chromatin and formation of apoptotic bodies. The comet assay revealed DNA strand break(s), indicating that AA induces DNA damage which culminates in apoptosis  

The antioxidant capacities of *A. indica* methanol extract increased in a dose-dependent pattern. The ovatodiolide purified from the extract of *A. indica* inhibited melanogenesis in B16F10 cells. It was noticed by observing the inhibited mushroom tyrosinase activity (IC (50) = 0.253 mM), the compound also effectively suppressed intracellular tyrosinase activity (IC (50) = 0.469 mM) and decreased the amount of melanin (IC (50) = 0.435 mM) in a dose-dependent manner in B16F10 cells  

The effect of *A. malabarica* whole plants methanol extract (AMME) has been studied on cellular redox status during hamster buccal pouch carcinogenesis. Administration of AMME to DMBA - painted hamsters reduced the incidence of SCC and mean tumour burden in addition to preneoplastic lesions. In the buccal pouch, AMME reversed the susceptibility to lipid peroxidation while simultaneously increasing GSH-dependent antioxidant enzyme activities, whereas in the liver and erythrocytes, the extent of lipid peroxidation was reduced with elevation of antioxidants. Thus, modified oxidant status together with antioxidant adequacy in the target organ as well as in the liver and erythrocytes induced by AMME may significantly reduce cell proliferation and block tumour development in the HBP. AMME has been shown to prevent the increase in lipid peroxidation and protect against oxidative DNA damage by improving antioxidant defenses. Among the doses used in the present study, the medium dose and higher dose of AMME (250 mg/kg bw and 500 mg/kg bw) were found to be more effective in inhibiting HBP carcinogenesis compared to low dose. They also reported that the protective role of AMME against HBP carcinogenesis may be related to the antioxidant and anti proliferative properties of phytochemicals such as flavonoids present in the plant  

Hsu et al. reported that *A. indica* hexane extract (AIE) can induce cellular death in FaDu human pharynx squamous cancer cells by apoptosis. They found that AIE significantly inhibited migration and invasion of FaDu cells in a
The members of the genus *Anisomeles* have potent antioxidant properties. Among different plants tested for antioxidant properties, *Cassia occidentalis*, *Clitoria ternatea*, *Triandhema decandra*, *Capparis zeylanica*, *Anisomeles malabarica* and *Plumbago zeylanica* exhibited strong antioxidant characteristics, when compared to other plants. The ethyl-cetate extract of leaves from *A. malabarica* was tested for antioxidant property by using Free radical scavenging assays such as hydroxyl, superoxide anion radicals, 2,2-diphenyl-1-picryl hydrayl (DPPH) and 2,2'-azinobis-(3-ethyl-enzothiazoline-6-sulfonic acid) (ABTS) radical scavenging. They found that the extract effectively scavenged hydroxyl and superoxide anion, DPPH and ABTS radicals. All the concentrations of leaf extract have prominent free radical scavenging and antioxidant power and the preventive effects were in a dose-dependent manner. The methanolic extract of whole plant of *A. malabarica* contains significant quantities of the antioxidant principles compared to the other solvent extracts. The antioxidant capacities of *A. indica* methanol extract was evaluated by using DPPH assay, ABTS radical scavenging, assay, reducing capacity and metal ion chelating capacity and found that the antioxidant capacities of *A. indica* methanol extract increased in a dose-dependent pattern. Methanolic extract of *A. indica* shows the better free radical scavenging property when compared to aqueous extract. The antioxidant activity of whole plant (*A. malabarica*) was assessed by hydroxyl radical scavenging activity, FRAP assay, nitric oxide radical scavenging activities. The significant free radical scavenging activity was found in methanolic extract than that of standard. The radical scavenging activity of the extract was increased with increasing concentration. Prominent *in vitro* antioxidant potential of ethanolic extract of *A. malabarica* was reported. Significant antioxidant activity of the essential oil obtained from *A. indica* was reported. *In vitro* antioxidant assay by DPPH free radical scavenging activity and reducing ability with leaf extracts of *A. malabarica* was evaluated. It was found that methanol and hexane extracts showed good potency of antioxidant property. Comparative study of these crude extracts revealed that methanol extract was more effective than hexane. Methanol and hexane leaf extracts of *A. malabarica* showed good potency of antioxidant activity. Comparative study of these crude extracts revealed that methanol extract was more effective than

