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# *Acorus calamus*: Scientific Validation of Ayurvedic Tradition from Natural Resources

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## Abstract

*Acorus calamus* Linn. (Araceae), commonly known as “sweet flag” or “calamus”, is a semiaquatic, perennial, aromatic herb with creeping rhizomes. The plant is found in the northern temperate and subtropical regions of Asia, North America, and Europe. The plant exhibits polyploidy. Many ethnomedicinal and ethnobotanical uses have been ascribed to the rhizomes of the plant. *A. calamus* Linn. (AC) has been used as traditional Chinese and Indian prescriptions for its beneficial effects on memory disorder, learning performance, lipid peroxide content, and anti-aging and anticholinergic activity. Moreover, pharmacological studies have revealed that *Acorus* rhizome and its constituents, particularly  $\alpha$ - and  $\beta$ -asarone, possess a wide range of pharmacological activities such as sedative, CNS depressant, behavior modifying, anticonvulsant, acetylcholinesterase inhibitory, memory enhancing, anti-inflammatory, antioxidant, antispasmodic, cardiovascular, hypolipidemic, immunosuppressive, cytoprotective, antidiarrheal, antimicrobial, anthelmintic, insecticidal, adulticidal, diuretic, antioxidant, genotoxic, and mutagenic activities. This review is an effort to explore the different phytoconstituents and pharmacological activities of *Acorus calamus*.

**Keywords:** *Acorus calamus*, anticonvulsant activity,  $\alpha$ -asarone,  $\beta$ -asarone, essential oil, rhizomes, sweet flag.

## Introduction

*Acorus calamus* (AC) Linn. (Araceae), commonly known as “sweet flag” or “calamus”, is a species of semiaquatic, perennial, aromatic herb with creeping rhizomes. The plant is found in the northern temperate and subtropical

regions of Asia, North America, and Europe. The plant prefers swampy or marshy habitats. The plant exhibits polyploidy and three karyotypes. The diploid karyotype ( $2n = 24$ ) grows in North America and in parts of Asia (Siberia); the triploid karyotype ( $3n = 36$ ) is present in Central Europe and Kashmir, India; the tetraploid karyotype ( $4n = 48$ ) is found in India, East Asia, and Japan. In India, the plant is found growing wild as well as cultivated up to an altitude of 2200 m in the Himalayas. It is plentiful in the marshy tracts of Kashmir, Himachal Pradesh, Manipur, and Naga hills, and is regularly cultivated in Karnataka (Cavazza, 1976).

*Acorus calamus* Linn. (AC) possesses grass-like or sword-shaped, long slender leaves that fan out from a pinkish base and grow up to 1.5 m in length. The midvein is usually off-center. Cut or bruised leaves produce a sweet, tangerine-like scent. The flower stem, or scape, arises from the base of the outer leaves. The slightly curved spadix is crowded with small yellowish-green to brown flowers. The plant bears green, angular, 1 to 3 seeded berries. The seeds are oblong in shape (Anonymous, 2001). The rhizomes of the plant are the most important part. The rhizomes are woody, branched, light-brown or occasionally orange-brown in color, cylindrical to flat with distinct nodes and internodes. The rhizomes possess strong, characteristic, and aromatic odor and are bitter in taste (Anonymous, 2001). The transversely cut surface of the rhizome is cream in color with pinkish tinge and differentiated into narrow cortical and large stelar regions. Microscopically, epidermis has radially elongated cells with heavily thickened walls. The cortical region consists of thin-walled parenchymatous cells in chains, having large intercellular spaces, sheathed collateral vascular bundles, and patches

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Address correspondence to: Dr. Pulok K. Mukherjee, School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India. Tel.: +91-33-24298313; Fax: +91-33-24146046; E-mail: pknatprod@yahoo.co.in

of fibers. The stelar region has single barrel-shaped endodermal cells with abundant starch grains. Endodermal-cells are barrel-shaped and possess abundant starch grains. Large oil cells with yellowish content, cells containing dark-brown oleoresin content, and starch grains are scattered in the ground tissue of both cortex and stele. Solitary polygonal crystals of calcium oxalate are present in each cell of the storied row of cells running parallel to the fibers.

Various therapeutic potentials have been attributed to AC rhizomes in the traditional system of medicine as well as in folklore practices. Various bioactive phytoconstituents and pharmacological activities of the plant have been reported over the years. Hence, this review is directed toward exploring the various pharmacological activities of AC rhizomes as well as the phytoconstituents.

## Medicinal Uses

### Uses ascribed in traditional medicine

In the Ayurvedic system of medicine, the rhizomes of AC are considered to possess aromatic, stimulant, bitter tonic, emetic, expectorant, emmenagogue, aphrodisiac, laxative, diuretic, antispasmodic, carminative, and anthelmintic properties. They are used for the treatment of a host of diseases such as mental ailments like epilepsy, schizophrenia, and memory disorders, chronic diarrhea and dysentery, bronchial catarrh, intermittent fevers, tympanitis, colic, otitis media, cough, asthma, and glandular and abdominal tumors (Kirtikar & Basu, 1987; Anonymous, 2001). They are also used traditionally for flatulent colic and chronic dyspepsia. They are also employed for kidney and liver troubles, rheumatism, and eczema. The skin of the rhizomes is said to be hemostatic. The rhizomes are used in the form of powder, balms, enemas, and pills and also in ghee preparations (Kirtikar & Basu, 1987; Anonymous, 2001).

### Uses described in folklore medicine

The rhizome is used as an emetic (Srivastava et al., 1986) and to kill lice (Megoneitso & Rao, 1983). The leaves are used on wounds to kill worms (Kapur, 1993). The stem is used in cough, cold (Megoneitso & Rao, 1983), and toothache. The rhizome is useful in diseases of nervous system, throat, and diarrheal diseases and as an antitumor agent (Viswanathan, 1995; Jha & Verma, 1996). It is also used for protection against smallpox (Jain, 1989), ringworm (Bajpai et al., 1995), in respiratory and gastrointestinal tract diseases and snakebite (Bist & Badoni, 1990; Singh, 1995; Mohanty et al., 1996), in gout and rheumatism (Jain & Puri, 1994), and in dysmenorrhea (Borthakur & Goswami, 1995). The root is used in intermittent fever, as an antipyretic and antitussive

(Dobriyal et al., 1997). It is also found useful in stomach-ache (Mao, 1993), and root bark is used as an antidote to snakebite (Singh & Prakash, 1994). The tribals in the Garhwal region of the Himalayas take a decoction of the rhizome as a nonalcoholic beverage. The fresh rhizomes are chewed to prevent intoxication from alcohol. The decoction is also given to children for gastroenteritis. The rhizome pieces, are tied around the belly for jaundice (Bist & Badoni, 1990). Natives of Tirumala hills also use the rhizomes for the treatment of dental disorders (Balaji Rao et al., 1996).

