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Pharmaceutical and Biotechnological Potential of *Acorus Calamus* Linn.: An Indigenous Highly Valued Medicinal Plant Species

Neha Mittal, H. S. Ginwal* and V. K. Varshney[1]

Division of Genetics and Tree Propagation, Forest Research Institute, Dehradun, Uttarakhand (India) (e mail: neha_mittal87@yahoo.com, ginwalhs@icfre.org)
[1]Chemistry of Forest Products Division, Forest Research Institute, Dehradun, Uttarakhand (India) (varshney2000@yahoo.com)
[*]Author for correspondence: ginwalhs@icfre.org, ginwalhs@rediffmail.com

ABSTRACT

***Acorus calamus* Linn.** is an highly valued herbal medicinal plant in India and other European countries. From very early times it is used as an ingredient of several drugs of the Unani, Ayurveda and Local Health Care Systems. The rhizome, roots and leaves of this plant have been used from ancient times in India for the treatment of a variety of ailments and as a repellent and toxicant. The rhizome contains active ingredients possessing insecticidal, antifungal, antibacterial and allelopathic properties. The extract of *A. calamus* rhizome is used in the formulations of many general tonics. In India, the powdered rootstock and rhizome are used as an antispasmodic, anthelmentic and insecticide. The fragrant oil obtained from the rhizome is also used as flavorings in alcoholic beverages and as fragrant essences in perfumes and sacred oils. The major disadvantage of this oil is the presence of high quantity of carcinogenic β-Asarone compound, due to which this species is not being optimally used in India. The essential oil obtained from this species was found to be very effective against some pathogenic and non-pathogenic fungi (shows strong antifungal activity) and towards the gram negative as well as gram-positive bacteria *in vitro*. The multifarious properties of *A.calamus* make it as a potential plant species for various industrial applications. The present review described the applications of *A.calamus* in various pharmaceutical and biotechnology-based industries.

KEY WORDS: *Acorus calamus*; β-Asarone; Carcinogenic; Essential oil; Rhizome.

INTRODUCTION

The history of medicinal and aromatic plants and their uses in India is as old as human civilization. The Indian system of medicines comprises of Ayurveda, Siddha and Unani in which specifically Ayurveda is about 5000 year old and predominantly used medicinal plants for the drug preparations and formulations (1). A vast diversity of these medicinal and aromatic plants is also found in India because of its agro climatic conditions. Due to these reasons it is considered as "Botanical Garden of the World" has more than 2,200 species of medicinal and essential oil containing plants (1). Medicinal plants are the active constituent of medicines and are viewed as a possible bridge between sustainable economic developments, affordable or traditional health-care and conservation of vital biodiversity (2). Out of the thousands of medicinal and aromatic plants, which commonly, exist in India, one of the largely existing medicinal plant species is *A.calamus*.

A. calamus is one of the highly valued herbal, medicinal and economically important plant species and used since the time of Ayurveda. It belongs to the family Acoraceae and commonly known as Bach (Hindi) and Sweet flag (English). It is a native plant of Northern Asia from the Black sea to China and Japan. In India it is often cultivated in damp marshy places and is fairly common in Manipur, the Naga Hills, Kashmir and Sirmon. Its presence is very much plentiful near the marshy tracts of Kashmir and the Sirmour district of Himachal Pradesh and its cultivation practices are very common in Koratagere taluk district, Karnataka. The Systematic assessment of the Red List Status of the prioritized selected medicinal plant species of Southern India conducted by Foundation for Revitalization of Local Health Traditions (FRLHT) observed that the *A.calamus* species is endangered in Kerala and vulnerable in Tamil Nadu and it is enlisted in 100 red listed medicinal plants of conservation concern in Southern India (3).

It is a semi-aquatic, perennial, aromatic herb with creeping rhizomes, growing wild and also cultivated throughout India, ascending to an altitude of 2,200m in the Himalayas. Leaves look grass-like or sword shaped. They are simple, narrow, thick, glossy, bright green in color or closely arranged (3). The rhizome is the main part of the plant, horizontal, jointed, vertically compressed, spongy, 1.25-2.5 #cm in thickness. It is pale to dark brown, occasionally orange brown in color. All the different parts (leaves, root and rhizomes) possess an essential oil, commonly called the Calamus oil having several medicinal and aromatic properties but it possesses some restrictions too because of the presence of a carcinogenic compound (i.e. β -asarone) in it.

Geographical distribution

A. calamus L. is very commonly found up to the 5000 ft elevation near the marshy places or along the streams and water springs, especially at Chamba (4), Sherpur, Chowari and Shiunta districts (5). It has been also reported in the range of Garsa place (6) and on way to Pulga district (7) at the Parbati valley. Occurrence of *Acorus* is also reported in other districts

of Himachal Pradesh like Kinnaur, Kullu, Mandi, Shimla, Kangra, Sirmour etc. near the river beds, water courses and lakes up to the 2,600 m and found that the Khajjair lake having the floating land mess strewn with *A.calamus* rhizomes. Frequent collection of *A.calamus* is found in Pabbar catchment between Simoli and Zundi (1600-1800m), Rohru, Kharshali (2400m) and from the Beas valley in Kullu (8). In Uttarakhand state, it is commonly found in the area of Gopeshwar-Tungnath, Dist: - Chamoli. Abundant occurrence of *A.calamus* has been reported at the palces of Sagar, Gangolgoan and Batarni-Dhara (1200-1500#mt) (9). It is found very commonly at the Mothronwala swamp in the Doon valley, Dehradun (10). It's rhizomes had been collected from three districts namely Pithoragarh (Vill. Darkot), Almora (Syali) and Chamoli (Kimdal) (11) as well as it also abundantly found in the foot hills of Haldwani and Nainital districts (12-13).). It is found one of the industrially important aromatic plant species of the Sikkim region (14). It is also very commonly found at the Jammu and Kashmir regions near the marshy places and the riverbanks. As well as, its existence is commonly found in natural locality of Manasbal region with in the Kashmir state (15). Natural occurrence of this species has also been observed in southern part in the Nilgiris district of Tamil Nadu, and at the Kothagiri, Coonor etc. places of the Coimbatore district. It is also commonly found in the Pilli- Budarasingi place of the Sirakulum district; Andhra Pradesh (16) and the Belgum district of the Karnataka state (17).

Phenology

A. calamus rarely set flowers and seeds. The blooming period occurs from spring to early summer and lasts about a month. Flowers are small white or yellow green in color, fragrant and arranged in an elongate spadix (3). Perianth condition is found and the total six numbers of tepals generally arranged in two whorls, commomnly occurs (18). Pollens are monosculate and psilate. The carpels are alternate with the inner stamens. The ovary is trilocular and synascidiate and composes the lower half of the gynoecium. Mucilage rich ovaries enclose the placenta and the ovules. The placenta bears long trichomes surrounding three to six pendent orthotropous ovules per locule on the side facing away from the pollen tube transmitting tract, and the micropyles point toward the ovary wall. Characteristic feature i.e. the presence of papillae are commonly found on to the inner integuments, which are generally, protrude from the outer integuments like a beak (18-19).

The flowers of *A.calamus* are generally protogynous in nature, i.e. gynoecium matures first. Pollens are not powdery and they are blown off as single grains by wind and not transported far by the wind. After the fusion of male and female gametes development of the young berries takes place. The young berries are found pale green in color and glossy. Berries are generally found angular in shape, fleshy and having 1-3 seeds. These seeds are generally found oblong in shape (18-19).

Domestication

There are two common methods for multiplication of sweet flag i.e. macro (*in vivo*) and micro (*in vitro*) propagation. Macro propagation is mainly achieved vegetatively by using the rhizomes and top shoot cuttings and micro by using the small part of the plant as an explant and growing them on to the artificial media under aseptic and controlled conditions.

Rhizome cuttings of 2 to 3 nodes are planted during June-July in sunken moist beds of clayey loam to light alluvial soils and flooding the bed regularly waters them. It was propagated vegetatively by using sprouted rhizomes or shoot tips as a planting material obtained from the vigorously growing mother plants (20) and find out that the *Acorus* could be harvested about 10 months after the planting. Besides all it was found that the application of urea significantly increased the tyrosine and beta-asarone contents (by ~ 30%) but it decreased those of phenylalanine and alpha-asarone (21).