**ANTIOXIDANT ACTIVITY**

Western blotting analysis revealed that AIE treatments inhibited the expression of matrix metalloproteinase-9 (MMP-9) and MMP-2 proteins dose-dependently. Moreover, according to enzyme-linked immunosorbent assay (ELISA) and gelatin zymographic assay. The expression of MMP-9 and MMP-2, both proteins declined significantly with an increasing AIE dose. AIE can inhibit the migration and invasion of FaDu cells by suppressing the expression of MMP-9 and MMP-2. AIE also exhibited potent cytotoxicity toward FaDu cells in a time-dependent manner (IC50 = 60.1 μg/ml for 24 h treatment and 29.7 μg/ml for 72 h). In comparison, AIE displayed lower sensitivity to normal lung fibroblast MRC-5 cells (IC50 = 102.7 μg/ml for 24 h treatment). They also observed typical morphological changes of apoptosis, such as cell shrinkage, rounding, apoptotic vacuoles and forming majority of the floating cells through microscopically. Cytometric analysis revealed that FaDu cells were arrested at the G2/M phase. Moreover, AIE induced FaDu cell death mainly via the apoptosis pathway and partly via the necrosis pathway. The extract induced FaDu cell apoptosis by down-regulating Bcl-2 and Bcl-xL protein expression, up-regulating Bax and Bak protein expression, and activating caspase-9 and caspase-3. The anti-metastatic potential of *A. indica* aqueous extract (AI) and its isolated compounds apigenin, ovatodiolide, β-sitosterol and acteoside in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced human breast adenocarcinoma MCF-7 cells was evaluated. Among the test agents, crude extract AI and pure compound apigenin potently suppressed the TPA-induced MCF-7 cells migration and invasion. In addition, AI and apigenin time- and dose-dependently down regulated the matrix metalloproteinase (MMP)-9 enzymatic activities and its mRNA expression. Furthermore, AI and apigenin also down regulated the nuclear factor (NF)-κB subunit p65, and activator protein (AP)-1 subunit c-Fos proteins expression in nucleus and transcriptional activity of NF-κB and AP-1. The anti-rheumatic and immunomodulatory properties of the methanolic extract of the plant *A. malabarica* was evaluated. The three extracts i.e., aerial parts, leaves and roots inhibited TNF-α production in 1 μg/mL concentration. 38.75% inhibition of TNF-α was noticed at 200 μg extracts of the aerial parts of the plant followed by 17.64 and 14.94% by the roots and leaves respectively.
hexane. Methanol extract showed very good antiradical activity and reductive ability.  

**ANTI-EPILEPTIC AND ANTICONVULSANT ACTIVITY**  
The anticonvulsant potential of chloroform, ethyl acetate and methanol extracts of leaves of *A. malabarica* against pentylenetetrazole (PTZ) and maximal electroshock (MES) induced convulsions was tested. All the three extracts were administered (i.e. 100, 200, 400 mg/kg, p.o.) for 7 days and at the end of the treatment convulsions were induced experimentally. High doses (400 mg/kg, p.o.) of chloroform and ethyl acetate extracts both significantly decreased the extent of MES- and PTZ-induced convulsions. On the other hand, ethyl acetate extract at lowest and medium selected doses (i.e. 100 mg/kg, p.o. and 200 mg/kg, p.o., respectively, for 7 days) had also significantly attenuated PTZ-induced convulsions. However, methanol extract at any of the doses used (i.e. 100, 200 and 400 mg/kg, p.o.) did not show any significant effect on PTZ- and MES-induced convulsions. Single dose pretreatment with total flavonoids fraction of *A. malabarica* (25 and 50 mg/kg, i.p.) has found to be effective against both maximal electroshock and pentylenetetrazole - convulsions, but associated with a marked decrease in locomotor activity and motor activity performance (i.e., neurotoxic effects), similar to that of diazepam treatment. Interestingly, chronic treatment with total flavonoids fraction at lower doses (6.25 and 12.5 mg/kg, i.p., 1 week) has also produced significant antiepileptic activity, but without causing neurotoxic effects. Pretreatment with ethyl acetate extract of *A. indica* at concentrations of 200, 400 mg/kg, po, for 1 week showed significant antiepileptic activity against pentylenetetrazole induced convulsions. Isolated flavonoid fraction showed more potent antiepileptic activity as compared to ethyl acetate extract, without any neurotoxic effect.  