## Ethnobotanical Uses Other Than Medicinal Uses

Since antiquity, AC rhizome has been used for medicinal baths, in incense, and for tea. The powdered rhizome is used as an insecticide for the destruction of fleas, bedbugs, moths, lice, and so forth. The rhizomes are used in incense sticks (Vashist & Handa, 1964). It is effective in killing insect pests in stored rice and is considered to be better than chemicals for this purpose as it shows no residual effect. The root is used as an insecticide and for protection from insect attacks (Jain & Suri, 1980; Singh et al., 1996).

### Chemical constituents

A wide variety of chemical constituents have been reported from the rhizomes of AC. The oil of AC rhizomes has been analyzed by various workers for their chemical constituents (Oprean et al., 2001; Raina et al., 2003). The oil was found to contain varying concentrations of  $\alpha$ -asarone (1),  $\beta$ -asarone (2),  $\gamma$ -asarone (3), calamene, calamenenol, calameone (4),  $\alpha$ -pinene (5),  $\beta$ -pinene (6), camphene, *p*-cymene, eugenyl acetate, eugenol (7), isoeugenol (8), methyl isoeugenol (9), calamol, azulene (10), eugenol methyl ether, dipentene (11), methyleugenol, asaronaldehyde (12), terpinolene (13), 1,8-cineole (14), camphor (15),  $\alpha$ -caryophyllene (16), and hydrocarbons (Fig.1) (Nigam et al., 1990; Srivastava et al., 1997; Mukherjee, 2002). The oil also contains fatty acids such as palmitic acid and its ester, heptylic acid, an ester of butyric acid (Chaudhury et al., 1957).

Sharma and Dandiya (1969) first reported the synthesis of asarone from 1,2,4-trimethoxybenzene. Fractionation from the volatile oil by gas chromatography resulted in the isolation of  $\alpha$ -asarone and  $\beta$ -asarone, which are the *trans*- and *cis*-isomers, respectively, of 2,4,5-trimethoxy-1-propenylbenzene (Baxter et al., 1960). Other constituents identified in the rhizome were cyclobutanolignan acoradin, 2,4,5-trimethoxybenzaldehyde, 2,5-dimethoxybenzoquinone, galangin (5,7-dihydroxyflavanol), along with sitosterol and acoramone (17) (Patra & Mitra, 1981).

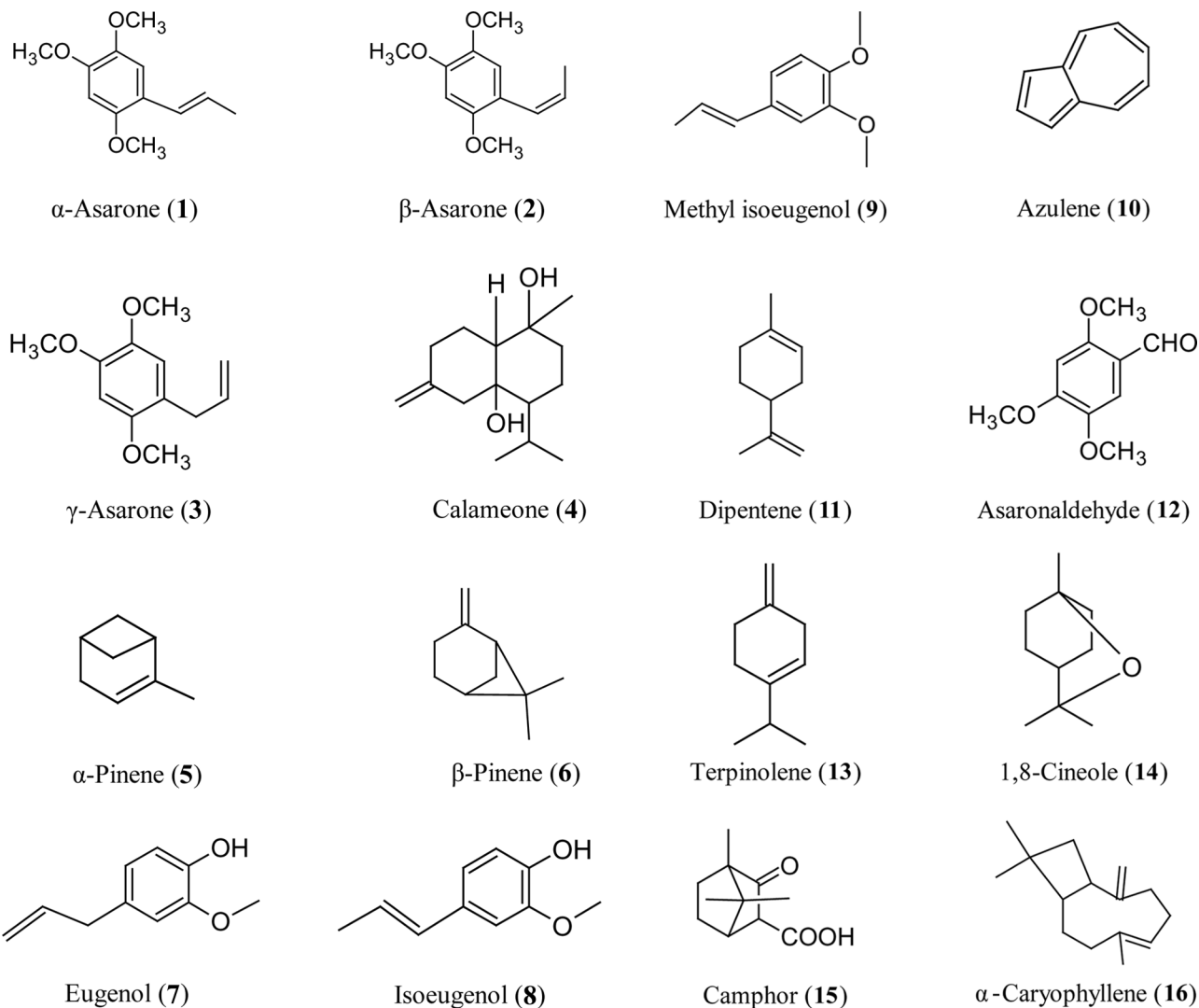


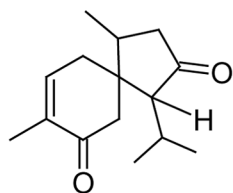
Figure 1. Phytoconstituents of *Acorus calamus* L.