Several *in vitro* culture techniques were also standardized for the mass propagation of this medicinally important plant species. Multiple shoot formation was induced using shoot tip culture methodology in this amphibious monocot (22-27). They obtained maximum shoot multiplication frequency on Murashige and Skoog's medium supplemented with 6-benzyl amino purine and indole-3-acetic acid. Regenerated shoots were excised and rooted *in vitro* or directly transferred in to the sterile soil. After acclimatization and transplantation, 80% of the *in vitro* derived plants showed healthy growth in *ex vitro* condition. It was also evident that 40g/l sucrose showed the best results for shoot proliferation from shoot tip explants of *in vitro* proliferated shoots of *A.calamus* (27).

Genetic diversity

In case of *A.calamus* DNA based markers play an important role for the selection of the desired germplasm. They can be directly utilized for the identification of the required variety but instead of this quality these markers i.e. RAPD, RFLP, ISSR, SSR etc. can also be utilized for the analysis of Intra and Inter specific genetic variation. In this context RAPD profile by using OPA (1-20) primers (13) and ISSR profile by using three polymorphic primers (28) were made for *A.calamus*. Positive response of all the OPA primers were found except the OPA-6, OPA-19 and OPA-20 for RAPD analysis and two hundred bands were produced by using the three selected ISSR primers. The number of fragments, which were generated by each primer, varied between 100bp to 5,540bp. The index for evenness of genetic diversity among and within population showed that the individuals within a population were homogenous in terms of their genetic composition and a very little variation was found among the population individuals. Similarly in *A.gramineus* another species of *Acorus* shows the similar kind of genetic variation patterns when studied by the DNA based RAPD marker (29). Genetic variation in 255 samples from 17 sampling sites employing RAPD marker in the populations of *A.gramineus* growing along the six river systems in Western Taiwan were studied. Further, 34 high-intensity and highly reproducible polymorphic fragments were used for the AMOVA statistical analysis. The results of AMOVA showed the total variation of 46.84% among the river systems, 16.88% differences among sampling sites and 36.28% differences among individuals within sampling sites. They also conducted the cluster and principal coordinated analysis and which revealed that the sampling

sites for each river system formed distinct clusters and the individuals with in the sampling site show little genetic variation. The results of the above study concluded that the population genetic structure of the plants of different river systems is highly diversified, which seems to imply that the gene flow among them is very limited.

Pharmaceutical and Biotechnological potential

a) *Phytochemistry*

A brownish, yellow color, essential oil obtained from different parts of the plant is commonly called the 'Calamus oil' (30), contains several chemical constituents. Though all these components are commonly found in the essential oil obtained from the different parts of the plant i.e. roots, leaves and rhizomes, the phenolic ether called 'Asarone' is found as a major (up to 96%) and active constituent of the essential oil (31) (Table-I). It is commonly found in three isomeric forms viz. alpha (α), beta (β) and gamma (γ). Among these three forms, 'β-Asarone' [(Z)-1, 2, 4 trimethoxy-5-prop-1-enylbenzene] is considered the most characteristic component of the oil and its concentration in calamus oil generally depends on the parts from which the oil has been extracted and on to the ploidy level of the plant. The yield of the extracted rhizome oil was found between 0.67 to 1.1% (11), 4.5% (12) and 2.23% (47). Similarly the yield of leaf oil was found in the range of 0.56-1.01% (32).

Table-I: Chemical composition of "Calamus oil" obtained from different parts of plant.

Compound Name	Leafs	Roots	Rhizomes	Reference cited
Calamenone	-	+	+	(30)
α-pinene	-	-	+	(30)
Calamine and Calamol	-	+	-	(30)
Azulene	-	+	-	(30)
Isoeugenol	-	+	-	(30)
Camphor	-	+	-	(30)
Palmitic and butyric acids	-	+	+	(31)
Asaronic acid	-	-	+	(31)
Eugenol, Eugenol methyl-ether				(31)
Asarylic aldehyde, calamene, calamenol, calamenone	-	-	+	(31)
Heptylic acid	-	+	+	(32)
Isocalamendiol and Preisocalamendiol	-	-	+	(32)
Aliphatic and Oxygenated monoterpenes	+	-	-	(32)
n-heptanic acid	-	-	+	(33)
Dehydroabietic acid, Acetic acid, linolenic acid, Nonanoic acid, a-Ursolic acid	-	-	+	(34)
Furyl ethyl ketone, Galagravin, Retusin, Sakuranin, Dehydrodiisoeugenol, Elemicin, Epiedesmin Lysidine, Borneol, Borynl acetate	-	-	+	(34)
Methyl eugenol, cis-methyl eugenol	-	-	+	(35)
Geranyl acetate, β-Farnesene, Shyobunone, Epishyobunone and Iso-shyobunone	-	-	+	(35)
Asaronaldehyde, Acorenone, Calamendiol	-	-	+	(33, 36)
Z-3-(2-,4,5-trimethoxy phenyl)-2 propenal	-	-	+	(37)
Phenyl indane	-	-	+	(37)
Phenyl propane, carbonyls, phenols, aliphatic compounds, alkaloids, carbohydrates and resins.	-	-	+	(38)
Calamusenone and it's Isomer	-	-	+	(39)
Asarone and it's Isomer	+	+	+	(31, 32, 40- 41)
Acorgaermacrone	-	-	+	(42- 44)

Elemene, Caryophyllene, Cadalene and Humulene	-	-	+	(42- 44)
Calamenene	-	-	+	(33, 42- 44)
Acolamone and Isoacolamone	-	-	+	(45)
N^6-Methyl-N^9-octyl-3-oxa-tricyclo[3.2.2.02,4] nona-1 (8), 5(9), 6-triene-6, 9-diamine	-	-	+	(46)

Note: - (+) = showing presence and (-) =showing absence of particular compound.

Table-II: Biological activity of *Acorus calamus* essential oil.

Biological activity	Pathogenecitiy Against Pathogens namely	Reference
Antifungal Activity	*Candida albicans* and *Cryptococcus neoformans*	(67)
	Epidermophyton floccosum, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *T. rubrum*.	(68)
	Ascosphaera apis	(69)
	Aspergillus oryzae, A.nidulans, A.fumigates, Penicillum aculactum, Phomopsis destuctum	(70)
	Curvularia lunata and *Alternaria alternata*	(71)
	Macrophomina phaseolina	(71- 72)
	Fusarium moniliforme and *Trichosporium vesiculosum*	(72)
	Helminthosporium oryzae	(73)
	Anthracnose disease occurred in Mango	(74)
Antibacterial Activity	*Aeromonas hydrophila*	(75)
	Bacillus cereus, *Bacillus subtilis*, *Shigella dysenteriae*, *Shigella flexneri*, *Vibrio cholera*, *Salmonella paratyphi*	(76)
	Bacillus cereus, *Pseudomonas - pseudoalcaligenes*.	(76)
	Bacillus proteus, Aerobic spore bearers, *Staphylococcus pyogens* and the *Shigella shiga*	(70)
	Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and *Klebsiella pneumoniae*	(76- 78)
Insecticidal Activity	*Culex quinquefasciatus* and *Callosobruchus chinesis*	(79)
	Musa domestica L.	(80- 82)
	Sitotroga cerealella Oliv.	(83)
	Callosobruchus phaseoli (Gyllenhal)	(84).
	Nilaparvata lugens Stal	(85- 86)
	Diamondback moth, *Plutella xylostella* (L.)	(87)
	Callosobruchus maculates (F.)	(88)
	Tobacco caterpillar *Spilarctia oblique* Walker	(89)
	Attagenus unicolor japonicus	(90)

	Caryedon serratus (Olivier)	(91)
	Trialeurodes vaporariorum	(92)
	Spodoptera litura (Fab.)	(93)
	Rice bug *Lepticorisa acuta*	(94)
	Tribolium castaneum	(95)
	Ants	(96)
	Trogoderma granarium	(97- 99)
	Dysdercus konenigii F	(100- 103)
	Callosobruchus chinesis	(104)
	Bactrocera cucurbitae (COQ)	(105)
	Bactrocera dorsalis	(105)
Antidiarrhoeal Activity	*Pongamia glabra* [*Pongamia pinnata*], *Aegle marmelos* immature fruits and *Strychnos nux-vomica* in castor oil-induced diarrhea in male Swiss albino mice.	(106)
Nematicidial activity	*Meloidogyne incognita*	(107-108)
Miticidal Activity	Ear canker caused by *Psoroptes cuniculi* in White Giant rabbits	(109)
Anti-Methicillin Activity	Synergistic interaction against antibiotics tetracycline, chloramphenicol, ciprofloxacin, cefuroxime and cefitidizime	(110)
Biopesticidal Activity	*Oryzaephilus surinamensis*, *Lasioderma serricorne*, *Araecerus fasciculatus* and *Tribolium castaneum*	(111)
	Maize borer Prostephanus truncates (Horn)	(112)
	Rice weevil Sitophilus oryzae	(113)
Antifeediant Activity	*Spodoptera litura* F.	(114)
	Attagenus unicolor japonicus	(90)

Table- III: Concentration of β –asarone compound (%) in calamus oil.

