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Insulin Plant, *Costus speciosus*: Ethnobotany and Pharmacological Updates

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Abstract

This review paper highlights current proven pharmacological research updates of *Costus speciosus*, a traditional medicinal herb known for many phytochemicals and ethnobotanical uses. The rhizome of these plants are used as an alternative source for diosgenin and generally used to control diabetes. Therefore, *Costus speciosus* is commonly called as Insulin plant (antidiabetic plant). *Costus speciosus* showing antidiabetic activity is of significant interest for ethnobotanical community presenting varying degree of hypoglycemic and antihyperglycemic activity. Furthermore, *Costus speciosus* also plays an important role as a herbal medicine for the treatment of various health ailments. In general, plant based medicines are safe and there are no side effects compared to other drugs and more effective treatment of health disorders. Because of the popularity of herbal medicines, there is a growing demand for natural plant based medicines in both developing countries and international market. In India, the herbal preparations of *Costus speciosus* are presently used by local people in tea bags (herbal-chai), supplements, beverages, and in food items (not under any brand names) which regulates body sugar and cholesterol levels. However, due to urbanization, industrialization and increase in commercial demand of *Costus speciosus*, this medicinal plant has been over exploited for its innumerable medicinal properties from natural habitat. Therefore, *Costus speciosus* has become endangered and now-a-days *Costus* species are available only in the wild habitat. Hence conservation of *Costus speciosus* using *in vitro* micropropagation protocols plays a significant role in preserving this endangered plant species in the natural habitat and alternative for the production of valuable secondary metabolite.

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Introduction

Insulin plant, *Costus speciosus* (Koen.) Sm (Family: Zingiberaceae) commonly known as 'Spiral ginger' is a rhizomatous perennial herb with pinkish white flowers in reddish bracts (Malabadi, 2002, 2005; Malabadi et al., 2004, 2005a, 2012b; Malabadi and Vijayakumar, 2005; Prakash and Mehrotra, 1996). It is distributed below 1500m altitude in tropical forests throughout India (Malabadi, 2002, 2005; Malabadi et al., 2004, 2005a, 2012b). *Costus speciosus* (Pushpamoola in Kannada) flowers during the months of July and August, and the aerial parts withering away during winter months (Rajesh et al., 2009). The plant serves as an ornamental and the rhizome serves as a source of antihelmintic compounds and an alternative source of diosgenin along with tigogenin, dioscin and gracillin (Chopra et al., 1956; Dasgupta and Pandey, 1970; Rathore and Khanna, 1978; Malabadi, 2002, 2005; Gupta et al., 1981; Malabadi et al., 2004, 2005a, 2012b; Prabhu et al., 2014).

In Ayurveda, *Costus speciosus* is used to subdue *vata* and *kapha* and promotes complexion (Rani et al., 2012). Insulin plant, *Costus speciosus* is a traditional medicinal herb with various well known pharmacological actions such as antidiabetic, hypolipidemic, anticholinesterase, hepatoprotective, antioxidant, adaptogenic, diuretic, antibacterial, antifungal, antiviral, antifertility, estrogenic, anticariolytic, antispasmodic, anti-inflammatory, antituberculosis, carminative, digestive, antiseptic, antipyretic activities, ecbolic agent, and anabolic properties (Srivastava et al., 2011a, 2011b; Prabhu et al., 2014; Daisy et al., 2008; Rajesh et al., 2009; Eliza et al., 2009a, 2009b; Sulakshana and Rani, 2014; Rajesh et al., 2009; Daisy et al., 2008; Prabhu et al., 2014; Vijayalaxmi and Sarada, 2008; Bhattacharya et al., 1972; Singh and Srivastava, 1992). It is also used locally for diabetes and jaundice (Srivastava et al., 2011a; Bhuyan and Zaman, 2008; Malabadi et al., 2004, 2005b). The plant is used internally for eye, ear infections, diarrhoea (sap from leaves, young stems), cold, catarrhal fever, cough, dyspepsia, treating boils, and formally used in Malaysia for small pox (Srivastava et al., 2011a; Rani et al., 2012).