Two phenyl indanes, viz., 2,3-(2,4,5-trimethoxyphenyl)-2-propenal and 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3 (2,4,5-trimethoxyphenyl) indane (Saxena, 1986) and two new triterpenoid saponins, 1 $\beta$ ,2 $\alpha$ ,3 $\beta$ , 19 $\alpha$ -tetrahydroxyurs-12-en-28-oic acid-28-*O*-{( $\beta$ -D-glucopyranosyl (1-2))- $\beta$ -D galactopyranoside and 3- $\beta$ , 22- $\alpha$ -24,29-tetrahydroxyolean-12-en-3-*O*-( $\beta$ -D arabinosyl (1,3))- $\beta$ -D-arabinopyranoside, were characterized in the ethanol extract of the rhizome (Rai et al., 1998). The petroleum ether and benzene fractions of the rhizome were reported to yield thymol, cumin, and phellandrene, while the chloroform fraction yielded a triterpene alcohol and a triterpene acid (Negi & Joshi, 1981). The root was found to contain 13 amino acids of which arginine, lysine, phenylalanine, threonine, and tryptophan were essential amino acids. The other amino acids identified were  $\alpha$ -alanine, asparagine, aspartic acid, glutamic acid, norvaline, proline, and tyrosine (Vashi & Patel, 1987). The ethanol (50%) extract of the rhizome was found to

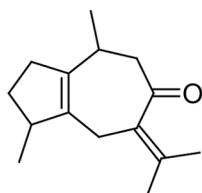
contain glycosides, sterols, and terpenoids (Tewari et al., 1984). Two sesquiterpene ketones of the guaiane-type calamusenone (18) and its isomer were isolated from sweet flag oil. Sesquiterpenes, namely shyobunone (19), isoshyobunone (20), isocalamendiol (21), dehydroxyisocalamendiol, and epishyobunone (22), were isolated from AC, in addition to calameone. The thermal isomerization of shyobunone (23), an elemene type sesquiterpene, resulted in the formation of preisocalamendiol (24), a germacrone-type compound, and acorone (25) (Yamamura et al., 1971; Nawamaki & Kuroyanagi, 1996). The structures of the major chemical constituents of *Acorus* rhizomes are shown in Figure 1.

### Pharmacological Activities

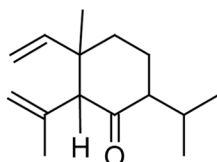
AC has been screened for various pharmacological activities. It has significant CNS actions such as



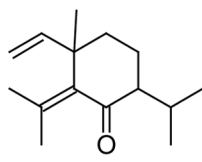
Acoramone (17)



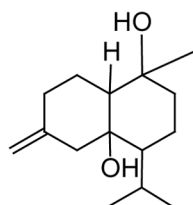
Calamusenone (18)



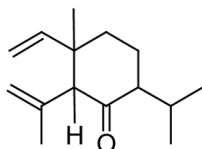
Shyobunone (19)



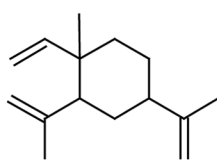
Isoshyobunone (20)



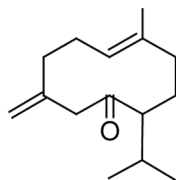
Isocalamendiol (21)



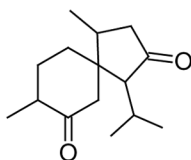
Epishyobunone (22)



Elemene (23)



Preisocalamendiol (24)



Acorone (25)

Figure 1. Continued.

anticonvulsant, sedative, hypnotic, tranquilizing, and memory enhancing, which justifies its use in some CNS diseases in the Ayurvedic system of medicine. It also has effective acetylcholinesterase inhibitory, antispasmodic, antimicrobial, anti-inflammatory, anthelmintic, and insecticidal effects. The various pharmacological activities of AC are shown in Table 1.

### Sedative and hypnotic effect

The volatile oils from AC showed potentiation of the sedative activity of pentobarbitone in mice. The active principle responsible for the activity resided in the hydrocarbon fraction of the oil or in an oxygenated component out of various fractions of the oil (Dandiya et al., 1959a). The steam volatile fractions prolonged the sleeping time in mice with pentobarbital, hexobarbital, and ethanol. The sedative potentiating activity was highest in the volatile fraction of the petroleum ether extract (Dandiya & Cullumbine, 1959). Pretreatment of mice with lysergic acid diethylamide (LSD) partly prevented the hypnotic potentiating action of the volatile oil (Dandiya et al., 1959b). Study on the mechanism of the hypnotic potentiation of barbiturate-induced hypnosis in mice by *acorus* oil showed that the potentiating action was antagonized by lysergic acid diethylamide as well as dibenzylamine hydrochloride. The results proved that the hypnotic potentiating action might be mediated through serotonin and catecholamines (Malhotra et al., 1962). CNS of the essential oil showed sedative-tranquilizing action in rats, mice, cats, dogs, and forced motor activities in mice. The oil inhibited monoamine oxidase at a higher dose level (Dhalla & Bhattacharya, 1968). Essential oil from the rhizome of AC antagonized amphetamine-induced agitational symptoms and also inhibited the conditioned avoidance response in rats (Bhattacharya, 1968).  $\beta$ -Asarone exerts sedative and hypothermic effects in rats. In one study, the alcohol extract of AC showed potent sedative and analgesic properties. The essential oil, crude alcohol, and aqueous extracts showed depressant action in dogs (Bose et al., 1960).

### CNS depressant activities

The effect of the ethanol extract of AC was studied on spontaneous electrical activity and monoamine levels of the brain. In AC-treated rats, electrogram recording revealed there was an increase in  $\alpha$  activity together with an increase in the norepinephrine level in the cerebral cortex, but a decrease in the midbrain and cerebellum. The serotonin level was increased in the cerebral cortex but decreased in the midbrain. Similarly, the dopamine level was increased in the caudate nucleus and midbrain but decreased in the cerebellum. Thus, AC showed its depressive action by changing electrical activity and by differentially altering brain monoamine levels in different brain regions (Hazra & Guha, 2003). Tripathi and Singh (1995) performed a clinical study in 50 cases of depression. AC given for 6 weeks showed reduction in the degree of severity of depression and better rehabilitation and also a significant improvement in assessment based on the rating of symptoms on the Hamilton depression rating scale.