Plant Part Used	Concentration of Asarone (%)	Concentration of Asarone Isomers (%)			Reference cited
		α	β	γ	
	82	-	-	-	(128)
	-	3.95	42.47-79.35	-	(129)
Rhizome	-	-	81.7	-	(130)
	-	92.68	1.17	2.27	(12)
	-	-	76.29; 88.64; 78.44;	-	(11)
	-	-	~ 85	-	(14)
	-	-	75.8	-	(131)
	80.1	-	-	-	(31)
	-	79.54	8.47	-	(60)
Leaf	-	-	27.4-45.5	-	(32)

b) *Pharmaceutical applications*

The word '*Acorus*' is derived from Kore, means pupil and refers to the alleged ophthalmic virtues of the plant and has been long used as a medicinally important plant species. This herb in Sanskrit is commonly called the "Vacha" which means, strengthening of word power, and it is generally prescribed for improving voice and intellectual capacity. *A.calamus* is found as a component in over 77 ayurvedic preparations like Aswagandharistam, Sara watharishtam, Dhanwantharaishtam, Nimbamrithasavam, Hinguevachadi chroornam, Cheriyanarayanathailam, Gugguluthiktha kamquatham, Manasami thravatakom etc. (20) as well as in the preparation of Chooram, Ghritham, Tailam, Kashayam and the presence of above compounds in oil was detected through TLC method analysis (48). According to Sushruta Samhitaa, juice of this herbal plant species is used for intellectual vigour and longevity as well as its rhizome preparations can be used in the treatment of asthma, pustular eruptions and heart diseases. In Charaka Samhitaa, this has

been described as a resuscitative (Samjnasthapana) and according to the Tibbi or Unani literature the rhizome has a very bitter taste but useful as a brain tonic and general debility (49).

This plant has several uses and importance in the modern medicinal system as well; its rhizome extract is commonly used in the formulation of many general tonics and its powder is used in the preparation of P-tab tablets. The rhizome has several medicinal properties and used as a stimulant, bitter, tonic, aphrodisiac, laxative, emetic, expectorant, emmenagogue, and diuretic (50). It is also used as a remedy for chronic diarrhea. It forms an important ingredient for a number of paragonic mixtures. Rhizome part is also used in the treatment of insomnia, melancholia, neurosis, remittent fevers, epilepsy, delirium, hysteria, dyspepsia and in loss of memory (49, 51). In the Indigenous system of medicine it is used in the treatment of infectious diseases of chest pain (52), urinary tract and for leucoderma. Fresh rhizomes are commonly inhaled in simple colds as an anti-allergic and its small pieces chewed in empty stomach for curing asthma (8). It is also used as an antitumor agent and antidepressant compound (53).

The alcoholic extract possesses sedative and analgesic properties as well as causes a moderate depression in the blood pressure and in respiration system (50). Ethanolic extract use to improve the adipocyte differentiation in 3T3-L1 cells (54). It also showed significant antisecretory and antiulcerogenic activities (55). The methanolic extract possesses significant immunopotentiating activity. Protective effect of both ethyl acetate, methanolic extract compounds, also was found against the noise stress (56). It is also found a component of the skin care bath preparations and the hair care preparations (20). The major biomedical application of the *A.calamus* is the F-3 bioactive fraction; which is, commonly obtained from this plant and shows the cholesterol-reducing effects via inhibition of cholesterol biosynthesis and promotion of cholesterol excretion. This F-3 bioactive fraction has a potentially beneficial effect in atherosclerosis associated with hyperlipidemia (57). Two novel lectins namely ACL and AGL were purified from rhizomes of sweet flag and found the potent mitogenic activity towards mouse splenocytes and human lymphocytes. Both ACL and AGL also significantly inhibited the growth of J774, a murine macrophage cancer cell-line and to lesser extent WEHI-279, a B-cell lymphoma (58).

The main commercially utilized preparation of this species is the 'Calamus' drug, commonly prepared from the dried rhizomes (59). The other commercial preparations obtained from the plant are Bacha-Churna, Sarasvata-churna, Ashwagandha-rishta, Yogaraja-guggulu, Sanjivanivati, Chandraprabhavati, Unmadgajakesarirasa, Mahashankhavati (8). It is also utilized as a component in the formulation of Jintu- Ayurvedic Syrup; this syrup is commonly used as a unicough syurp and made by the Universal Medicaments of Nagpur, India (A leading manufacturer of Ayurvedic products). Asarone compound obtained from the plant is used in the manufacturing of the *Acorus* tablets (Institute for Traditional Medicine, Portland, Oregon and AXM Pharma Inc, China), which is generally used in the treatment of the upper respiratory infections and more recently these tablets developed by the ITM for the treatment of the ADD. The powdered extract of *A.calamus* is used in the gelatin capsules and sold in the form of Asarone pills in the market at moderate prices. β-asarone, showed the maximum *in vitro* Acetyl cholinesterase enzyme inhibitory potential. Owing to inhibition property of this compound it has been approved to be used in the drugs which are commonly used in the treatment of the mild to moderately severe Alzhemier's disease (60).

Pioneer Enterprise used *A.calamus* in the Vekhand (Herbal extract) product in the ratio of 5:1 and also as a raw material for the product PH2318 Asarone (Yick-Vic Chemicals and Pharmaceuticals Pvt. Ltd., Hongkong). Its other products are the Infusum calami, B.P.C. - Infusion of Calamus. Tinctura Calami, B.P.C. - Tincture of Calamus and Hyidextractum Calami, U. S. P. - Fluidextract of Calamus. Commercially it is used as a major constituent in the formulations of mosquito repellent and also extensively utilized as an aromatic agent in the agarbatties, dhups and havan samagaries. It also used to protect clothes from insect attacks and prevent to destroy them from the houseflies, bed bugs and lice (49).

c) *Applications in food industry*

The water ethanolic extract of this species exhibits antioxidant property since the time of Ayurveda (61-62), and used in the food-industry as a fat-oxidant (20). It is found to be used in alcoholic beverages, as a fragrant essence as well as in perfumes and scared oils (63). Calamus oil is also used in foods such as frozen desserts, yogurts, cakes, confectionery and desserts at levels of 0.2, 0.2, 0.3, 0.3 and 3.0 mg/Kg respectively (64).

d) *Biological activity*

Current research investigates sweet flag value as an insecticidal, antibacterial, antifungal and nematocidal agent (65) and as a biopesticide (66). The essential oil obtained from this species was found to be very effective against some pathogenic and non-pathogenic fungi (shows strong antifungal activity) and towards the gram negative as well as gram-positive bacteria *in vitro*. An compound Azaron or 1, 2, 4-trimethoxy-5- (1-propenyl) Benzene, isolated from the rhizome extract of *A.calamus* was screened for antifungal activity as well as an enzyme isolated from the *A. calamus* leaf protein i.e. Heam peroxidase, found toxic for the fungal hyphal growth (Table-II) . Similarly, the root essential oil of Indian and European varieties were reported to be repellent and toxic to clothes moths, house flies, fleas, several species of mosquitoes, lice and several species of stored grain insects etc. (115).

