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A REVIEW ON MEDICINAL BOON: COSTUS SPECIES

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ABSTRACT

Costus species is an important medicinal and ornamental plant used to cure different diseases. The plant has been found to possess many pharmacological activities such as antibacterial, antifungal, anticholineesterase, antioxidant, antihyperglycemic, anti-inflammatory, analgesic, antipyretic, antidiuretic, antistress and estrogenic activity. The rhizomes of Costus species are bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelminthic, depurative, febrifuge, expectorant, tonic, improve digestion, and is a stimulant herb that clears toxins. It also has anti-fertility, anabolic properties. The present review reveals information about distribution, morphology, constituents, traditional, pharmacological and medicinal uses of Costus species, need for its conservation and different conservation methods using biotechnological approaches.

1. INTRODUCTION

Higher plants are major sources of therapeutic agents and extensively utilized throughout the world in traditional as well as modern system of medicine. Currently more than 2000 plants are used as single drugs (1000) and compound formulations (8000) [6].In India, the agro-climatic conditions provide an ideal habitat for the growth of more than 9500 medicinal plants. Thus, there is an enormous scope for India to emerge as major player in medicinal plant sector. Plant products contain many important chemicals with medicinal and valuable pharmacological properties. There is a growing demand for natural products of medicinal/pharmaceutical importance in both domestic and international market. The current demand for the plant based products in medicine and industry has resulted in extensive investigation of the plants for potential therapeutic agents.*Costus*Linn.is a tropical herbaceous plant from family Costaceae under the order Zingiberales. The leaves are large and spirally arranged on stems which differentiate it from its nearest relative, the zingiber or true ginger. Hence these plants are often referred as the spiral ginger or Crepe ginger. The *Costus spp* are commonly grown as medicinal and ornamental plants. The rhizome is the major source of diosgenin, which is anti-diabetic in nature and is used in the treatment of diabetes mellitus [7, 8]. The most popular species in the genus is *C. speciosus,* which has emerged as an important antidiabetic plant [9].

There are more than 100 species of the *Costus*. The different species of *Costus* vary in flower colour. Some varieties with flowers and bracts look like compact cones, while others are shaped like pineapple or soft crepe coming out of green cones. Some leaves are pubescent on abaxial surface, while others are smooth and purplish. About seven species of the genus *Costus* Linn. are known from India. Other cultivated species of this genus are *C.barbatus, C.chartaceus, C. cuspidatus , C. giganteus, C. igneus, C. spectabilis, C.Pictus*[26].

Costus speciosus (Family: Costaceae*)* is an important medicinal plant widely used in several indigenous systems of medicine for the treatment of various ailments. *Costus speciosus* (Koening) sm., is native to the Malay Peninsula of Southeast Asia. In India the plant naturalizes in Sub-Himalayan tract, in parts of central India and in the Western Ghats of Maharashtra, Karnataka and Kerala [10]. The rhizome of these plants are used as an alternative source for diosgenin and generally used to control diabetes. Nurseries are now promoting this exotic species as an ‘antidiabetic plant’ which lowers the blood sugar levels. Presently these plants are mostly collected from wild habitat only. Due to indiscriminate collection from natural habitat *C. speciosus* has become endangered. To overcome the problem an effort

should be made for conservation and systematic cultivation of *C. speciosus*. The present review deals with habit and habitat, cultivation, uses, medicinal properties, future research needs involving *C. speciosus*[26].

Costus igneus Nak (syn. *Costus pictus* D. Don, *Costus mexicanus* Liebm ex Petersen or *Costus congenitus* Rowle), commonly known as fiery costus, Step ladder or Spiral flag or Insulin plant, is native to South and Central America. This is a recent introduction to India from America as an herbal cure for diabetes and hence commonly called as 'insulin plant' [12]. It is widely grown in gardens as ornamental plant in South India and also run wild in many places [13]. It is used in India to control diabetes, and it is known that diabetic people eat one leaf daily to keep their blood glucose low [14]. Leaves of *C. igneus* were one among the plants known to be effectively used for treating diabetes by the tribal people of Kolli hills of Namakkal district, Tamilnadu [15]. In Mexican folk medicine, the aerial part of *C. pictus* D. Don is used as an infusion in the treatment of renal disorders [16].

The plant belongs to the family Costaceae. The Costaceae was first raised to the rank of family by Nakai on the basis of spirally arranged leaves and rhizomes being free from aromatic essential oils. Before the elevation to family status, Engler and Prantl recognized Costoideal as a subfamily under Zingiberaceae. Several anatomical and morphological features support this isolated position including well developed aerial shoot with distinct, rigid, and commonly branched stems. The leaves are inserted in a low spiral with divergences. The family Costaceae consists of four genera and approximately 200 species. The genus Costus is the largest in the family with about 150 species that are mainly tropical in distribution.[13,17]The present review deals with the recent research carried out in the area of phytochemistry, pharmacological, biological activities, and safety of *Costus igneus* Nak[27].