Table 1. Pharmacological activities of the rhizome of *Acorus calamus* L.

Activity	Active compound/extract activity	References
Sedative and hypnotic effect	Essential oil, $\alpha$ -asarone, $\beta$ -asarone, alcohol extract, aqueous extract	Bhattacharya, 1968; Bose et al., 1960; Dandiya et al., 1959a,b; Dandiya & Cullumbine, 1959; Dhalla & Bhattacharya, 1968; Malhotra et al., 1962
CNS depressant activities	Essential oil, $\alpha$ -asarone, $\beta$ -asarone, alcohol extract, aqueous extract	Dandiya & Menon, 1964, 1965; Menon & Dandiya. 1967; Sharma et al., 1961; Tripathi & Singh, 1995; Zanoli et al., 1998; Hazra & Guha, 2003
Anticonvulsant activity	Essential oil, aqueous extract, alcohol extract, $\alpha$ -asarone	Dandiya & Cullumbine, 1959; Dandiya & Menon, 1963; Dandiya & Sharma, 1962; Khare & Sharma, 1982; Madan et al., 1960; Manis et al., 1991; Sharma et al., 1961
Behavioral changes	Essential oil, aqueous extract, alcohol extract, $\alpha$ -asarone	Chak & Sharma, 1965; Dandiya et al., 1958; Dandiya & Cullumbine, 1959; Dandiya & Menon, 1963, 1964; Dandiya & Sharma, 1962; Dasgupta et al., 1977; Dhalla et al., 1961; Malhotra et al., 1961; Shipochliev, 1968; Singh, 1989; Shukla et al., 2002, 2006; Vohora et al., 1990
Acetylcholinesterase inhibitory and memory-enhancing effect	Hydroalcohol extract, methanol extract, essential oil, $\alpha$ -asarone, $\beta$ -asarone	Kirtikar & Basu, 1954; Howes & Houghton, 2003; Nishiyama et al., 1994; Zhang et al., 1994; Houghton et al., 2006a,b; Mukherjee & Wahile, 2006a
Anti-inflammatory	Essential oil	Varde et al., 1988; Vohora et al., 1989
Antioxidant	Ethyl acetate extract	Acuna et al., 2002; Govindarajan et al., 2003; Manikandan et al., 2005; Manikandan & Devi, 2005
Actions on cardiovascular system	Essential oil, $\alpha$ -asarone, $\beta$ -asarone	Arora, 1965; Chopra et al., 1954; Madan et al., 1960; Mangain & Singh, 1994; Moholkar et al., 1975
Hypolipidemic activity	$\alpha$ -Asarone, 50% ethanol extract	Garduno et al., 1997; Parab & Mengi, 2002; Mukherjee, 2003
Actions on respiratory system	Alcohol extract	Bose et al., 1960; Chandra, 1980; Rajasekharan & Srivastava, 1977
Anticancer activity	Lectins, ethanol extract	Bains et al., 2005; Mehrotra et al., 2003
Immunosuppressive activity	Ethanol extract	Mehrotra et al., 2003
Anti-inflammatory	Coconut oil extract	Varde et al., 1988; Vohora et al., 1989
Antulcer and cytoprotective properties	Ethanol extract	Rafatullah et al., 1994

(Continued)

Table 1. Continued.

Activity	Active compound/extract activity	References
Antidiarrheal	Methanol, aqueous extracts	Shoba & Thomas, 2001
Antispasmodic	Essential oil, water-soluble fraction of dried alcohol extract, $\alpha$ -asarone	Agarwal et al., 1956; Bhakuni et al., 1988; Bose et al., 1960; Chopra et al., 1954; Das et al., 1962; Gilani et al., 2006
Antibacterial	Alcohol, ether, acetate buffer, dilute sulphuric acid, hexane, acetone extract, essential oil	Alankarao & Rajendra Prasad, 1981; Chopra et al., 1954; Jain et al., 1974; Joshi & Magar, 1952; Kapil et al., 1983; Valsaraj et al., 1997; Vashi & Patel, 1987; Vijaya & Ananthan, 1994; Vohora et al., 1989
Antifungal	Essential oil, alcohol extract $\beta$ -asarone, asaraldehyde, acoradin	Alankarao & Rajendra Prasad, 1981; Kapil et al., 1983; Saxena et al., 1990; Vashi & Patel, 1987
Antiviral	Alcohol extract, $\beta$ -asarone	Badam, 1995
Anthelmintic	Alcohol extract, essential oil	Chaudhari et al., 1981; Kaleysa Raj, 1974; Sharma et al., 1985; Singh et al., 1991
Insecticidal	Essential oil, $\beta$ -asarone, cold alcohol, ether, hexane, acetone, petroleum ether extracts	Agarwal et al., 1973; Ahmad et al., 1991; Khan, 1986; Koul, 1987; Mathur & Saxena, 1975; Pandey et al., 1977; Saxena et al., 1977; Singh & Mehta, 1998; Saxena & Rohdendorf, 1974; Saxena & Srivastava, 1972; Sharma et al., 1994; Subrahmanyam, 1949; Komalamisra et al., 2005
Piscicidal	Alcohol extract	Virdi, 1982
Adulticidal activity	Methanol extract	Hidayatulfathi et al., 2004
Diuretic	50% ethanol extract	Aswal et al., 1996
Genotoxicity and mutagenicity	$\alpha$ -Asarone, $\beta$ -asarone	Abel, 1987; Goeggelmann & Schimmer, 1983; Jin et al., 1982; Mukherjee & Wahle, 2006b