The root extract acts as an astringent, aphrodisiac, depurative, purgative, anthelmintic, febrifuge, expectorant, and is useful in catarrhal fever, coughs, skin diseases, snake bites, and appetite stimulant properties (Khanna et al., 1977; Rathore and Khanna, 1978; Rastogi and Mehrotra 1991; Srivastava et al., 2011a; Rani et al., 2012; Malabadi et al., 2004, 2005a, 2012b; Malabadi, 2002, 2005; Anonymous, 2007). In addition to this, an alkaloid extracted from *Costus speciosus* rhizomes had a papaverine-like smooth muscle relaxant and antispasmodic activities in laboratory animals (Prabhu et al., 2014; Srivastava et al., 2011a; Rani et al., 2012).

Rhizomes are bitter, astringent, acrid, cooling, aphrodisiac, anthelmintic, depurative, febrifuge, expectorant, tonic and useful in burning sensation, constipation, pneumonia, leprosy, gout rheumatism, bronchial asthma, dropsy, urinary diseases, jaundice cooling and relief from headache, to treat mental disorders, worm infection skin disease, improves digestion, act as stimulant, and a herb that clears toxins (Srivastava et al., 2011a; Verma and Khosa, 2012; Prabhu et al., 2014). Rhizomes exhibit cardiotonic, hydrochloretic, diuretic and CNS depressant activities (Srivastava et al., 2011a; Prabhu et al., 2014). Rhizome juice is given with sugar internally to treat leprosy, used as antivermin and for abortion (Srivastava et al., 2011a; Rani et al., 2012). Diosgenin content up to 3.37% has been reported in rhizome of *Costus speciosus* which is a steroidal sapogenin used for synthesis of sex hormones, cortisone and oral contraceptives (Roy and Datta, 1977; Sarin et al., 1974, Sarin et al., 1982; Indrayanto and Setiawan, 1994; Selim and Jaouni, 2015). Diosgenin is a precursor of sex hormones (progesterone), corticosteroids (corticosone) and contraceptives (Selim and Jaouni, 2015). Leaf infusion or decoction is utilized as a sudorific or in a bath for patients with high fever (Srivastava et al., 2011a). Bruised leaves of *Costus speciosus* were also applied in fever; decoction of stem is used in fever and dysentery (Srivastava et al., 2011a; Rani et al., 2012). Leaves are used for scabies and stomach ailments (Srivastava et al., 2011a; Rani et al., 2012).

Rhizomes are the major source of diosgenin, curcumin and curcuminoids (Rani et al., 2012; Srivastava et al., 2011a). Various compounds like α-amyrinsterate, β-amyrin and lupeol Palmitates were isolated from the leaves of *Costus speciosus* (Pai and Kulkarni, 1977; Rani et al., 2012; Srivastava et al. 2011a). Two new quinones dihydro phytyl plasto quinone and its 6-methyl derivatives and α- tocopherol were isolated from seeds (Gupta et al., 1981; Rani et al., 2012; Srivastava et al., 2011a). Five new compounds-tetradecyl 13-methyl pentadecanoate, tetradecyl-11-methyltridecanoate, 14-oxotricosanoic acid, 14-oxoheptacosanoic acid and 15-oxooctacosanoic acid were isolated from rhizomes (Gupta et al., 1981; Rani et al., 2012; Srivastava et al., 2011a). From the roots, 31-norcycloartanone, cycloartanol, cycloartenol and cyclolaudenol were isolated (Rastogi and Mehrotra, 1999). Methyl 3-(4-hydroxyphenyl)-2E propenoate was isolated from rhizomes (Pai and Kulkarni, 1977; Srivastava et al., 2011a). Seed oil (6.0%) consists of palmitic acid (55.97%), oleic acid (23.75%), linoleic, stearic, myristic and lauric acids (Rani et al., 2012; Srivastava et al., 2011a). Defatted seeds contained diosgenin, glucose, galactose and rhamnose. Tubers and roots of *Costus speciosus* contain 5α-stigmasten-3b-ol, sitosterol-β-D-glucoside, dioscin, prosapogenins A and B of dioscin, gracillin and quinines (Mahato et al., 1980; Rani et al., 2012; Srivastava et al., 2011a). Tigogenin and diosgenin (2.6%) have been isolated from rhizomes (Gupta et al., 2008; Rani et al., 2012; Srivastava et al., 2011a). Saponins were also reported from rhizomes, including seeds and roots. Saponins isolated from seeds were reported to possess hypotensive and spasmolytic effect (Mahmood et al., 1984; Rani et al., 2012; Srivastava et al., 2011a). Two active sesquiterpenoids, costunolide and eremanthin, have been isolated from hexane extract of *Costus speciosus* (Prabhu et al., 2014). Eremanthin isolated from *Costus speciosus* possesses hypoglycemic and antihyperlipidimic activities (Eliza et al., 2009a, 2009b).