Limitations

Though the Calamus oil obtained from *A.calamus* has several medicinal, aromatic and Industrial properties but the Food and Drug Administration (FDA) banned its use in the food formulations and in the other therapeutic preparations (20) due to the carcinogenic and toxic properties of the β -asarone compound (116), which is found as a major and active

constituent in the oil. The European Union (EU) has also recommended the limits of calamus oil utilization i.e. 0.1 mg/kg in food and beverages and 1mg/Kg in spirits and spices used for snacks. Its use is prohibited by the United States and Canada. Likewise, CEFS (Council of European Committee of Experts on Flavoring Substances) encouraged the use of only those varieties, which have low contents, or free of β-asarone and proposed the limits of 0.05 mg/kg for foods and beverages and 0.5 mg/kg for alcoholic beverages traditionally flavored with calamus.

β-asarone generally shows the carcinogenic, toxic, acute-toxic, sub-acute toxic, subchronic toxic and chronic toxic properties. It is also found genotoxic and neurotoxic in nature (64). The chromosomes of the human lymphocytes cells get also affected by the carcinogenic properties of this compound (117). As well as its effects is also found on to the rats, i.e. death, growth depression, hepatic and heart abnormalities and serious effusion in abdominal or peritoneal cavities. This compound also possesses the hypnotic and the sedative pharmacological activities (118) as well as its alcoholic and aqueous extracts produce the fall in blood pressure, heart depression both in rate and amplitude, depressant effect on the tone and rhythimicity in the isolated intestine from the guinea pigs and rabbits. The essential oil depressant action was also estimated on the rat uterus (119). The toxic effects of the calamus oil was also shown in rats and found that the carcinogenicity is due to the presence of the β- asarone compound in it and they had also found the development of the malignant tumors after 59 weeks when rats were fed 500-5000 mg/Kg of the calamus oil. Due to the highly carcinogenic and toxic properties of the β-asarone compound FDA, CEFS and the European commission suggests that varieties of this plant species having low β-asarone or absence of this compound should be used in the food-stuffs, alcoholic beverages as well as in the medicines. By keeping above all in view it can be concluded that the utilization of high β-asarone containing variety of *A. calamus* in medicines, food-stuffs and Industries is it's a limitation. So, the identification and evaluation of the low β-asarone compound containing variety/germplasm is pre-requisite, for its commercial utilization. There are different ways to come out from this challenge like evaluation of germplasm based on different morphological, biochemical or DNA based marker.

Germplasm characterization

Morphological variations were investigated in *A.calamus* populations collected from the Southeast Ohio (28). The study suggested that the *A.calamus* is a stress tolerator that displays plasticity in rhizome length and shoots density with respect to nutrient availability. As well as different morphometric traits i.e. rhizome length, rhizome diameter, fresh rhizome yield, plant height, oil content (%), concentration of β -asarone (%), leaf length, leaf diameter, leaf color etc, was also used to study the variations in three different clones of *A.calamus* collected from, Someshwar valley (S-2-6), Baheri (Bh-2-1) and Bangalore (B-1-5) (8) and in North American diploid variety and the European triploid variety (120). Based on the different observations it was found that the clone S-2-6 i.e. from Someshwar valley produced the highest fresh rhizome yield (0.504 Kg/hill) followed by Bh-2-1 (0.407 Kg/hill) and B-1-5 (0.403 Kg/hill) as well as the dry rhizome yield and the production of oil content was also found highest in the clone S-2-6 followed by B-1-5 and Bh-2-1 respectively. The concentration of β-asarone content was also found lower in the S-2-6 clone as compared to the other clones. Based on the above results and the superior performance in evaluation trial, the clone S-2-6 was released as a 'CIM-Balya' variety having high yield with better oil content. Similarly, the rhizome length, rhizome diameter was found superior in North American Diploid variety and only a remarkable characteristic difference between the diploid and triploid accessions was found i.e. in the leaf color. It was found light green in diploid accessions and dark green in the triploids. Generally four varieties of *A.calamus* exist in nature, i.e. diploid, triploid, tetraploid, hexaploid and the variations among them are generally found in their chromosome numbers and in the chemical constitution of the essential oil. The basic chromosome number of *A.calamus* is found 12 and the four varieties contains 2x=24 (diploid), 3x=36 (triploid), 4x=48 (tetraploid) and 6x=72 (hexaploid) (121) but it was also reported 2n=18 number of chromosomes in *A.calamus* (122). Several authors have also been reported different chromosome number in this plant species and correlate its ploidy level with the concentration of β-asarone compound. For example: The Jammu plant variety of *A.calamus* contains a tetraploid 2n=48=4x number of chromosomes and 80% β –asarone, 13% α-asarone content in their oil whereas, the Kashmir variety having the hexaploid 2n=54=6x number of chromosomes and only 5.2% of the asarone content in their oils (123-125). Likewise the rhizomes and leaf part of the diploid variety 2n=24= 2x characterized by the absence/<7% of β-asarone compound (125-126) and the triploid accessions 2n=36=3x characterized by the presence of 3-19% β-asarone in the rhizome oil and 31-44% in the leaf top oil; whereas, the tetraploids contained up to 96% of β-asarone in their rhizome oils and 60-70% in their leaf-top oils (38) and 60.92-8.0% (127). Above all, the tetraploids of Japan and far-east Russian (East Siberia) are characterized by the presence of 10-40% of β –asarone in rhizomes and 20-50% β –asarone in their leaf top oils. The other authors have all also been quantified the concentration of asarone compound in the calamus oil obtained from different parts of the plant using GC-MS methodology (Table-III). Instead of the GC-MS analysis of the essential oil, some authors have also applied other techniques for the identification and quantification of the β –asarone compound. In the above context, concentration of β-asarone content was rapidly assessed using micellar electrokinetic capillary chromatography (MEKC) (41). This method was mainly based on a running buffer and provides a baseline separation of α-asarone and β-asarone compounds. The results indicated that the raw product originated from India contained 4.4% w/w β-asarone, while that from the United States contained 0.2% w/w β-asarone. Quantitative detection and monitoring of β–asarone compound in this species using HPTLC a technique was also carried (132) and it

was found that this is a precise (average recovery=99.6%, C.V. < 0.5%), sensitive (limit of quantification = 120 ng) specific and reproducible (C.V. < 2%) methodology (133).

The above morphological parameters can be used as a preliminary step for the identification of lowest β-asarone containing variety of *A.calamus* but this method is not very much accurate and precise. So, the next methodology that can be used for the identification of low β -asarone containing variety/varieties is the quantification of this compound through GC-MS analysis and identification of its ploidy level by counting the total number of chromosomes. The above cytogenetical and biochemical tool for the identification of the low β -asarone containing variety/varieties is a valuable tool but it is a very time consuming and lengthy process. By keeping above all in view a more precise and reliable method for the identification of the low β –asarone or β –asarone free diploid varieties by using molecular approach was established. Amplification of the 700 of bp sequence of the non-transcribed spacer (NTS) region in the 5S-rRNA gene of the two varieties i.e. diploid and triploid for the *A.calamus* was carried out. A clear-cut differentiation in the spacer region of the gene in two varieties was recorded. The PCR amplified 5s-rRNA gene spacer region was digested with the Eco-RI restriction enzyme, from the identified sequences in the diploid cytotypes the Eco-RI site was absent but found present in the triploid cytotype at 586 bp position. Two fragments of 586 bp and 127 bp, on the gel were represented as a triploid variety whereas undigested fragment were observed in the diploid accession (40, 134). For the confirmation of ploidy level of diploid and triploid varieties they quantified the total concentration of β-asarone compound in both the varieties. This compound was found absent in diploids and the higher percentage (11%) was found in triploids. For further conformation they also counted the total number of chromosomes and found 24 number of chromosomes in the diploid and 36 in triploids. On the basis of information and data collected they concluded that the diploid variety (2n=24) of *A.calamus* contains low or no β–asarone and gives a single band on the gel by the amplification of the 5s-rRNA gene spacer region. The inter and intra specific varaiation based on the 5S-rRNA gene spacer region between the *A. gramineus* and six chemotypes of *A.calamus* plant species was also demonstrated (134). They amplified the 5S-rRNA gene spacer region from six *A.calamus* plants (2 from chemotype A, 2 from chemotype B and 2 from intermediate type M) and one from *A.gramineus* plant and the amplified sequenced region spans 713bp for *A.calamus* and 695bp for *A.gramineus*. With in the six individuals of *A.calamus* plant species, the amplified sequences corresponding to the coding region were completely identical having slight intra specific varaiation at ten nucleotide sites (nucleotide positions 99, 143, 194, 206, 248, 249, 333, 482, 610, 689) whereas, the interspecific variation showed the unique pattern in which there was no base difference between the two plants of *A.calamus* (C1 and C2) belonging to chemotype A and between the two plants (C5 and C6) belonging to chemotype B. In contrast of this, the base differences were found between the two plants (C3 and C4) belonging to the intermediate plant type individuals (i.e. type M).