$\alpha$ -Asarone and  $\beta$ -asarone showed many pharmacodynamic actions similar to some well-established tranquilizers.  $\alpha$ -Asarone and  $\beta$ -asarone significantly enhanced the anesthetic activity of pentobarbitone, hexobarbitol, and ethanol in mice.  $\beta$ -Asarone appeared to be more active (Sharma et al., 1961). The mechanism of tranquilizing action of  $\alpha$ -asarone was also studied.  $\alpha$ -Asarone was not found to cause any change in the noradrenaline content of whole brain of rat, and pretreatment of  $\alpha$ -asarone failed to block the effect of reserpine on the spontaneous motor activity and ptosis of mice, as well as the conditioned avoidance response of trained rats. It was found that the sedative effect of  $\alpha$ -asarone was dependent on the depression of the ergotropic division of the hypothalamus (Menon & Dandiya, 1967).  $\beta$ -Asarone administered in association with a cannabinomimetic drug was shown to potentiate some of the typical behavioral activities induced in animals by cannabinoids (Zanoli et al., 1998).  $\alpha$ -Asarone reduced spontaneous motor activity and caused reduction in anxiety without dulling the perception in rats. It produced a prolonged calming effect in monkeys (Dandiya & Menon, 1964).  $\alpha$ -Asarone also partially antagonized tremorine-induced tremors in mice but was found to be inferior to atropine in this respect. The tranquilizing effect of  $\alpha$ -asarone was responsible for its antagonism to mescaline and amphetamine, while the anticholinergic effect accounted for the partial protection offered to tremorine-treated mice (Dandiya & Menon, 1965).

### Anticonvulsant activity

The steam volatile fractions of rhizome of AC exacerbated tonic seizures provoked by metrazol in rats and also potentiated the action of reserpine in reducing amphetamine toxicity in aggregated mice (Dandiya & Cullumbine, 1959). *Acorus* oil investigated for its antianaleptic activity was used as a saline suspension, given 1 h prior to production of convulsions in adult albino mice. It successfully prevented seizures in maximal electroshock seizures test (Khare & Sharma, 1982).  $\beta$ -Asarone caused generalized convulsion and potentiated metrazol seizures in rats, while  $\alpha$ -asarone showed a tendency to protect against metrazol convulsions and modified electroshocks (Sharma et al., 1961). In a study using electroconvulsions,  $\alpha$ -asarone increased the percentage mortality of animals treated with chlorpromazine but not of those treated with reserpine (Dandiya & Sharma, 1962; Dandiya & Menon, 1963). The aqueous and alcohol extracts were found to reduce the severity of maximum electric shock-induced seizure in rats. Further, the extracts significantly increased the pentylenetetrazole-induced seizure latency (Manis et al., 1991). The essential oil showed a protective effect against electroshock seizures in rats (Madan et al., 1960).

### Behavioral changes

The steam volatile fractions of the rhizome of AC produced a fall in the blood pressure of anesthetized cats, caused hypothermia in mice, and potentiated the action of acetylcholine, histamine, and barium chloride on the isolated gut of guinea pigs (Dandiya & Cullumbine, 1959). The *in vitro* studies of the oil on the respiration of rat brain revealed that it inhibited the oxygen uptake of brain tissues (Dhalla et al., 1961). The oil affects the 5-HT and noradrenaline contents of brain similar to reserpine (Malhotra et al., 1961). Estimation of brain 5-HT content of rat revealed that neither the oil nor its active principles liberated 5-HT. These compounds also did not cause additional decrease of the brain 5-HT content in reserpine-treated animals (Dandiya & Sharma, 1962; Dandiya & Menon, 1963;). The oil also caused hypothermia in mice and, even in low doses, counteracted LSD-induced hyperpyrexia.  $\alpha$ -Asarone failed to cause release of 5-HT from the brain and also prevented the depletion of adrenal ascorbic acid in rats subjected to cold stress (Dandiya & Menon, 1964). The effect of  $\alpha$ -asarone was studied on experimentally induced conflict neurosis in rats, and it was found to increase the number of shocks accepted by the animals (Chak & Sharma, 1965).

In one study,  $\alpha$ -asarone antagonized the hyperactivity and hallucinogenic effect of mescaline in rats and offered protection to aggregated mice treated with *dextro* amphetamine. Intraperitoneal administration of a flavone isolated from the chloroform extract of the rhizome produced profound behavioral changes in the rhesus monkey. This flavone also produced a calming effect on conscious animals, *viz.*, mice, rats, and rabbits. The flavone showed an activity similar to *Cannabis indica* (Dasgupta et al., 1977). A study was conducted with crude powder administered for 60 days to investigate the effects of the plant on escape/avoidance conditioning and general motor activity of rats. It enhanced learning performance, especially in the females, whereas the effect of the plant on general activity was not significant (Singh, 1989). The ethanol extract of the rhizome was screened for CNS activity in mice and rats. The extract exhibited a large number of actions similar to  $\alpha$ -asarone but differed from the latter in several respects including the response to electroshock, apomorphine and isolation-induced aggressive behavior, amphetamine-toxicity in aggregated mice, and behavioral despair syndrome in forced swimming (Vohora et al., 1990). In one study, the essential oil of AC was tested for its effect on motor activity, the position of the eyelids, and the general state. General depression without ataxia was observed (Shipochliev et al., 1968).

In one study, exposure of rats to acrylamide caused hind limb paralysis in 58% of the animals on day 10 and decreased behavioral parameters, namely distance traveled, ambulatory time, stereotypic time, and basal



stereotypic movements compared with the control group. Treatment with the ethanol:water (1:1) extract of the rhizomes of AC increased the GSH content and GST activity in the corpus striatum where as insignificant changes were observed in other parameters. The rats also showed a partial recovery in other behavioral parameters. The levels of GSH content and GST activity increased in the corpus striatum, where as the dopamine receptors decreased compared with the AC-treated rats. The results proved that the neurobehavioral changes produced by acrylamide was prevented with the treatment by ACrhizomes (Shukla et al., 2002). The neuroprotective potential of ethanol:water (1:1) extract of rhizomes of AC was reported in middle cerebral artery occlusion (MCAO)-induced ischemia in rats. Ischemic rats treated with AC exhibited a significant improvement in neurobehavioral performance, *viz.*, rota-rod performance and grid walking. Extract treatment significantly decreased malonaldehyde levels in cortex, increased reduced glutathione levels and SOD activity in both cortex and corpus striatum, and neurologic function score was also improved in the AC-treated rats (Shukla et al., 2006).