2. MORPHOLOGY

It is a perennial, upright, spreading plant reaching about two feet tall, with the tallest stems falling over and lying on the ground. Leaves are simple, alternate, entire, oblong, evergreen, 4-8 inches in length with parallel venation. The large, smooth, dark green leaves of this tropical evergreen have light purple undersides and are spirally arranged around stems, forming attractive, arching clumps arising from underground rootstocks. Beautiful, 1.5-inch diameter, orange flowers are produced in the warm months, appearing on cone-like heads at the tips of branches [18]. Fruits are inconspicuous, not showy, less than 0.5 inch, and green-colored.

Figure no 1- Insulin plant

Costus species are perennial rhizomatous herbs with erect or spreading stems. Leaves are simple, smooth, persistent, spirally arranged around the trunk. The leaves are sub sessile and appear dark green in colour, elliptic or obovate in shape. Leaves are simple, alternate, entire, oblong, evergreen, 4-8 inches in length with parallel venation. There is some variation in different species of *Costus* like flower colour and texture of the leaves **(Table.1).** In case of *C.igneus*, the leaves have light purple underside whereas *C.speciosus* had silky texture beneath. The inflorescence is a spike around 10 cms long with large bracts in sub terminal position. Bracts are ovate or mucronate forming a cone like structure. In *C.pictus*, bracts are green with yellow coloured flower with red stripes **(Fig 2).** *C.igneus*, bracts are green in colour with orange flowers **(Fig 3).** In *C.speciosus*, bracts are bright red coloured with white flowers having cup-shape labellum and crest yellow stamens **(Fig 4).** Fruit is a capsule ellipsoidal in shape. Seeds are black, five in number with a white fleshy aril (Stone and Benjamin, 1970).The flowers appear in late summer or early fall. The flowers look like crepe paper, thus commonly called crepe ginger. After the flowers fade away, the attractive cone-shaped bracts remain. Generally, the stems sprout during the month of April and flowering commences during July and continues till the end of September. The flowers ripen during middle of the November after which the leaves shed off and majority of stems start drying up. The underground portions (rhizomes) remain dormant from December to March or even April (Sarin et al, 1982)

Table 1: Comparative account of the three species of *Costus*

Parameter	***C.pictus***	***C.speciosus***	***C.igneus***
Habit	Perennial herb	Perennial herb	Perennial herb
Leaves	Narrow with wavy Edges	Large, pubescent and dark green	Large, smooth, dark green with purple undersides

Flower	Yellow with reddish Stripes	White ,crepe paper	Orange in colour
Bracts	Large, greenish	Large, reddish	Large, greenish
Seed	Minute, black with white fleshy aril	Minute, black with white fleshy aril	Minute, black with white fleshy aril

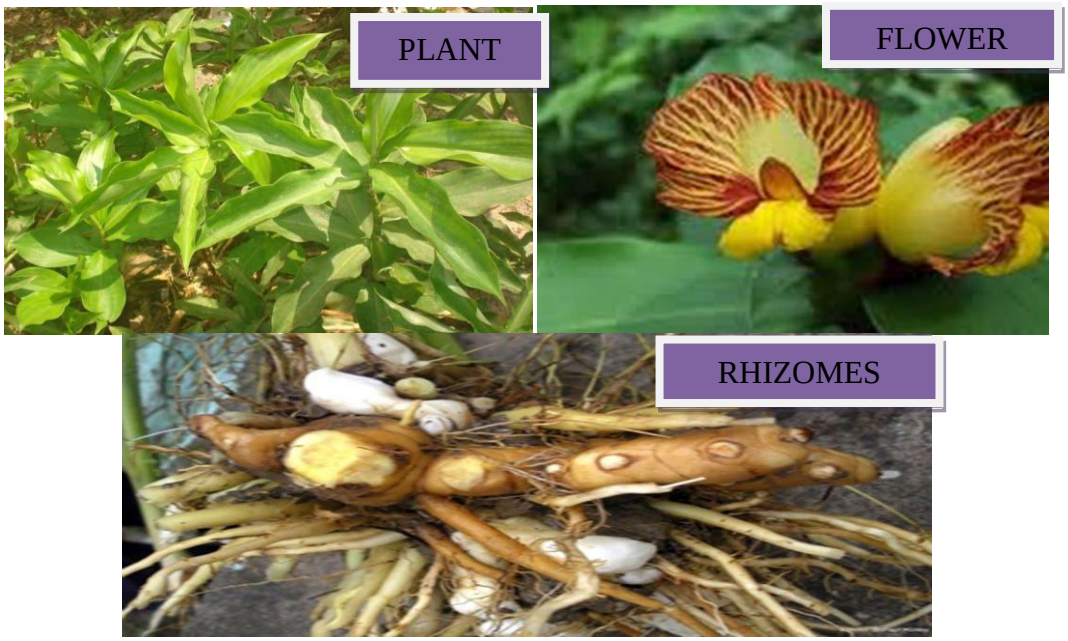


Figure no 2- *Costus pictus* plant with flowering

Figure no 3- *Costus igneus* plant with flowering

Figure no 4 - *Costus speciosus* plant with flowering

3. MACROSCOPIC ANALYSIS

Macroscopic observations of the plant were done. The shape, size, surface characters, texture, colour, odour, taste, etc were noted. It is a technique of qualitative evaluation based on the study of morphological & sensory profiles of herbs [5].