Costus speciosus: *In vitro* micropropagation

Costus speciosus is an important medicinal plant widely used in several indigenous systems of medicine for the treatment of various ailments (Rani et al., 2012; Srivastava et al., 2011a). The use of herbal preparations made from medicinal plants is widespread in developing countries (Rani et al., 2012; Srivastava et al., 2011a). The plant is conventionally propagated by vegetative techniques using rhizome and sucker segments and through seeds (Malabadi, 2002; Malabadi et al., 2004; Malabadi et al., 2005b, 2012b; Srivastava et al., 2011a). These techniques are very slow for large-scale propagation. Due to indiscriminate collection of its medicinal rhizome, *Costus speciosus* is rapidly disappearing from its natural habitat. Roy and Pal (1991, 1995), and Chaturvedi et al. (1984) reported shoot multiplication in *Costus speciosus* but only limited success has been achieved and the described protocol gives rise to a very low percentage of shoot regeneration. However, the existence of this species has been threatened due to deforestation, jhum cultivation, habitat disturbance for conversion of wetland ecosystem into agricultural land and uncontrolled plucking/uprooting of these plants (Punyarani and Sharma, 2010). Therefore, there is an urgent need to develop methods for the mass propagation and conservation of this Threatened/Endangered species (Malabadi, 2002; Malabadi et al., 2004; Malabadi et al., 2005a, 2012b; Robinson et al., 2009; Punyarani and Sharma, 2010). Conservation of herbal medicine is very important for the welfare of rural and tribal communities for the treatment of conventional illness (Roy and Pal, 1991, 1995; Srivastava et al., 2011a; Rani et al., 2012).

The successful regeneration of 4-5 shoots was observed from the *in vitro* cultured rhizomes of *Costus speciosus* (Malabadi, 2002). In this study, only rhizomes cultured on modified MS basal medium supplemented with 8.87 μM BA, 9.29 μM KN and 5.37 μM NAA (shoot induction medium) induced shoot formation (Malabadi, 2002). Further elongation was observed when newly formed shoot buds along with parental rhizome were cultured on modified MS basal medium supplemented with 8.87 μM BA, 9.29 μM KN and 5.37 μM NAA and 100 mg l^{-1} adenine sulphate with 10% coconut water (Malabadi, 2002). In another study, the best shoot regeneration was achieved in *Costus speciosus* by using thin rhizome sections. Efficient initiation of shoot buds was observed when thin rhizome sections were cultured on modified Gamborg-B5 basal medium (1968) supplemented with 18.16μM thidiazuron (Malabadi et al., 2004). Shoots with two to three leaves were successfully rooted on modified half strength Gamborg-B5 basal medium containing 5.37μM NAA. The rooted plantlets were transferred to soil and acclimatised showing normal growth. Survival rate was 100% (Malabadi et al., 2004). Furthermore a study conducted by Malabadi et al. (2005a) also reported that the highest percentage of shoot regeneration of *Costus speciosus* using thin rhizome sections and triacontanol (TRIA) (Malabadi et al., 2005a). According to this study, initiation of shoot buds was observed when rhizome thin sections were cultured on B5 basal medium supplemented with 5μg^{-1} TRIA (Malabadi et al., 2005a). Shoots with two to three leaves produced roots when cultured on B5 basal medium supplemented with 2 μg^{-1} TRIA (Malabadi et al., 2005a). The well-rooted shoots were hardened and transferred to soil where they showed normal growth and a 100% survival rate was achieved. Therefore, this study showed that TRIA can be used as an effective growth regulator in the micropropagation and conservation of *Costus speciosus* (Malabadi et al., 2005a).