Thus, it can be easily concluded that the low β-asarone containing variety having several applications in different fields of biotechnology due to less carcinogenicity (generally diploid) in comparison to the higher carcinogenic varieties. This makes it a highly valuable germplasm of *A.calamus*: An indigenously highly valued medicinal plant species and it can be preliminary identified by the leaf color variation (morphological marker), quantification of β-asarone compound mainly through the GC-MS analysis (biochemical marker) and can be easily identified by the PCR-RFLP methodology (DNA based marker).

CONCLUSION

Acorus calamus basal monocotyledon (sister group to the other monocots), anoxia tolerator and highly valued medicinal plant species used in the medicines as well as in the food-stuffs since long time but having some restrictions too i.e. the presence of carcinogenic compound in its essential oil. So in the different fields of biotechnology it can be utilized by the evaluation of desired germplasm using different DNA and oil based techniques.

REFERENCES

1. C.K. Kokate, A.P. Purohit and S.B. Gokhale, Pharmacognosy, (31st Edn., Nirali Prakashan, Pune, India, 2005) 1-57.
2. U. Dhar, S. Manjkhola, M. Joshi, A. Bhatt, A.K. Bisht and M. Joshi. Current status and future strategy for development of medicinal plants sector in Uttaranchal, India. *Current Science*. **83(8):** 956-964 (2002).
3. S. Maiti. Harnessing herbal heritage of India. *NEWSLETTERS-National Research Center for Medicinal and Aromatic plants*. **6(1):** 1-6 (2005).
4. R. Gupta. Survey record of medicinal and aromatic plants of Chamba forest division, Himachal Pradesh. *Indian Forester*. Vol. 454-463 (1964).
5. S.R. Shabnam. Medicinal plants of Chamba. *Indian Forester*. 50-53 (1964).
6. S.P. Jain and H.S. Puri. An ethno medico botanical survey of Parbati valley in Himachal Pradesh (India). *Journal of Economic and taxonomic Botany*. **18(2):** 321-327 (1994).
7. S.K. Jain and R.C. Bharadwaja. On a Botanical trip to the Parbati valley. *Indian Forester*. 302- 305 (1949).
8. N.S. Chauhan, Book on Medicinal and Aromatic plants of Himachal Pradesh, (Indus Publishing Company, New Delhi, 1984) 73-76.
9. G.P. Kimothi and B.C.L. Shah. Some medicinal plants of Gopeshwar-Tungnath region of Uttar Pradesh. *Ancient Science of Life*. **8 (3 and 4):** 283-292 (1989).
10. N. Gupta, A. Anthwal and A. Bahuguna. Biodiversity of Mothronwala Swamp, Doon Valley, Uttaranchal. *The Journal of American Science*. **2(3):** 33-40 (2006).
11. V.K. Srivastava, B.M. Singh, K.S. Negi, K.C. Pant and P. Suneja. Gas chromatographic examination of some aromatic plants of Uttar Pradesh hills. *Indian perfumer*. **41(4):** 129-139 (1997).
12. M.C. Nigam, A. Ahmad and L.N. Misra. GC-MS examination of essential oil of *Acorus calamus*. *Indian Perfumer*. **34(4):** 282-285 (1990).
13. H.S. Chauhan. A high rhizome and oil yielding variety "CIM-Balya" of Vach (*Acorus calamus*). *Journal of Medicinal and Aromatic plant Sciences*. **28:** 632-634 (2006).
14. S.G. Agarwal, R.K. Thappa and T.N. Srivastava. Some industrially important aromatic plants of Sikkim. *Indian Perfumer*. **31(2):** 113-115 (1987).
15. S.D. Sharma, A.M. Chishti and M.K. Kaul. Phytochemical survey of plants from Kashmir-II. *Indian Drugs*. **22(4):** 187-195 (1985).
16. K.P. Rao and S.H. Sreeramulu. Ethnobotany of selected medicinal plants of Srikakulam district, Andhra Pradesh. *Ancient Science of Life*. **4(4):** 238-244 (1985).
17. R.B. Malabadi, G.S. Mulgund and K. Natraja. Ethnobotanical survey of medicinal plants Belgum district, Karnataka, India. *Journal of Medicinal and Aromatic Plant Sciences*. **29:** 70-77 (2007).