#### Acetylcholinesterase-inhibitory and memory-enhancing effect

In Ayurveda, herbal medicines with *rasayana* effects are believed to be restorative, to attain longevity, intelligence, and freedom from age-related disorders. AC is regarded in Ayurvedic medicine as promoting rasayana effects and has been used to treat memory loss (Kirtikar & Basu, 1954; Mukherjee & Wahile, 2006a). AC is used in Ayurvedic medicine on a regular basis for the treatment of memory loss and other mental disorders (Kirtikar & Basu, 1954; Howes & Houghton., 2003). AC extract has also been used as traditional Chinese prescription, and its beneficial effects on memory disorder, on learning performance, lipid peroxide content, and anti-aging effects in senescence have been reported (Nishiyama et al., 1994; Zhang et al., 1994). The *in vitro* acetylcholinesterase (AChE) inhibitory effect of hydro-alcohol extract and essential oil of AC rhizomes was reported based on Ellman's method in 96-well microplates using bovine erythrocytes. The essential oil showed stronger inhibition than the hydro-alcohol extract (Houghton et al., 2006a). Methanol extracts of AC showed significant acetylcholinesterase enzyme inhibition at a concentration 200 µg/mL (Oh et al., 2004). Houghton et al. (2006b) reported the *in vitro* acetylcholinesterase inhibitory effect of  $\beta$ -asarone and  $\alpha$ -asarone.  $\beta$ -Asarone is at least an order of magnitude more active than its *trans*-isomer  $\alpha$ -asarone. The AChE-inhibitory activity of the oil can be ascribed to  $\beta$ -asarone. Because cognitive performance and memory are related to acetylcholine levels, the AChE-inhibitory effect of the plant may account for its traditional use.

#### Anti-inflammatory activity

Varde et al. (1988) studied the anti-inflammatory activity of AC in rats using acute and chronic experimental models. The oral administration of the extract showed inhibition of the carrageenin-induced paw edema, inhibition of cotton pellet granuloma formation, and inhibition of croton oil granuloma pouch inflammatory response. The rhizomes extract showed significant anti-inflammatory effect in acute, chronic, and immunologic models of inflammation (Vohora et al., 1989).

#### Antioxidant activity

The ethyl acetate extract of AC was found to be a potent antioxidant by inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Acuna et al., 2002). *In vitro* antioxidant activity by DPPH scavenging at three different concentrations (0.2, 0.1, and 0.01 g/mL) showed a maximum activity of 86.43% at 0.2 g/mL (Govindarajan et al., 2003). The ethyl acetate and methanol extracts of AC protected most of the changes of oxidative stress status in the rat brain induced by noise-stress. The results of this study proved that during exposure to noisy environment, ROS generation led to increase in corticosterone, LPO, and SOD, but a decrease in CAT, GPx, GSH, protein thiols, and vitamins C and E levels. Both the ethyl acetate and methanol extracts of *Acorus calamus* protected against most of the changes in the rat brain induced by noise-stress (Manikandan et al., 2005).

Manikandan and Devi (2005) demonstrated antioxidant properties of  $\alpha$ -asarone against noise-stress-induced changes in the rat brain. In their study,  $\alpha$ -asarone, one of the active principle components, was administered intraperitoneally one-half hour before the animals were exposed to noise-stress for 30 days; and they investigated whether a 30-day exposure to noise can produce an oxidative stress. The antioxidant was verified by measuring the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), levels of reduced glutathione (GSH), vitamin C, vitamin E, protein thiols and lipid peroxidation (LPO) in different regions of the rat brain.  $\alpha$ -Asarone had an effective protective role by normalizing the increased SOD and LPO, and decreasing CAT, GPx, GSH, vitamins C and E, and protein thiols due to noise exposure.

#### Actions on cardiovascular system

In one study, Chopra et al. (1954) reported that the essential oil of AC caused a moderate depression of blood pressure. The essential oil showed quinidine-like activity in combating auricular fibrillation, auricular flutter, and ventricular arrhythmias after two-stage coronary ligation in dogs. It further resembled quinidine qualitatively in causing a prolongation of conduction time and

refractory period in isolated rabbit auricles (Madan et al., 1960). The essential oil was reported to have negative inotropic and antiarrhythmic properties.  $\alpha$ -Asarone and  $\beta$ -asarone showed cardiac depressant activity on frog and rabbit heart perfusion experiments and also possessed a moderate amount of hypotensive activity in anesthetized dogs (Arora, 1965). The alcohol extract (50%) of AC exhibited a dose-dependent hypotensive action on dog blood pressure (Moholkar et al., 1975). Mangain and Singh (1994) performed a clinical trial on 45 patients of ischemic heart disease. The efficacy of the AC was tested and showed a significant improvement in the AC-treated groups. AC was found to be effective in the improvement of chest pain, dyspnea on effort, reduction of body weight index, improving ECG, decreasing serum cholesterol, decreasing SLDL (serum low-density lipoproteins), and increasing SHDL (serum high-density lipoproteins).

### Hypolipidemic activity

Administration of the ethanol (50%) extract of the rhizomes of AC (100 and 200 mg/kg) as well as saponins (10 mg/kg) isolated from the extract demonstrated significant hypolipidemic activity. On the contrary, the aqueous extract showed hypolipidemic activity only at a dose of 200 mg/kg (Parab & Mengi, 2002).  $\alpha$ -Asarone obtained from *Acorus* rhizomes was also reported to possess hypolipidemic activity in mice (Garduno et al., 1997; Mukherjee, 2003).

### Actions on respiratory system

The alcohol extract of rhizome possesses a bronchodilator effect (Bose et al., 1960). In a clinical trial of patients with moderate to severe bronchial asthma, the fresh rhizome of AC was administered by a chewing method for 2–4 weeks. The AC was found to have antiasthmatic potential without any side effects (Rajasekharan & Srivastava, 1977). In another study, small pieces of the rhizome were administered to asthmatic patients by a chewing method, and significant effect in relieving of bronchospasm was observed without any side effects (Chandra, 1980).

### Anticancer activity

Two lectins purified from the rhizomes of two sweet flag species, namely AC, by affinity chromatography, showed potent antimitogenic activity toward mouse splenocytes and human lymphocytes. Both lectins also significantly inhibited the growth of J774, a murine macrophage cancer cell line and, to a lesser extent, WEHI-279, a B-cell lymphoma (Bains et al., 2005). The *in vitro* anticellular property of the ethanol extract of AC rhizome was evaluated, and it was found that the extract inhibited

proliferation induced by the mitogen phytohemagglutinin. In addition, AC extract inhibited growth of several cell lines of mouse and human origin. It also inhibited production of nitric oxide, interleukin-2, and tumor necrosis factor- $\alpha$  (Mehrotra et al., 2003).