4. MICROSCOPIC ANALYSIS

Microscopic studies are the initial step in quality control of medicinal plants in ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro & microscopic identification & chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. Microscopic evaluation is one of the simplest & cheapest methods for the correct identification of the source of the materials. The microscopic analysis of the powder of the leaf showed fragments of the simple unicellular trichomes, hexacytic stomata; abaxial solitary bundle has single, wide, circular thin walled xylem elements & small clusters of phloem elements located on the upper side .The vascular bundle is surrounded by wide layer of parenchymatous bundle sheath, mesophyll cells containing starch grains, Calcium oxalate crystals of minute particle were aggregated into large masses in the leaf mesophyll cells containing starch grains. Calcium oxalate crystals of minute particle are aggregated into large masses in the leaf mesophyll cells. Tracheids with spiral thickenings & epidermal cells were observed [5].

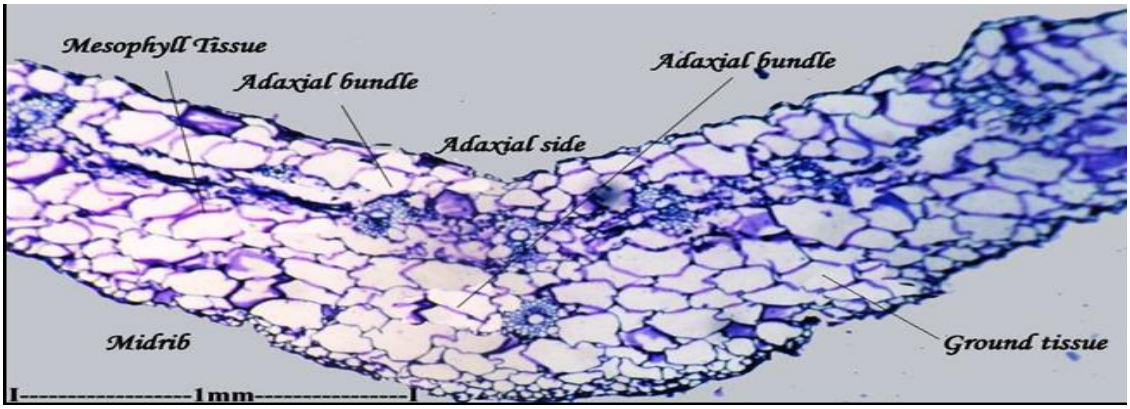


Figure no 5- T.S. of Leaf through Adaxially depressed Midrib

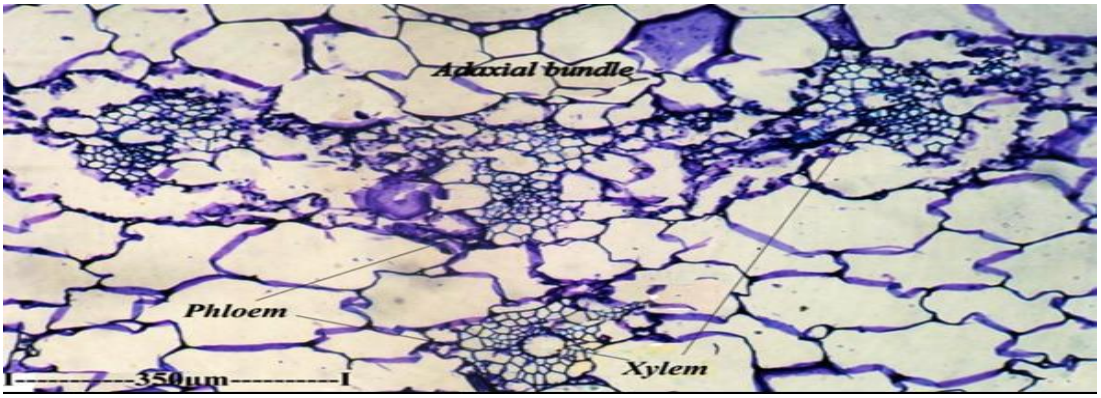


Figure no 6- Midrib- Vascular Bundle enlarged

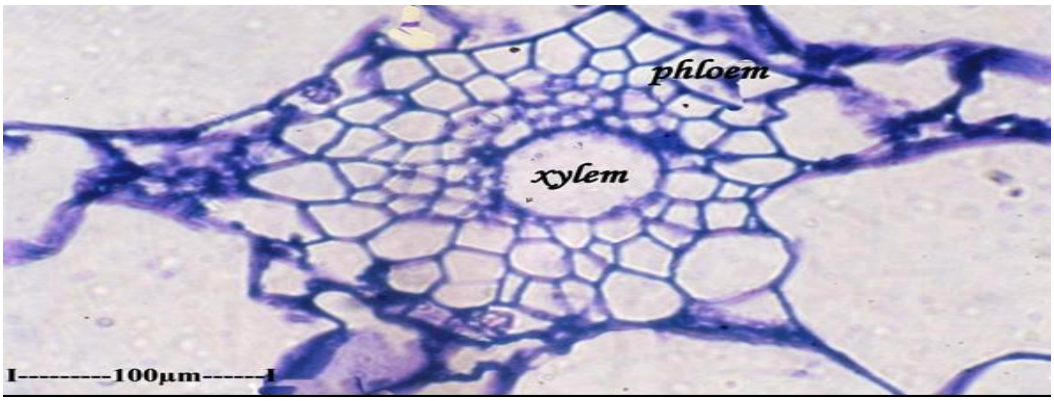


Figure no 7- The Adaxial Median Bundle enlarged

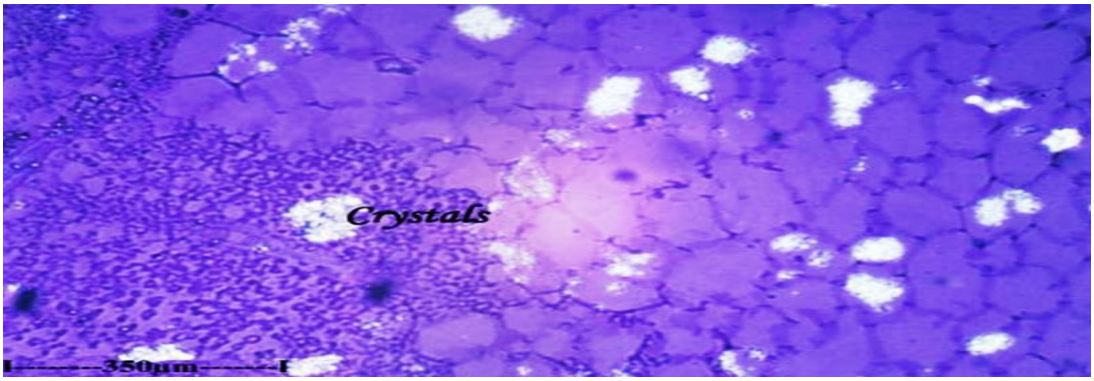


Figure no 8-Crystal distribution in the Mesophyll

5. PHYSICOCHEMICAL ANALYSIS

The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts & or silica(earthy matter).Acid insoluble ash provides information about non-physiological ash produced due do adherence of inorganic dirt, dust to the crude drug. Increased acid insoluble ash indicates adulteration due do dirt, sand (or) soil. The extractive values are primarily useful for the determination of exhausted or adulterated drug & helpful in the detection of adulteration. Total ash, acid insoluble ash, water soluble ash, loss on drying, extractive values, foaming index, swelling index &moisture content were determined [5].

Table 2: Standardization parameters of leaves of Costus species

Sr.No	Parameters*	Values* expressed as
1.	**Moisture content**	12.37±0.032
	Ash value	
	Total ash	15.28±0.140
2.	Acid insoluble ash	3.17±0.080
	Water soluble ash	9.70±0.110
3.	**Foreign organic matter**	0.06±0.080
4.	**Extractive values**	
	Petroleum ether	25.74±0.150

	Chloroform	7.19±0.300
	Ethyl acetate	8.60±0.030
	Ethanol	5.10±0.110
	Methanol	16.25±0.040
	Water	11.12±0.600
	Benzene	5.76±0.080
5.	**Foaming index**	Less than 100
6.	**Swelling index**	**Expressed as ml**
	Initial volume	3.2±0.10
	Final volume	7.6±0.140

6. LEAF CONSTANTS