18. M. Buzgo and P.K. Endress. Floral structure and development of Acoraceae and its systematic relationships with basal angiosperms. *International Journal of Plant Sciences*. **161(1):** 23-41 (2000).
19. P.J. Rudall and C.A. Furness. Systematics of *Acorus*: ovule and anther. *International Journal of Plant Sciences*. **158(5):** 640-651 (1997).
20. P.N. Ravindran and I. Balachandran. Under utilized medicinal spices. *Spice India*. **17(12):** 2-14 (2004).
21. A. Menghini, N. Pocceschi, G. Venanzi and T.B. Palladini. Effect of nitrogen fertilization on photosynthesis rate, nitrogenous metabolites and alpha and beta asarone accumulation in triploid *Acorus calamus* L. leaves. *Flavour and Fragrance Journal*. **13(5):** 319-323 (1998).
22. A. Hettiarachchi, K.K.S. Fernando and A.H.M. Jayasuriya. *In vitro* propagation of wadakaha (*Acorus calamus* L.). *Journal of the National Science Council of Sri Lanka*. **25(3):** 151-157 (1997).
23. V.M. Kulkarni and P.S. Rao. *In vitro* propagation of sweet flag (*Acorus Calamus*, Araceae). *Journal of Medicinal and Aromatic Plant Sciences*. **21:** 325-330 (1999).
24. K.N. Harikrishnan and M. Hariharan. *In vitro* clonal propagation of sweet flag (*Acorus calamus* L.)- A medicinal plant. *Plant tissue culture and biotechnology:-emerging trends. Proceedings of a symposium held at Hyderabad, India*. 220-222 (29-31 January, 1997).
25. S.A. Rani, V.V. Subhadra and V.D. Reddy. *In vitro* propagation of *Acorus calamus* Linn. - a medicinal plant. *Indian Journal of Experimental Biology*. **38(7):** 730-732 (2000).
26. X. CaiXia, T. GenGuo and Y. Jun. Study on tissue culture and rapid propagation of *Acorus calamus* 'Variegatus'. *Journal of Zhejiang Forestry Science and Technology*. **26(2):** 23-25, 29 (2006).
27. M.B. Ahmed, M. Ahmed, R. Salahin, R. Sultana, M. Khatun, M.A. Razvy, M.M. Hannan, R. Islam and M. Hossain. Standarization of a suitable protocol for *in vitro* clonal propagation of *Acorus calamus* L. an important medicinal plant in Bangladesh. *American Eurasian Journal of Scientific Research*. **2(2):** 136-140 (2007).
28. A. Pai and B.C. McCarthy. Variation in shoot density and rhizome biomass of *Acorus calamus* L. with respect to environment. *Castanea*. **70(4):** 263-275 (2005).
29. L.C. Liao and J.Y. Hsiao. Relationship between population genetic structure and riparian habitat as revealed by RAPD analysis of the rheophyte *Acorus gramineus* Soland. (Araceae) in Taiwan. *Molecular Ecology*. **7:** 1275-1281 (1998).
30. S.S. Chaudhry, S.K. Gautam and K.L. Handa. Composition of calamus oil from calamus roots growing in Jammu and Kashmir. Drug research laboratory, Jammu. 183-186 (1957).
31. N.C. Kelkar and S.B. Rao. Essential oil from the rhizomes of *Acorus calamus* Linn. *Journal of Indian Institute of Science*. **17A:** 25-31 (1933).
32. R. Venskutonis, Petras, and A. Dagilyte. Composition of essential oil of sweet flag (*Acorus calamus* L.) leaves at different growing phase. *Journal of Essential Oil Research*. 1-5 (2003).
33. L.J. Mc Gaw, A.K. Jager and J. Staden. Isolation of β-asarone, an antibacterial and anthelmintic compound from *Acorus calamus* in South Africa. *South African Journal of Botany*. **68:** 31-35 (2002).
34. J.M. Chapman, R. Meyer, K. Kindscher and S. Niemann. Chemical constituents of *Acorus calamus* from a Kansas Prairie. Rockhurst University, Kansas City, MO, University of Kansas, Lawrence, KS, CSS Analytical Company, Shawnee, KS. 2006.
35. L.C.M. Rost and R. Bos. Biosystematic investigations with *Acorus* L. 3. Communication. Constitutents of essential oils. *Planta Medica*. **36:** 350-361 (1979).
36. V. Lander and P. Schreier. Acorenone and gamma-asarone: indicators of the origin of calamus oils (*Acorus calamus*, L.). *Flavour and Fragrance Journal*. **5(2):** 75-79 (1990).
37. D.B. Saxena. Phenyl Indane from *Acorus calamus*. *Phytochemistry*. **25(2):** 553-555 (1986).
38. V.K. Raina, S.K. Srivastava and K.V. Syamasunder. Essential oil composition of *Acorus calamus* L. from the lower region of the Himalayas. *Flavour and Fragrance Journal*. **18:** 18-20 (2003).
39. M. Rohr, P. Naegeli and J.J. Daly. New sesquiterpenoids of sweet flag oil (*Acorus calamus*). *Phytochemistry*. **18:** 279-281 (1979).
40. C.M. Bertea, C.M.M. Azzolin, S. Bossi, G. Doglia and M.E. Maffei. Identification of an EcoRI restriction site for a rapid and precise determination of β – asarone free *Acorus calamus* cytotypes. *Phytochemistry*. **66:** 507-514 (2005).
41. K.M. Hanson, M. Gayton-Ely, L.A. Holland, P.S. Zehr and B.C.G. Soderberg. Rapid assessment of β-asarone content of *Acorus calamus* by micellar electrokinetic capillary chromatography. *Electrophoresis*. **26:** 943-946 (2005).
42. M. Iguchi, H. Nishiyama Koyama, S. Yamamura and Y. Hirata. Isolation and structure of three new sesquiterpenes. **Tet. Lett No. 51:** 5315-5318 (1968).
43. M. Iguchi, H. Nishiyama Koyama, S. Yamamura and Y. Hirata. Isolation and structure of isocalamendiol. **Tet. Lett. No. 42:** 3729-3732 (1969).
44. M. Iguchi, S. Nishiyama Yamamura and Y. Hirata. Preisocalamendiol, a plausible precursor of isocalamendiol. **Tet. Lett. No. 11:** 855-857 (1970).
45. M. Niwa, M. Nishiyama, Iguchi and S. Yamamura. Selinane type sesquiterpenes: Acolamone and isoacolamone. **Chem. Lett.:** 823-826 (1972).
46. A.D.R. Nurdjaman, K.D.R. Akiyama, T.D.R. Fujita and H. Hayashi. Laboratory of Natural Product Chemistry, Department of Applied Biological Chemistry, Faculty of Agriculture, Osaka Prefecture University, Japan. 2000.
47. A.R. Chowdhury, R.C. Gupta and M.L Sharma. Essential oil from the rhizome of *Acorus calamus* Linn. raised on alkaline soil. *Indian Perfumer*. **41(4):** 154-156 (1997).
48. A. Thankamma, L.G. Radhika and C. Soudamini. Detection of *Acorus calamus* in ayurvedic preparations. *Ancient Science of Life*. **14(4):** 276-280 (1995).
49. I.C. Chopra, K.L. Handa and B.K. Abrol. Indian calamus: Its scope in modern therapeutics. *Journal of Scientific and Industrial Research*. **17A:** 8-11 (1958).
50. L.D. Kapoor, Handbook of ayurvedic medicinal plants, (CRC press, LCC, Washington D.C., New York, 2001) 18-19.
51. S.B. Vohora, S.A. Shah and P.C. Dandiya. Central nervous system studies on an ethanol extract of *Acorus calamus* rhizomes. *Journal of Ethnopharmacology*. **28(1):** 53-62 (1990).
52. B. Lal, S.K. Vats, R.D. Singh and A.K. Gupta. Ethnobiology in human welfare: - abstracts of the fourth international congress of ethnobiology, Lucknow, Uttar-Pradesh, India.17-21 November, 1994, 143.
53. C.P. Bopaiah, N. Pradhan and B.S. Venkataram. Pharmacological study on antidepressant activity of 50% ethanol extract of a formulated ayurvedic product in rats. *Journal of Ethnopharmacology*. **72(3):** 411-419 (2000).
54. W. HaoShu, L. YingYin, W. LinJia, Z. ChangXin, H. QiaoJun and L. YiJia. A fraction of *Acorus calamus* L. extract devoid of beta asarone enhances adipocyte differentiation in 3T3-L1 cells. *Phytotherapy Research*. **21(6):** 562-564 (2007).
55. S. Rafatullah, M. Tariq, J.S. Mossa, M.A. Yahya, M.S. Said and A.M. Ageel. Anti-secretagogue, anti-ulcer and cytoprotective properties of *Acorus calamus* in rats. *Fitoterapia*. **(1):** 19-23 (1994).
56. S. Manikandan, R. Srikumar, N.J. Parthasarathy and R.S. Devi. Protective effect of *Acorus calamus* Linn on free radical scavengers and lipid peroxidation in discrete regions of brain against noise stress exposed rat. *Biological and Pharmaceutical Bulletin*. **28(12):** 2327-2330 (2005).
57. T.D. Souza, S.A. Mengi, S. Hassarajani and S. Chattopadhayay. Efficacy study of the bioactive fraction (F-3) of *Acorus calamus* in hyperlimidemia. *Indian Journal of Pharmacology*. **39(4):** 196- 200 (2007).
58. J.S. Bains, V. Dhuna, J. Singh, S.S. Kamboj, K.K. Nijjar, and J.N. Agrewala. Novel lectins from rhizomes of two *Acorus* species with mitogenic activity and inhibitory potential towards murine cancer cell lines. *International Immunopharmacology*. **5(9):** 1470-1478 (2005).
59. Wealth of India (Raw Material), Vol. I (A), (Council of Industrial and Scientific Research, New Delhi, 1985) 63-65.
60. P.K. Mukherjee, V. Kumar, M. Mal and P.J. Houghton. *In vitro* acetyl cholinesterase inhibitory activity of the essential oil from *Acorus calamus* and its main constituents. *Planta medica*. **73:** 283-284 (2007).
61. R. Govindarajan, A.K. Agnihotri, S. Khatoon, A.K.S. Rawat and S. Mehrotra. Pharmacognostical evaluation of an antioxidant plant - *Acorus calamus* Linn. *Natural Product Sciences*. **9(4):** 264-269 (2003).
62. R. Govindarajan, M. Vijayakumar and P. Pushpangadan. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. *Journal of Ethnopharmacology*. **99(2):** 165-178 (2005).
63. T.J. Motley. The ethnobotany of sweet flag *Acorus calamus* (Araceae). Economic Botany. **48(4):** 397-412 (1994).