Nodal segments of *Costus speciosus* containing single axillary buds were cultured on Murashige and Skoog medium (MS medium) supplemented with plant growth regulators for inducing plantlets (Punyarani and Sharma, 2010). The most effective media for breaking axillary bud dormancy was 5 µM BAP, 1 µM NAA, 50 gl^{-1} sucrose and 10 µM AdS supplemented medium (Punyarani and Sharma, 2010). The propagules from 40-70gl^{-1} sucrose produced roots in shoot multiplication medium, that 10 µM AdS, 1 µM NAA, 50gl^{-1} sucrose and 3-11 µM BAP supplemented medium (Punyarani and Sharma, 2010). The best response for shoot multiplication was on 10 µM AdS, 1 µM NAA, 50g l^{-1} sucrose and 7 µM BAP (Punyarani and Sharma, 2010). The well-rooted shoots were hardened and transferred to the soil where they showed 95% survival rate (Punyarani and Sharma, 2010). These results confirmed that axillary bud can be used for the micropropagation of *Costus speciosus* (Punyarani and Sharma, 2010).

Costus speciosus: Pharmacological activities

1) *Costus speciosus* had been documented as an antidiabetic plant (Insulin plant) in Indian Ayurvedic literature (Daisy et al., 2008; Rajesh et al., 2009; Sulakshana and Rani, 2014). Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels (Srivastava et al., 2011a). Hyperglycemia is the main cause of complications such as coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and premature death (Srivastava et al., 2011a; Sulakshana and Rani, 2014). The rhizome is the major source of diosgenin, which is anti-diabetic in nature and is used in the treatment of diabetes mellitus (Rani et al., 2012; Sulakshana and Rani, 2014). Species of *Costus* are known to contain a steroidal saponin-diosgenin as a major bioactive component, which is utilized as a precursor for the synthesis of various drugs (Srivastava et al., 2011a; Sulakshana and Rani, 2014; Shetty et al., 2010). Because of its immense potential in antidiabetic properties, systematic study was conducted with an objective to evaluate the antihyperglycemic activity of petroleum ether, chloroform, methanolic, and aqueous extracts of *Costus speciosus* rhizomes on over night fasted, Streptozotocin (STZ) induced diabetic rats (Rajesh et al., 2009). Furthermore, antihyperglycemic activity of a drug is the ability of the drug to lower very high blood sugar levels to acceptable lower levels (Rajesh et al., 2009). During this experimental study, blood glucose level (BGL) monitored at 0, 30, 60, 120 and 240 minutes confirmed that all extracts of *Costus speciosus* resulted in the reduction of blood glucose level (BGL) significantly except pet ether extract (Rajesh et al., 2009). Aqueous extract and methanolic extracts reduced initial blood glucose level (BGL) of 387 to 120 mg/dl and 303 to 161 mg/ dl respectively at the end of 240 minutes (Rajesh et al., 2009). Therefore, the aqueous extract and methanolic extracts of *Costus speciosus* were highly effective in bringing down the blood glucose level (BGL) from 590 to 96 mg/dl and 570 to 128 mg/dl respectively at the end of 240 minutes, which was on par with the glibenclamide (Rajesh et al., 2009). Results from multiple dose studies wherein the drug was administered for 14 days also confirmed the above findings and the serum lipid profiles high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were found to be optimum in aqueous or methanolic extracts on par with normal healthy rats or standard drug glibenclamide treated rats (Rajesh et al., 2009).

2) Diuretics, either alone or in combination with other drugs are valuable in the treatment of hypertension, congestive heart failure, ascites and pulmonary edema (Prabhu et al., 2014). Diuresis has two components: increase in urine (water secretion) and a net loss of solutes (i.e. electrolytes) in the urine (Prabhu et al., 2014). However, two widely used diuretics, thiazides and the high ceiling loop diuretic, furosemide, have been associated with a number of adverse effects, such as, electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the renin-angiotensin-neuroendocrine systems and impairment of sexual function (Prabhu et al., 2014). Therefore, there is an attempt to discover the newer drugs with lesser adverse effects is ongoing, and herbal medicines plays an important role in controlling the human health disorders. Recently a study was conducted to explore the diuretic activity of aqueous-ethanolic extract of the leaves of *Costus speciosus* in healthy Wistar albino rats (Prabhu et al., 2014). This study was conducted in saline primed Wistar albino rats (n=6) using frusemide (20 mg/kg per oral) as the reference diuretic drug with two oral doses of aqueous-ethanolic extract of *Costus speciosus* 200mg/kg and 400mg/kg respectively (Prabhu et al., 2014). Urine volume and electrolytes (Sodium, Potassium and Chloride) excretion was estimated at the end of 24 hours (Prabhu et al., 2014). This study showed that *Costus speciosus* extract significantly increased the volume of urine (10 ± 3ml/100g/24hr and 14 ±2.5ml/100gm/24hr), increasing the diuretic index to 1.25 and 1.75 for 200mgkg and 400mg/kg dose ranges respectively ($p<0.05$). The test drug, when compared to the control group, showed a significant increase in the excretion of sodium,