Quantitative analytical microscopy is useful in measuring the cell contents of the crude drugs & its aids in identification, characterization & standardization. Leaf constants such as vein islet numbers, vein terminal number, stomatal number & stomatal index were determined.

Table 3: Quantitative analytical microscopical parameters of the *Costus species*

Sr.No	Parameters*	Value obtained
1.	Stomatal number(lower epidermis)	13±0.27
2.	Stomatal index(lower epidermis)	10.37±0.23
3.	Vein islet number	4.1±0.057
4.	Vein termination number	3.9±0.088

*mean of 6 readings ± SEM

7. CULTIVATION

Costus species are usually grown in fertile, organic, moist, well-drained soils in shade (Whistler, 2000). Tropical climate with high humidity and minimum temperature of 13^{o} C is best for its cultivation. *Costus* or Crepe ginger grows from thick fleshy structures called rhizomes. A single rhizome will produce new shoots and increase to a 3ft wide clump in the second year under ideal growing conditions. *Costus* reproduces vegetatively by rhizomes, division of culms, stem cuttings. It can also be grown through seeds, but the percentage of seed germination was found to be low (Merina Benny, 2004).Therefore, there is a need for development of standard cultivation methods for species of *Costus.*

8. GROWTH AND PROPAGATION

Spiral flag grows in either full sun or partial shade. It needs fertile soil and ample moisture, and is often planted near water. Propagation is by division of the clumps, cuttings, or by separating the offsets or plantlets that form below the flower heads. Mites and nematodes can be a problem, especially on light, sandy soil. Plant has no diseases are of major concern [18].

9. PHYTOCHEMICAL STUDY

Sequential screening for phytochemicals of *C. igneus* leaves revealed that it is rich in protein, iron, and antioxidant components such as ascorbic acid, α-tocopherol, β-carotene, terpinoids, steroids, and flavonoids [19, 20]. It was revealed in another study that methanolic extract was

found to contain the highest number of phytochemicals such as carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids[21]. Preliminary phytochemical evaluation of Insulin plant (*C. pictus*) revealed that the leaves contain 21.2% fibers. Successive extracts gave 5.2% extractives in petroleum ether, 1.06% in cyclohexane, 1.33% in acetone, and 2.95% in ethanol. Analysis of successive extracts showed presence of steroids in all extracts. The ethanol extract contained alkaloid also. The rhizomes of the genus *Costus* are the major source of a compound, diosgenin (Sarin et al, 1974). Dasgupta and pandey (1970) reported diosgenin as the major constituent isolated from rhizomes of *Costus* species. Diosgenin was also reported from other parts of *Costus* such as leaves, stems and flowers. Other constituents isolated from *Costus* species are Tigogenin, dioscin, gracillin β- sitosterol glucoside [28, 29].