64. Opinion of the scientific committee on food on the presence of β-asarone in flavorings and other food ingredients with flavoring properties. *European Commission*. 2-15 (2002).
65. M.A. Ali. Isolation and characterization of nematocidal constituents from gourbach (rhizome of *Acorus calamus* L.). *Bangladesh Journal of Scientific and Industrial Research*. **29(2):** 87-93 (1994).
66. A.S. Rani, M. Satyakala and U.S.N. Murty. *Acorus calamus* Linn: an important medicinal plant and source of a potent biopesticide. *Proceedings of the National Academy of Sciences India Section-B, Biological Sciences*. **73(1):** 1-15 (2003).
67. S. Thirach, K. Tragoolpua, S. Punjaisee, C. Khamwan, C. Jatisatienr and N. Kunyanone. Antifungal activity of some medicinal plant extracts against *Candida albicans* and *Cryptococcus neoformans*. *Acta Horticulturae*. **597:** 217-221 (2003).
68. C. Jatisatienr and A. Jatisatienr. An ethnomedico botanical survey of Parbati valley in Himachal Pradesh (India). *Acta-Horticulturae*. **501:** 87-93 (1999).
69. P. Chantawannakul, T. Puchanichanthranon and S. Wongsiri. Inhibitory effects of some medicinal plant extracts on the growth of *Ascosphaera apis*. *Acta Horticulturae*. **678:** 183-189 (2005).
70. G.S.J.G. Alankararao and R. Prasad. Antimicrobial property of *Acorus calamus* Linn. *In vitro* studies. *Indian Perfumer*. **15 (3 and 4):** 4-6 (1981).
71. J. Begum, H. Sohrab, Md. Yusuf, J.U. Chowdhury, M.M. Husain, H.A. Begum, and M.N. Anwar. *In-vitro* antifungal activity of azaron isolated from the rhizome extract of *Acorus calamus* L. *Pakistan Journal of Biological Sciences*. **7(8):** 1376-1379 (2004).
72. M. Ghosh. Antifungal properties of Haem Peroxidase from *Acorus calamus*. *Annals of Botany*. **98:** 1145-1153 (2006).
73. D.B. Saxena, S.S. Tomar and R.P. Singh. Fungitoxicity of chemical components and some derivatives from *Anethum sowa* and *Acorus calamus*. *Indian Perfumer*. **34(3):** 199-203 (1990).
74. C. Rattanakreetakul, R. Korpraditskul, T. Romphophak and V. Korpraditskul. Proceedings of 41st Plants and Agricultural Extension and Communication (Kasetsart University) 2003, 371-378.
75. R. Bhuvaneswari and C. Balasundaram. Traditional Indian herbal extracts used *in vitro* against growth of the pathogenic bacteria *Aeromonas hydrophila*. *Israeli Journal of Aquaculture Bamidgeh*. **58(2):** 89-96 (2006).
76. A.K.A. Chowdhury, T. Ara, M.F. Hashim and M. Ahmed. A new phenyl propane derivative from *Acorus calamus*. *Pharmazie*. **48(10):** 786-787 (1993).
77. J. Parekh, N. Karathia and S. Chanda. Screening of some traditionally used medicinal plants for potential antibacterial activity. *Indian Journal of Pharmaceutical Sciences*. **68(6):** 832-834 (2006).
78. J. Rajendhran, A.M. Mani and K. Navaneethakannan. Antibacterial activity of some selected medicinal plants. *Geobios*. **25:** 280-282 (1998).
79. S.B. Bhonde, S.G. Deshpande and R.N. Sharma. Toxicity of some plant products to selected insect-pests and its enhancement by combinations. *Pesticide Research Journal*. **13(2):** 228- 234 (2001).
80. S.M. Ahmed, H. Chander and J. Pereira. Insecticidal potential and biological activity of Indian indigenous plants against *Musca domestica* L. *International Pest Control*. **23(6):** 170, 172, 174-175 (1981).
81. P.B. Deshmukh and D.M. Renapurkar. Insect growth regulatory activity of some indigenous plant extracts. *Insect Science and its Application*. **8(1):** 81-83 (1987).
82. A.C. Mathur and B.P. Saxena. Induction of sterility in male houseflies by vapors of *Acorus calamus* L. oil. *Naturwissenschaften*. **62(12):** 576-577 (1975).
83. T.P.S. Teotia and G.C. Tewari. Insecticidal properties of drupes of dharek (*Melia azedarach*) and rhizomes of sweet flag (*Acorus calamus*) against adults of *Sitotroga cerealella* Oliv. *Indian Journal of Entomology*. **39(3):** 222-227 (1978).
84. M.M. Rahman and G.H. Schmidt. Effect of *Acorus calamus* (L.) (Araceae) essential oil vapours from various origins on *Callosobruchus phaseoli* (Gyllenhal) (Coleoptera: Bruchidae). *Journal of Stored Products Research*. **35(3):** 285-295 (1999).
85. D. Cheramakara, K. Sombatsiri and S. Choowaew. Laboratory investigation on efficacy of extract from sweetflag (*Acorus calamus* Linn.) rhizomes on brown planthopper (Nilaparvata lugens Stal.). Department of Agriculture, Bangkok (Thailand). Agricultural Toxic Substances Div. 1. Technical conference of Agricultural Toxic Substances Division. Kan prachum wichakan kong watthu mi phit kan kaset khrang thi 1. Bangkok (Thailand), 1995: 9-15.
86. Z. Kavitharaghavan, C. Vijayaraghavan and R. Rajendran. Synergistic effect of plant extracts on the biology of rice brown plant hopper, *Nilaparvata lugens* Stal. *Journal of Plant Protection and Environment*. **3(2):** 6-8 (2006).
87. M.S. Murthy, P.S. Jagadish and V.T. Sannaveerappanavar. Efficacy of plant products and new insecticides against diamondback moth, *Plutella xylostella* (L.) (Yponomeutidae: Lepidoptera) on cabbage. *Journal of Plant Protection and Environment*. **3(2):** 1-5 (2006).
88. L.G. Lakshmi and M.S. Venugopal. Effectiveness of powdered plant products as grain protectants against the pulse beetle, *Callosobruchus maculatus* (F.). *Journal of Entomological Research*. **31(1):** 75-78 (2007).
89. A. Dubey, R. Gupta and B.S. Chandel. Efficacy of *Acorus calamus*, *Vitex negundo* and *Ageratum conyzoides* against tobacco caterpillar *Spilarctia obliqua* Walker. *Indian Journal of Entomology*. **66(3):** 238-240 (2004).
90. H. Mikyeong, K. Soonll and A. YoungJoon. Insecticidial and antifeediant activities of medicinal plant extracts against *Attagenus unicolor japonicus* (Coleoptera: Dermestidae). *Journal of Stored Products Research*. **42(1):**15-22 (2006).
91. V.B. Joshi and S.A. Ghorpade. Evaluation of some plant products and chemicals against *Caryedon serratus* (Olivier) infesting stored groundnut. *Journal of Insect Science Ludhiana*. **14(1/2):** 88-89 (2001).
92. R. Gorski. Evaluation of the effectiveness of natural essential oils in the monitoring of the occurrence of greenhouse whitefly (*Trialeurodes vaporariorum* Westwood). *Journal of Plant Protection Research*. **43(4):** 393-397 (2003).
93. N. Raja, K. Elumalai, M. Jayakumar, A. Jeyasankar, C. Muthu and S. Ignacimuthu. Biological activity of different plant extracts against armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Journal of Entomological Research*. **27(4):** 281-292 (2003).
94. 94. S.J. Nelson, N. Murugesan, V.K.R. Sathiyanandam, Y.S. Johnson, T. Edward and M. Muthusamy. A new botanical insecticide for managing rice bug. *International Rice Research Notes*. **28(1):** 44 (2003).
95. 95. M. Joseph, S.N. Mukherjee and R.N. Sharma. Growth inhibition and impairment of reproductive potential in *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) by commercially available plant extracts. *Insect Sci. Appl.* **15(2):** 197-202 (1994).
96. G.H. Schmidt and D. Borchers. Studies on the sterilizing effects of Indian calamus oil on ants. *Mitteilungen der Deutschen Gesellschaft fur Allgemeine und angewande Entomologie*. **3(1/3):** 201-213 (1981).
97. O. Koul, K. Tikku and B. P. Saxena. Follicular regression in *Trogoderma granarium* due to sterilizing vapours of *Acorus calamus* L. oil. *Current Science*. **46(20):** 724-725 (1977).
98. 98. K. Tikku, O. Koul and B.P. Saxena. The influence of *Acorus calamus* L. oil vapours on the histocytological pattern of the ovaries of *Trogoderma granarium* Evert. *Bull. Grain. Technol.* **16(1):** 3-9 (1978).
99. S. Nair and J. Thomas. Evaluation of the chemosterilant effect of *Acorus calamus* L. extracts on Melon fly, *Bactrocera cucurbitae* COQ. *Journal of Tropical Agriculture*. **39:** 145-148 (2001).
100. B.P. Saxena and J.B. Srivastava. Effect of *Acorus calamus* L. oil vapours on *Dysdercus koenigii* F. *Indian Journal of Experimental Biology*. **10:** 391-393 (1972).
101. B.P. Saxena and A.C. Mathur. Loss of fecundity in *Dysdercus koenigii* F. due to vapours of *Acorus calamus* L. oil. *Experientia*. **32:** 315-316 (1976).
102. B.P. Saxena, K. Tikku and O. Kaul. Spermatogenesis in *Dysdercus koenigii* and induced sterility by *Acorus calamus* oil vapours. *Acta Entomology Bohemoslov*. **74:** 381 (1977b).
103. B.P.O. Saxena and K. Tikku. Inhibition of embryonic development by oil of *Acorus calamus* L. treated males in *Dysdercus koenigii* F. *Indian Perfumer*. **21:** 73-78 (1977c).
104. P. Kumari, P. Kumari and M.K. Verma. Effect of *Acorus calamus* as pulse grain protectant against *Callosobruchus chinensis*. *Journal of Applied Biology*. **9(2):**188-189 (1999).
105. S. Singh and S.S. Sehgal. Investigations on constituents of *Acorus calamus* root oil as attractants to melonfly, *Bactrocera cucurbitae* and oriental fruit fly, *Bactrocera dorsalis*. *Indian Journal of Entomology*. **63(3):** 340-344 (2001).
106. F.G. Shoba and M. Thomas. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. *Journal of Ethnopharmacology*. **76(1):** 73-76 (2001).
107. D.B. Saxena, B.K. Goswami and S.S. Tomar. Nematicidal activity of some essential oils against *Meloidogyne incognita*. *Indian Perfumer*. **31(2):** 113-115 (1987).