### Immunosuppressive activity

The *in vitro* immunomodulatory property of the ethanol extract of AC rhizome was evaluated. The extract was found to inhibit antigen (purified protein derivative) stimulated human peripheral blood mononuclear cells. Intracytoplasmic interferon- $\gamma$  (IFN- $\gamma$ ) and expression of cell surface markers, CD16 and HLA-DR, on human peripheral blood mononuclear cells were not affected on treatment with AC extract, but CD25 expression was downregulated (Mehrotra et al., 2003).

### Antiulcer and cytoprotective properties

The ethanol extract of AC was studied in rats for its ability to inhibit gastric secretion and to protect gastroduodenal mucosa against the injuries caused by pyloric ligation, indomethacin, reserpine, and cysteamine administration and cytotoxic agents including 80% ethanol, 0.6 M HCl, 0.2 M NaOH, and 25% NaCl. An oral dose of 500 mg/kg of the extract showed significant antisecretory and anti-ulcerogenic activity in rats subjected to pyloric ligation, indomethacin, reserpine, and cysteamine administration. The extract had a highly significant protective effect against cytotoxic agents. These findings support the use of calamus for the treatment of gastropathy in traditional medicine (Rafatullah et al., 1994).

### Antidiarrheal activity

A study was undertaken to evaluate the effect of aqueous and methanol extracts of AC rhizome for its antidiarrheal potential against castor oil-induced diarrhea in mice. The methanol plant extract was more effective than the aqueous plant extract against castor oil-induced diarrhea. The methanol extract significantly reduced induction time of diarrhea and total weight of the feces (Shoba & Thomas, 2001).

### Antispasmodic activity

The oil of AC rhizome produced antispasmodic action on the involuntary muscle tissue by inhibiting the excessive peristaltic movements of the intestines in rabbits and dogs (Chopra et al., 1954). The alcohol extract showed relaxation of the smooth muscles in an isolated preparation of rat intestines and caused negative inotropic action on frog heart (Agarwal et al., 1956). The essential oil was found to be more effective than the alcohol and aqueous extracts, as observed in lung perfusion and

isolated tracheal chain experiments (Bose et al., 1960).  $\alpha$ -Asarone and the essential oil from the rhizome showed a relaxant effect as well as antispasmodic effect against various spasmogens. The antispasmodic activity of  $\alpha$ -asarone indicated that the action was nonspecific and probably due to direct musculotropic action.  $\alpha$ -Asarone was more active than that of the essential oil (Das et al., 1962). In another study, the ethanol (50%) extract of the rhizome showed antispasmodic activity on isolated guinea pig ileum and gross behavioral effect, hypothermia, and analgesic activity in mice (Bhakuni et al., 1988).

Another study by Gilani et al. (2006) determined its possible pharmacological basis as an antispasmodic and antidiarrheal. In the isolated rabbit jejunum preparation, the crude extract of AC caused inhibition of spontaneous and high  $K^+$  (80 mM)-induced contractions, with respective  $EC_{50}$  values of  $0.42 \pm 0.06$  and  $0.13 \pm 0.04$  mg/mL (mean  $\pm$  SEM;  $n = 6$  to  $8$ ), thus showing spasmolytic activity, mediated possibly through calcium channel blockade (CCB). Results of the study suggest that the spasmolytic effect of the plant extract is mediated through the presence of CCB-like constituent(s), which is concentrated in the *n*-hexane fraction, and this study provides a strong mechanistic base for its traditional use in gastrointestinal disorders such as colic pain and diarrhea.

### Antibacterial activity

In one study, the alcohol extract showed antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus citreus*, *Bacillus megaterium*, *Salmonella paratyphi* A and B, *Salmonella marcescens*, *Proteus vulgaris*, and *Shigella dysomei* (Vashi & Patel, 1987). The alcohol, ether, acetate buffer, and dilute sulfuric acid extract of the root showed antibacterial activity against *Staphylococcus aureus* (Joshi & Magar, 1952). The extract of the rhizome showed moderate *in vitro* antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Diplococcus pneumoniae*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi* A and B, and *Shigella flexneri* (Vohora et al., 1989). The 80% ethanol extract showed inhibition of the two strains of *Bacillus subtilis* and *Staphylococcus aureus* using the agar diffusion method (Valsaraj et al., 1997).

The ether extract of AC in *in vitro* studies using the disk diffusion method showed antibacterial activity against enteropathogenic *Escherichia coli*, *Shigella sonnei*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Vibrio cholerae*. The extract showed antibacterial activity against *Klebsiella pneumoniae* and *Vibrio cholerae* using the tube dilution method (Vijaya & Ananthan, 1994). The essential oil from the rhizome showed a weak antibacterial activity against *Staphylococcus aureus*,

*Escherichia coli*, *Salmonella typhosum*, and *Shigella flexneri* (Chopra et al., 1954). The volatile oil inhibited the growth of *Mycobacterium tuberculosis* var. *hominis* and Gram-negative organisms. The oil showed antibacterial activity against *Staphylococcus albus*, *Corynebacterium diphtheriae*, *Salmonella typhi*, *Salmonella faecalis*, *Bacillus pumilus*, *Streptococcus pyogenes*, and *Pseudomonas solanacearum* (Jain et al., 1974). The essential oil exhibited *in vitro* antibacterial activity against *Bacillus proteus*, *Escherichia coli*, *Staphylococcus pyogenes*, *Shigella shiga*, *Shigella boydii*, *Salmonella typhi*, and *Salmonella paratyphi* (Alankararao & Rajendra Prasad, 1981; Kapil et al., 1983).

### Antifungal activity

The essential oil showed antifungal activity against *Aspergillus oryzae*, *A. nidulans*, *A. fumigatus*, *Penicillium aculeatum*, *Phomopsis destuctum*, citrus decay pathogens *Penicillium digitatum*, *P. italicum*, *Diplodia natalensis*, *Alternaria tenuis*, *Candida albicans*, *Epidermophyton cressens*, and *Microsporum gypseum* (Alankararao & Rajendra Prasad, 1981; Kapil et al., 1983). The alcohol extract possesses antifungal activity against *Aspergillus niger*, *Penicillium selenium*, and yeast *Saccharomyces* (Vashi & Patel, 1987). The fungitoxicity data of the oil with those of its major constituents  $\beta$ -asarone, asaraldehyde, acoradin (three asarone derivatives) against *Helminthosporium oryzae* indicated that  $\beta$ -asarone was the most active.  $\beta$ -Asarone present was primarily responsible for its fungitoxicity (Saxena et al., 1990).