The major component of the ether fraction was bis (2'-ethylhexyl)-1,2-benzenedicarboxylate (59.04%) apart from α-tocopherol and a steroid, ergastanol[22]. Stem showed the presence of a terpenoid compound lupeol and a steroid compound stigmasterol [23].Bioactive compounds quercetin and diosgenin, a steroidal sapogenin, were isolated from *C. igneus* rhizome [24]. Trace elemental analysis showed that the leaves and rhizomes of *C. pictus* contains appreciable amounts of the elements K, Ca, Cr, Mn, Cu, and Zn[25]. Steam distillation of stems, leaves, and rhizomes of *C. pictus* D. Don yielded clear and yellowish essential oils.

Table 4: The major constituents identified in the essential oil were enlisted

Stem oil (%)	**Leaf oil (%)**	**Rhizome oil (%)**
Hexadecanoic acid(28.3)	Hexadecanoic acid(24.51)	Hexadecanoic acid(25.26)
9,12-octadecadienoic acid(18.33)	2-pentanol(22.48)	9,12-octadecadienoic acid(7.74)
Dodecanoic acid(5.62)	Dodecanoic acid(3.96)	Dodecanoic acid(16.56)
Linalyl propanoate(6.03)	Is-ionone(8.69)	Tetradecanoic acid(10.20)
Tetradecanoic acid(4.82)	Farnesyl acetone(7.04)	Linalool(8.48)
A-eudesmol(3.55)	A-ionone(8.01)	α-terpineol(4.44)[27]

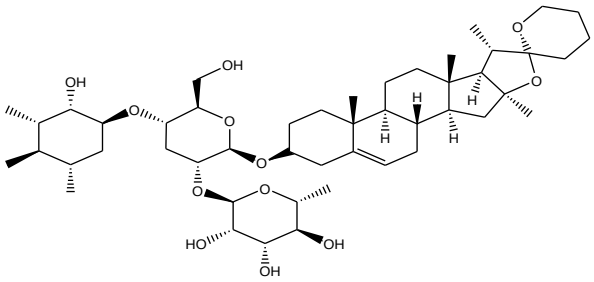
Rhizomes also reported to possess Prosapogenin B of dioscin, Diosgenone, Cycloartanol, 25-encycloartenol and octacosanoic acid (Yoganarasimhan, 2000; Chopra et al, 1999) **(Fig.8).** The rhizomes yield an essential oil which contains pinocarveol (59.9%), cadinene (22.6%), cineol (10.7%), p-methoxy benzophenone (3.3%) andcavacrol (1.3%) (Anonymous, 2007). Gupta et al (1986) isolated five new compounds (Oxo acids and branched fatty acids esters) like tetradecyl 13-methylpentadecanoate, tetra11-methyltriadecanoate, 4-oxotriaconsanoic

acid, 14–oxoheptacosanoic acid and 15-oxooctacosnoic acid from the rhizomes. Methyl 3-(4-hydroxyphenyl) -2E-propenoate and Bis (2-ethylhexyl) phthalate was also reported from the rhizomes (Rastogi and Mehrotra, 2004). Steroidal glycosides like methyl protodioscin, methylprotogracillin, protogracillin,26-O-β-D-glucopyranosyl-(25R)-furost-5-ene-3β,22,26-triol was noticed from the methanolic extracts of rhizomes (Inoue et al, 1995). Besides glycosides, amino acids, proteins and flavonoids were reported from the rhizomes of *Costus* by Saraswathi et al (2010). Saponins from the seeds yielded three genins and glucose on acid hydrolysis (Singh and Thakur, 1982.

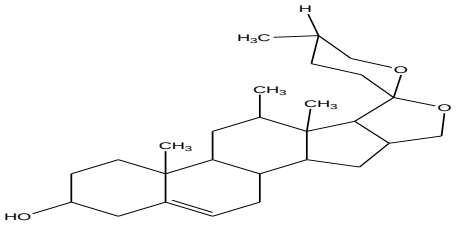
The major genin reported was diosgenin. Two newfurostanol saponins Costusosides I and J were characterized as 3-O-[β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl]-26-O-(β-D glucopyranosyl-22α-methoxy (25R) furost-5-en-3β, 26-diol and its 22-hydroxy derivatives respectively (Rastogi and Mehrotra, 2004; Singh and Thakur, 1982 b). B-sitosterol-β-D-glucoside, prosapogenins A and B of dioscin, gracillin, 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl]-26-O[βDlucopyranosyl]-22α-methoxy-(25R)furost-5-en-3β,26-diol,protodioscin and methylprotodioscin were isolated from seeds (Singh and Thakur, 1982 a). Mahmood et al (1984) reported dihydro phytyl plastoquinone and its 6-methyl derivative along with α- tocopherol quinone and 5α-stigmast-9(11) en-3β-ol from seeds. Methyl hexadecanoate, methyloctadecanoate and tetracosanyl octadecanoate were also isolated from seeds (Singh and Thakur, 1984). A tocopherol is noticed from seeds and identified as G2-tocopherol. Defatted seeds contain diosgenin, glucose, galactose and rhamnose (Rastogiand Mehrotra, 2004).