108. R.P. Singh, S.S. Tomar, C. Devakumar, B.K. Goswami and D.B. Saxena. Nematicidal efficacy of some essential oils against *Meloidogyne Incognita*. *Indian Perfumer*. **35(1):** 35-37 (1991).
109. D.A.P. Singh, M. Raman, M. Sasikala, M.A. Kumar, R. Sakthivadivu, M.E. Bharathi and V.R.S. Kumar. Miticidal property of *Acorus calamus* against ear canker in rabbits. *Indian Journal of Animal Sciences*. **77(1):** 27-30 (2007).
110. F. Aqil, I. Ahmad and M. Owais. Evaluation of anti methicillin resistant *Staphylococcus aureus* (MRSA) activity and synergy of some bioactive plant extracts. *Biotechnology Journal*. **1(10):** 1093-1102 (2006).
111. M.R.S. Reddy and P.V.R.M. Reddy. Vasa (*Acorus calamus*) - a botanical pesticide against turmeric beetles. *Insect Environment*. **6(1):** 8-9 (2000).
112. S. Pierce and G.H. Schmidt. Effect of ether extracts of the oil from rhizomes of *Acorus calamus* (L.) and beta-asarone on the larger maize borer *Prostephanus truncatus* (Horn). *Anzeiger fur Schadlingskunde Pflanzenschutz Umweltschutz*. **66(5):** 89-96 (1993).
113. Sunilkumar, A. Naganagoud and B.V. Patil. Evaluation of botanical powders against rice weevil (*Sitophilus oryzae*) in stored sorghum. *Karanataka Journal of Agricultural Sciences*. **18(4):** 1117-1120 (2005).
114. S.J. Nelson and M.S. Venugopal. Antifeedant and growth disruptive effects of various plant products on *Spodoptera litura* F. (Lepidoptera: Noctuidae). *Journal of Entomological Research*. **30(2):** 93-102 (2006).
115. M. Jacobson. Insecticides, Insect repellants and attractants from Arid/ Semi Arid- Land plants, Workshop Proceedings Plants: The Potentials for Extracting Protein, Medicines, and Other Useful Chemicals, 1976, 138-146.
116. M. Riaz, S. Qamar and F.M. Chaudhary. Chemistry of the medicinal plants of the genus *Acorus* (Family Araceae). *Hamdard Medicus*. **38(2):** 50-62 (1995).
117. G. Abel. Chromosome damaging effect on human lymphocytes by β-asarone. *Planta medica*. **53:** 251-253 (1987).
118. R.M. Baxter, P.C. Dandiya, S.I. Kandel, A. Okany and G.C. Walker. Separation of the hypnotic potentiating principles from the essential oil of *Acorus calamus* L., of Indian origin by liquid-gas chromatography. *Nature*. **185:** 466-467 (1960).
119. B.C. Bose, R. Vijayvargiya, A.Q. Saifi and S.K. Sharma. Some aspects of chemical and pharmacological studies of *Acorus calamus* Linn. *Journal of the American Pharmaceutical Association*. **49(1):** 32-34 (1960).
120. K. Dusek, B. Galambosi, E.B. Hethelyi, K. Korany and K. Karlova. Morphological and chemical variations of sweetflag (*Acorus calamus* L.) in the Czech and Finnish gene bank collection. *Horticultural Science (Prague)*. **34(1):** 17-25 (2007).
121. A. Krahulcova. Chromosome numbers in selected monocotyledons (Czeh Republic, Hungary and Slovakia). *Perslia Praha*.**75:** 97-113 (2003).
122. Matsuura and T. Suto. J. Fac.Sci., Hokkaido Univ., 1935. **5:** 33.
123. J.E.K. Ammal, S.N. Sobti and K.L. Handa. The interrelationship between polyploidy, altitude and chemical composition in *Acorus calamus*. *Current Science*. **Letters to the Editor:** 500 (1964).
124. M.M. Chopra, V.N. Vashist and K.L. Handa. Chromatographic estimation of asarones in Indian *Acorus calamus* oil (tetraploid variety). *Journal of Chromatography*. **17:** 195-196 (1965).
125. Usseglio-Tomasset. Gas chromatographic examination of some essential oils of *Acorus calamus*. *Indust, Agrar, (Florence)*. **4:**153-156 (1966).
126. G. Mazza. Gas chromatographic and mass spectrometric studies of the constituents of the rhizome of calamus: I. the volatile constituents of the essential oil. *Journal of Chromatography*. **328:** 179-94 (1985).
127. G. Ram, U. Jamwal, P. Koul, S.G. Aggarwal and M.K. Bhan. Evaluation of *Acorus calamus* germplasm for isolation and characterization of beta asarone deficient cultivars. *Journal of Tropical Medicinal Plants*. **6(2):** 209-213 (2005).
128. J.C. Gupta, G.N. Gupta and D.R. Dhingra. Essential oil from the rhizomes of *Acorus calamus*. *J. Proc. Oil. Technol. Assoc. (Kanpur) India*. **11:** 31-33 (1956).
129. V.B. Kapil and G.K. Sinha. Composition of oil of *Acorus calamus* raised under submountain and Tarai region of Kumaon and U.P. *Indian Perfumer*. **21:** 26-30 (1977).
130. R. Operan, M. Tamas, R. Sandulescu and L. Roman. Essential oil analysis. II. Mass spectra identification of terpene and phenylpropane derivatives. *Journal of pharmaceutical and biomedical analysis*. **24:**1163-1168 (2001).
131. V.N. Vashist and K.L. Handa. A chromatographic investigation of Indian calamus oils. *Soap Perfume Cosmet*. **37:** 135-139 (1964).
132. D.B.A. Narayana, P. Raghuvanshi, S. Agarwal and K.S. Srinivas. Quantitative detection of β-asarone in *Acorus calamus* using HPTLC. *Indian Drugs*. **32(6):** 254-257 (1994).
133. V. Widmer, A. Schibli and E. Reich. Quantitative determination of beta asarone in calamus by high performance thin layer chromatography. *Journal of AOAC International*. **88(5):** 1562-1567 (2005).
134. N. Sugimoto, F. Kiuchi, M. Mikage, M. Mori, H. Mizukami and Y. Tsuda. DNA profiling of *Acorus calamus* chemotypes differing in essential oil composition. *Biological and Pharmaceutical Bulletin*. **22(5):** 481-485 (1999).