**LARVICIDAL ACTIVITY**  
The acetone, chloroform, ethyl acetate, hexane, and methanol dried leaf, flower, and seed extracts of *Achyranthes aspera*, *Anisomeles malabarica*, *Gloriosa superba*, *Psidium guajava*, *Ricinus communis* and *Solanum torvum* were tested against the larvae of cattle tick *Rhipicephalus microplus*, sheep internal parasite *Paramphistomum cervi* at 2,000 ppm and fourth instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* at 1,000 ppm. All plant extracts showed moderate effects after 24 h of exposure; however, the highest parasite mortality was found in the leaf ethyl acetate extract of *A. aspera*, leaf methanol extract of *A. malabarica*, flower methanol extract of *G. superba* and leaf methanol extract of *R. communis* were potent against the larvae of *R. microplus* (LC$_{50}$ = 265.33, 95.97, 153.73 and 181.49 ppm; LC$_{90}$ = 1,130.18, 393.88, 1,794.25, and 1,829.94 ppm); leaf acetone and chloroform of *A. malabarica*, flower acetone extract of *G. superba*, leaf chloroform extract of *A. malabarica*, flower methanol of *G. superba* and leaf methanol extract of *R. communis* were active against the larvae of *A. subpictus* (LC$_{50}$ = 48.83, 135.36, 106.77, and 102.71 ppm; LC$_{90}$ = 225.36, 527.24, 471.90, and 483.04 ppm); and leaf ethyl acetate extract of *A. aspera*, leaf chloroform extract of *A. malabarica*, flower methanol extract of *G. superba*, and leaf methanol extract of *R. communis* were effective against the larvae of *C. tritaeniorhynchus* (LC$_{50}$ = 68.27, 95.98, 59.51, and 93.94 ppm; LC$_{90}$ = 306.88, 393.83, 278.99, and 413.27 ppm), respectively. The larvicidal activity of two indigenous plants, *A. malabarica* and *Phyllanthus emblica* against the larvae of economically important malarial vector, *Anopheles stephensi* under laboratory condition was evaluated and found that the methanol extract of both the plants showed significant larvicidal activity and also combined extracts (synergistic) exhibit highest larval mortality. The egg hatching and larvicidal effect of indigenous plant extracts were investigated against the sheep parasite, *Haemonchus contortus*. The efficacy of leaf, bark, and seed ethyl acetate, acetone and methanol extracts of *Andrographis paniculata*, *Anisomeles malabarica*, *Annona squamosa*, *Datura metel* and *Solanum torvum* were tested against *H. contortus* using egg hatch assay (EHA) and larval development assay (LDA). All plant extracts showed moderate parasitic effects after 48 and exposure for egg hatching and LDA, respectively. Dharmasiri et al., studied the gastroprotective effects of *Anisomeles indica*.  

**CONCLUSION**  
Members of the genus *Anisomeles* are widely used in traditional system of medicine from several decades in many countries like India. Now the scientific research on these species suggests that these plants have pharmaceutical importance.
Presence of chemicals which support various biological activities are presented in this paper providing evidence for use of these plants in developing new drugs for curing various ailments. There is a need to standardize methods for isolation of active principles in pure forms to produce new standardized drugs. The active principles which are present in these plants showing strong antioxidant, antiinflammatory and anticancer properties. In these days it is very essential to develop natural drugs against cancer. Development of natural antioxidants from these plants help the food and pharmaceutical industries.

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