The seeds possess 6% of pale yellow sweet smelling fatty oil. The fatty acid composition of the oil was reported to contain Palmitic- 55.97%; stearic- 8.3%;oleic-22.75%; linoleic- 6.8%; arachidic- 1.7% (Anonymous, 2007). The analysis of roots showed the presence of chemical constituents like 24- hydroxytriacontan-26-one and 24-hydroxytriacontan-27-one together with methyl triacontanoate, diosgenin, sitosterol 8-hydroxy triacontane-25-one and methyl triacontanoate (Gupta et al, 1982). 5α-stigmast-9(11)-en-3β-ol was also characterized by Madan Gupta et al, 1981. The roots of this plant also contain β-sitosterol-β-D-glucoside, prosapogenins A and B of dioscin, dioscin, gracillin (Gupta et al, 1983; Mahato et al, 1980). Other components identified from seeds were 31-norcycloartanone, cyloartanol, cycloartenol and cycloalaudenol (Rastogi and Mehrotra, 2004). Diosgenin, an aglycone is one of the popular steroidal sapogenins that is used as a raw material for the synthesis of steroidal drugs especially sex hormones such as progesterone, testosterone, dihydroandrosterone,

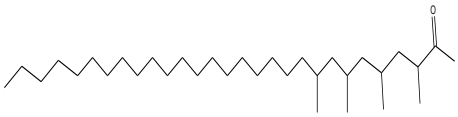
deoxycorticosterone and cortisone (Jaggi and Kapoor, 1992). Thus studies in *Costus* species may prove its role as therapeutic agents.



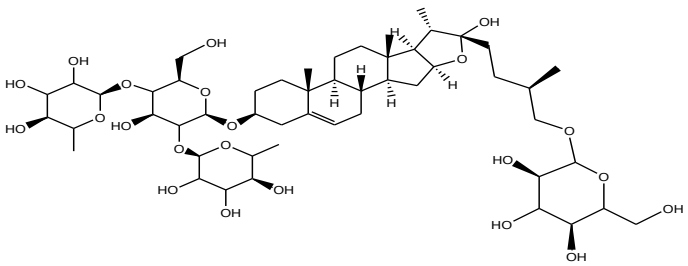
Dioscin



Diosgenin



Octasonic acid



Protodioscin

Figure 9: Structure of major chemical constituents of *Costus* species

Table 5: Preliminary Phytochemical tests for leaves of *Costus species*

S No	Natural product group	Test for natural products	Extract used for test	Presence(+)/Absence(-)
1	Alkaloids	(a) Dragendroff's test	Alcohol	+
		(b) Hager's test	Aqueous	-
		(c) Mayer's test	Alcohol	+
		(d) Wagner's test	Alcohol	+
		(e) Benedicts's test	Alcohol	+
		(f) Fehling's test	Aqueous	+
		(e)Molisch's test	Alcohol	+
2	Carbohydrates	(a) Benedict's test	Aqueous	+
		(b) Fehling's test	Aqueous	+
		(c) Molisch's test	Aqueous	+
3	Flavonoids		Alcohol	+
4	Phenols	(a) Ferric chloride test	Alcohol	+
5	Proteins	(a)Biuret's test	Aqueous	+
		(b)Ninhydrin	Aqueous	+
6	Saponin		Aqueous	+
7	Steroids	Salkowski reaction	Aqueous	+
8	Tannins	(a)Ferric chloride test	Aqueous	-
		(b)Lead acetate test	Aqueous	-
9	Fixed oils/Volatile oils		Petroleum ether	+
10	Glycosides		Aqueous	+
11	Starch		Aqueous	+

10. MEDICINAL PROPERTIES

Different parts of the *Costus* are used in treating various diseases **(Table.6).** The rhizomes are bitter and used mainly for treating diabetes. They show anthelmintic, astringent, expectorant properties. The extract of rhizome is used for treating burning sensation, constipation, leprosy, asthma, bronchitis, anaemia and other skin ailments (Bown Deni, 2008). Rhizomes of *Costus* are used as herbal remedy for fever and its paste is used for treating boils. It is also used to make sexual hormones and contraceptives (Warrier et al, 1994; Rastogi and Mehrotra, 1991). Leaves are used for scabies and stomach ailments. Leaves are ground into paste and applied to the fore head to bring down high fever. Besides rhizomes, stems are also used for treating blisters and burns. Roots are used against snake bite (Gruenwald et al, 2000; Rathore and Khanna, 1978).