### Antiviral activity

Badam (1995) reported that the alcohol extract of the rhizome showed potent antiviral activity against Herpes simplex virus HSV-1 and HSV-2 at a concentration well below the cytotoxic concentration. Pretreatment of Vero cells with the extract did not inhibit viral replication of HSV-1 and HSV-2. It shows that host cells were not affected by the extract.  $\beta$ -Asarone possesses strong inhibitory activity against the replication of both virus types. The crude alcohol extract and  $\beta$ -asarone showed toxicity to the host cells.

### Anthelmintic activity

The alcohol extract showed *in vitro* anthelmintic activity against human *Ascaris lumbricoides* (Kaleysa Raj, 1974). The essential oil inhibited the amplitude of rhythmic contractions of *Ascaris lumbricoides* within 5 min of exposure. The oil produced partial paralysis of the movements; the phenolic and nonphenolic fractions of the same oil, when tested separately, caused complete paralysis within 25 and 5 min, respectively (Chaudhari et al., 1981). In one study, the essential oil showed nematocidal activity against *Meloidogyne incognita* (Singh et al., 1991).

Sharma et al. (1985) performed a clinical trial on 147 children of age ranging from 5 to 11 years having round worm infestation; calamus powder 250mg three times daily was given for -3 days. As a result, 83% were completely cured, whereas 17% remained unchanged.

### Insecticidal activity

The essential oil showed insecticidal activity against houseflies *Musca domestica* (Singh & Mehta, 1998). The oil inhibited the interstitial cell activity of instar larvae of *Dysdercus koenigii*.  $\beta$ -Asarone with its antigonadal function represented a new type of antigonadal agent that might afford a new and safe approach toward insect control (Saxena et al., 1977). The essential oil showed antifeedant and growth inhibitory effects against third instar larvae of *Spodoptera litura* when used as emulsified foliage sprays (Koul, 1987) and was also effective in controlling the stored grain insect *Spodoptera litura* (Agarwal et al., 1973). The oil caused 100% mortality of *Aedes aegypti* and *Culex fatigans*. The oil vapors affected the hatching of eggs of all age groups of instar nymphs (Saxena & Srivastava, 1972). The oil vapors of the rhizomes caused sterility in male houseflies (Mathur & Saxena, 1975). The oil vapors also slowed morphologic changes in the ovaries of *Thermobia domestica* (Saxena & Rohdendorf, 1974).

The solvent extracts and the volatile principle of the AC rhizome were found to be toxic to housefly *Musca nebulosa* and to *Culex fatigans*. The powdered rhizome was found to be useful against bugs, moths, and lice (Subrahmanyam, 1949). The ethanolic extracts possessed the highest larvicidal activity against *Culex fatigans* (Komalamisra et al., 2005). The aqueous suspension and cold alcohol extract showed insecticidal activity against plant insects *Prodenia litura* and *Dactynotus carthami*. The powder of the rhizome exhibited repellent activity against pulse beetle *Callosobruchus chinensis* (Khan, 1986). The ether extract exhibited strong anti-feeding, repellent, and insecticidal activities against the third instar larvae of sawfly *Athalia proxima* (Pandey et al., 1977). The ethanol extract was found to have 100% mortality against the larvae of *Ailanthus webworm Atteva fabriciella* (Ahmad et al., 1991).

### Piscicidal activity

In one study, the crude extract of AC was screened for piscicidal activity against seven species of fishes found in the Doon valley. It showed strong piscicidal activity against seven species of fishes found in the Doon valley (Virdi, 1982).

### Adulticidal activity

In one study, the hexane fraction from the methanol extract of AC rhizome was screened for its adulticidal

activity with Standard WHO bioassay tests. The hexane fraction from the methanol extract of AC rhizome possessed the most effective adulticidal property, exhibiting  $LC_{50}$  and  $LC_{90}$  values of 0.04 mg/cm<sup>2</sup> and 0.09 mg/cm<sup>2</sup>, respectively (Hidayatulfathi et al., 2004).

### Diuretic activity

The ethanol (50%) extract of AC was screened for its diuretic activity in rats. The result of the study showed diuretic activity of AC (Aswal et al., 1996).

### Genotoxicity and Mutagenicity

AC is a mild co-carcinogen and may interfere with normal pregnancy inter-reactions. The effects of  $\beta$ -asarone on chromosomes were studied in human lymphocyte cultures. A very strong effect on the induction of structural chromosome aberrations was found after metabolic activation and cellular damage occurred. The results demonstrate clearly the genotoxic potency of  $\beta$ -asarone and suggested that only *Acorus* with low content of  $\beta$ -asarone should be used (Abel, 1987).  $\alpha$ -Asarone was mutagenic to *Salmonella typhimurium* in a concentration-dependent fashion.  $\alpha$ -Asarone-induced mutagenicity required a promutagen mixture containing liver S-9 fraction and NADPH. The mutagenicity of  $\alpha$ -asarone was comparable with that induced by aflatoxin. Apparently,  $\alpha$ -asarone is a positive mutagen (Jin et al., 1982; Mukherjee & Wahile, 2006b). In another study,  $\beta$ -asarone showed mutagenic activity in the *Salmonella*-mammalian microsome assay, and the results of the study suggested that only commercial drugs free from or with a low content of  $\beta$ -asarone should be used in human phytotherapy (Goeggelmann & Schimmer, 1983).

### Conclusions

For ages, AC rhizome has been used for the treatment of various ailments in traditional and folklore medicine. The plant exhibits polyploidy, and the composition of the essential oil obtained from the plant rhizome depends on the karyotype. Of the phytoconstituents reported from *Acorus* rhizomes,  $\alpha$ -asarone and  $\beta$ -asarone are the predominant bioactive constituents. Various pharmacological activities of AC rhizome such as sedative, CNS depressant, behavior modifying, anticonvulsant, anti-spasmodic, cardiovascular, hypolipidemic, immunosuppressive, anti-inflammatory, cytoprotective, antioxidant, antidiarrheal, antimicrobial, anthelmintic, insecticidal, and diuretic have been reported by different workers. Together with this, some untoward effects of AC rhizome and its constituents  $\alpha$ -asarone and  $\beta$ -asarone such

as genotoxicity and mutagenicity have also been reported, which limits its therapeutic usage. Thus, this rhizome is well-known as a CNS active herb from Ayurvedic tradition and requires further research to establish the molecular mechanisms of evaluate its activity.

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