Table 6: Medicinal importance of different parts of *Costus* species

Plant part used	Phyto-Constituent	Activity
Whole plant	Diosgenin	Astringent, aphrodisiac, purgative, anthelmintic, depurative and expectorant.
Roots	Diosgenin, sitosterol, dioscin, gracillin, cycloartanol, cycloartenol and cycloalaudenol.	Antibacterial, antifungal, tonic, expectorant and stimulant.
Rhizomes	Diosgenin, dioscin, gracillin and Beta-sitosterol.	Antispasmodic, antidiabetic, anti-inflammatory, antivermin, antiarthritic, cardiotonic, hydrochloretic, diuretic and CNS depressant.
Leaves	Diosgenin	Fever, dysentery, diabetes, eye and ear infections, diarrhorea and mental disorders[29].

11. TRADITIONAL USES

Costus is traditionally used as a medicinal herb mainly for its tonic, stimulant, carminative, diuretic, digestive and antiseptic properties. The rhizome is used internally in the treatment of abdominal pain, chest pains, liver problems, jaundice, gall bladder pain etc (Sivarajan and Balachandran, 1994). In Ayurveda, *Costus speciosus* is used to subdue vata and kapha and promotes complexion. It is reported to cure dyspepsia, fever, cough and other respiratory disorders. It is one of the constituent of indigenous drug "*amber mezhugu*" useful in rheumatism (Chopra et al, 1956). The rhizome possesses antifertility, anticholinesterase, anti-inflammatory and anthelminthic activities (Hussain et al, 1992). Essential oil from rhizomes showed antimicrobial activity (Asolkar et al, 1992). Steroid saponins andsapogenins from *Costus speciosus* were reported to possess antifungal activity (Singhand Srivastava, 1992).

The medicinal preparation further shows insulin potentiating action in addition to decreasing blood glucose. Similarly, the leaves of *Costus speciosus* are known to have hypoglycemic properties (Eliza et al, 2009).

12. OTHER USES

Costus species are widely used as an ornamental plant. In south -East Asia it is used as a food plant. Tender young shoots, fruits and rhizome are used as vegetables. In some parts of India, tubers are cooked in syrup and preserved (Nadkarni, 2009).

13. PHARMACOLOGICAL ACTIVITIES

The leaves and rhizomes of *Costus* species are reported as antidiabetic in nature and are used in the treatment of diabetes mellitus (Bhat Vishnu et al, 2010;). The epidemologic studies and clinical trials states that hyperglycemia is the main cause of complications such as coronary artery disease, cerebrovascular disease, renal failure, blindness, neurological complications and premature death (Eliza et al, 2008).Eremanthin and costunolide isolated from *Costus speciosus* was reported to possess hypoglycemic and hypolipidemic activities (Eliza et al, 2009 a and b). Similarly, Bavara and Narasimhacharya (2008) evaluated the antihyperglycemic, antihyperlipemic and antioxidant potency of an ethanol extract of *Costus speciosus* root in alloxan-induced diabetic male rats. The study exhibited that *Costus speciosus* can be beneficial in the management of diabetes and its complications. Daisy et al. (2008) investigated the possible protective effect of *Costus speciosus* rhizome extract on biochemical parameters in streptozotocin (STZ)-induced male diabetic Wistar rats. The hexane extract of the *C. speciosus* rhizome is known to possess antihyperglycemic and hypolipidemic activity and is able to decrease the serum glucose level and normalize other biochemical parameters in diabetic rats (Eliza et al, 2008). *Costus* plants possess anti-inflammatory, purgative, antiarthritic and antifungal activities (Bandara et al, 1988; Kirtikar and Basu, 2005). These plants are used internally for eye and ear infections which may be attributed to the anticholinesterase activity of the plant alkaloids (Mishra et al, 2009). Bhattacharya et al. (1972) confirmed that *C.speciosus* alkaloids possess anticholinesterase activity in both *in vitro* and *in vivo* methods. The hepatoprotective activity of the ethanolic extract of the rhizomes of *Costus speciosus* on carbon tetrachloride treated rats was reported (Bhuyan and Zaman, 2008; Verma and Khosa, 2009a). The biochemical evidence for anti-stress activity of alcoholic extract of *C. speciosus* rhizomes was provided by Verma and Khosa (2009 b). The antifertility activity of saponins isolated from the rhizomes of *Costus*

speciosus in rats was proved by Tewari et al (1973 a). Also the estrogenic activity of diosgenin isolated from *C. speciosus* was reported by Tewari et al (1973).

Singh et al (2008) showed anticarcinogenic activity of *Costus speciosus* and found that the minimum inhibitory concentration ranged from 0.78 to 10 mg/ml. The pharmacological aspects of different alkaloids and presence of anticholinesterase activity in *Costus speciosus* was noticed by Mandal and Chatterjee (1985). The ethanolic extract of the rhizome of *Costus speciosus* possesses anti-inflammatory and antipyretic properties (Binny et al, 2010).The importance of diosgenin has resulted in extensive search for its different sources. The report of Das Gupta and Pandey (1970) confirming the presence of diosgenin in the rhizomes of *Costus speciosus* raised interest of researchers in studying different species of *Costus* as a possible source. The research work has been conducted to evaluate the anti-diabetic effect of insulin plant. In a cross- sectional clinical study, patients consuming either one fresh leaf or 1 teaspoon of shade-dried powder/day of *C. igneus* in conjunction with other modalities of treatment had effectively produced glycemic control in diabetics.

However, an *in vitro* study of ethanolic extract of *C. pictus* leaf was analyzed to study GLUT4 translocation and glucose uptake activity, which showed no direct peripheral action at 300 µg/ml dose comparable with insulin and metformin. A study evaluated the ability of a tea made from the leaves of *C. spicatus* to alter glucose homeostasis in C57BLKS/J (KS) *db*/*db* mice, a model of obesity-induced hyperglycemia, with progressive beta-cell depletion. Intraperitoneal (IP) insulin tolerance testing after the 10-week study period showed that *C. spicatus* tea consumption did not alter insulin sensitivity, which suggested that at the dose given, tea made from *C. spicatus* leaves had no efficacy in the treatment of obesity-induced hyperglycemia.

14. TISSUE CULTURE STUDIES

In vitro propagation refers to the true-to type propagation of selected genotypes using plant tissue culture techniques. Plant Tissue Culture techniques offer an integrated approach for the production of standardized quality of phytopharmaceuticals through the mass production of consistent plant material. It is also used for successfully for *in vitro* regeneration of many medicinal plants. It is an alternative method of propagation (George and Sherrington, 1984) and being employed for the commercial propagation of a large number of plants with medicinal importance.

C.speciosus, C.pictus and *C.igneus* are the important medicinal plants having huge market demand in National and International market. Due to increased use, these plants are presently

collected from wild. This has created a pressure on forests, resulting in depletion and extinction of *Costus species* from natural habitat. The seeds of *Costus* are small in size and shows poor regeneration capacity, further the rhizomes are often prone to rotting in regions with frequent rainfall. The conventional methods for large scale propagation of *Costus* are not available due to low seed set, poor viability and germination. Thus the conservation and *in vitro* multiplication or micropropagation of *Costus* through *in vitro* technique will be much useful to meet the present market demand.

Micropropagation is a rapid method for large scale production of true to type plant material of selected genotypes. The standardization of protocols for micropropagation and multiple shoot production will result in production of large number of uniform plants.

15. ANTIBACTERIAL STUDIES

15.1 Bioassay:

Agar cup bioassays (Linday, 1962) were employed for testing antibacterial activity of plant extract. The medium were poured into petridishes under aseptic conditions in a laminar flow chamber & left to solidify. These petridishes were inoculated with 0.5ml of 24 hrs old cultures of test organisms. After inoculation, cups were scooped out with 7mm sterile cork borer & the lids of the dishes were replaced to each cup different concentrations of test solutions (50-2000µg) were added. Zone inhibitions were also studied for standard antibiotics, streptomycin & penicillinG. DMSO were used as control [3].

Table 7: Activity of antibiotics

Concentration in µg	Zone of inhibition in cms			
	E.coli	P. aeruginosa	B.subtilis	S. aureus
Penicillin G(100)	----------	----------	1.6	2.1
Streptomycin(100)	2.9	3.4	----------	----------

15.2 Minimum inhibitory concentration (MIC) test:

MIC studies reveals & studied by broth dilution method. Different concentrations (2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156 or 0.0078 mg/ml) of rhizome extract were prepared in 1ml working solution of extract (2mg/ml) were added to the test-tube 1, containing 1ml of nutrient broth. After through mixing, 1ml of solution was transferred to second test tube & the process were continued for succeeding transfers. The last test tube received no test solution & served as control.

For both tests, culture was kept in an incubator at 26°c & results were observed after 24hrs & 3-4 replicates were maintained for each treatment. [3]

Table 8: Antibacterial activity of rhizome extracts of Costus species

Test bacteria	Zone of inhibition in cms							
	Concentration of extract in µg							
	500	700	1000	1200	1400	1600	1800	2000
Activity of C.speciosus								
E.coli	---------	*0.18*	*0.4*	*0.56*	*0.74*	*0.9*	*0.95*	*1.15*
P.aeruginosa	*0.25*	*0.39*	*0.56*	*0.65*	*1.13*	*1.14*	*1.18*	*1.21*
B.subtilis	*0.18*	*0.25*	*0.38*	*0.4*	*0.58*	*0.74*	*0.8*	*1.1*
S.areus	*0.25*	*0.4*	*0.45*	*0.72*	*1.04*	*1.21*	*1.24*	*1.25*
Activity of C.igneus								
E.coli	---------	*0.17*	*0.39*	*0.52*	*0.6*	*0.78*	*0.92*	*1.11*
P.aeruginosa	*0.2*	*0.22*	*0.48*	*0.64*	*0.75*	*0.89*	*1.1*	*1.18*
B.subtilis	*0.17*	*0.21*	*0.28*	*0.56*	*0.62*	*0.83*	*0.97*	*1*
S.areus	*0.19*	*0.23*	*0.41*	*0.53*	*0.69*	*0.85*	*1.05*	*1.2*
Activity of C.pictus								
E.coli	---------	*0.46*	*0.53*	*0.53*	*0.66*	*0.78*	*0.85*	*1.18*
P.aeruginosa	----------	*0.21*	*0.24*	*0.65*	*0.74*	*0.84*	*1.08*	*1.21*
B.subtilis	*0.18*	*0.21*	*0.38*	*0.55*	*0.63*	*0.81*	*0.94*	*1.12*
S.areus	*0.2*	*0.25*	*0.41*	*0.54*	*0.69*	*0.79*	*1.12*	*1.26*

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