potassium and chloride excretion (Prabhu et al., 2014). The regulation of sodium, potassium balance is also intimately related to renal control of acid-base balance. The potassium loss that occurs with many diuretics may lead to hypokalemia (Prabhu et al., 2014). However, the electrolyte secretions were less when compared to frusemide indicating a weaker saluretic action. Therefore, these findings supported the use of *Costus speciosus* as a diuretic agent with an action similar to that of the loop diuretic, frusemide (Prabhu et al., 2014).

3) In another report, a study on secondary metabolite of *Costus speciosus* has been confirmed (Singh et al., 2013). Rhizome has been exploited for the extraction of an important bioactive metabolite diosgenin (up to 3.37% in the rhizome). Singh and co-workers (2013) reported the extraction of diosgenin from subcultured callus derived from *in vitro* derived explants of *Costus speciosus* (Singh et al., 2013). The presence of diosgenin in the callus was analyzed by performing TLC analysis observed under UV light (Singh et al., 2013). This work might help in generating a cost effective diosgenin extraction from an alternative source other than *Dioscorea* without uprooting the plant (Singh et al., 2013). During this diosgenin extraction method, dried powder of callus were ground with a 0.8 mm mesh sieve and dried for 2.5 h with a convection oven set at 60°C (Singh et al., 2013). The samples were defatted in a Soxhlet apparatus for 12 hrs with petroleum ether (bp 40-60°C) as solvent and double thickness cellulose extraction thimbles (Whatman) (Singh et al., 2013). The material in the thimble was air-dried, and then oven-dried at 60°C for 2hrs (Singh et al., 2013). Samples were stored in a desiccator at room temperature for overnight (Singh et al., 2013). Extraction experiments with (aqueous) 2-propanol were conducted in triplicate trials with 5 mL of solvent, heated at 80°C for 3 hrs in a capped test tube, followed by acid hydrolysis with sulfuric acid in 70% 2-propanol (Singh et al., 2013). The combined extracts were evaporated to dryness on a water bath. Dried sample dissolved in chloroform and ethanol and loaded on TLC plate (Taylor et al., 1997; Singh et al., 2013).

4) The leaf and rhizome extract juice of *Costus speciosus* were used with sugarcane juice along with other herbs to cure jaundice and other related liver diseases (Bhuyan and Zaman, 2008). Therefore, a study conducted to see if *Costus speciosus* alone offers hepatoprotection or not (Bhuyan and Zaman, 2008). During this study reported by Bhuyan and Zaman (2008), the methanolic extract of the rhizomes of *Costus speciosus* was evaluated for hepatoprotective activity by observing its effects on carbon tetrachloride (CCl4) induced hepatotoxicity in liver histoarchitecture and alteration in certain biochemical parameters (Bhuyan and Zaman, 2008). The biochemical parameters studied were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin and total protein (Bhuyan and Zaman, 2008). The crude extract was administered through the intraperitonial route in two dose groups, a lower dose group receiving 50mg/kg body weight/day and a higher dose group receiving 100mg/kg body weight/day (Bhuyan and Zaman, 2008). The crude extract administration led to reversal of the altered biochemical parameters in the group receiving the higher dose (Bhuyan and Zaman, 2008). These experimental results were compared with Silymarin, which was used as a standard, and hereby confirmed the presence of hepatoprotective activity in the methanolic extract of the rhizomes of the *Costus speciosus* (Bhuyan and Zaman, 2008).

5) Phytochemical evaluation of medicinal plants is one of the basic tool in the ethanobotanical study (Verma and Khosa, 2012). Furthermore, high performance thin layer chromatography (HPTLC) has been emerged as an important tool for the qualitative, and semi-quantitative phytochemical analysis of herbal drugs and formulations (Verma and Khosa, 2012). This includes developing a thin layer chromatography (TLC) fingerprint profiles, and estimation of chemical markers and biomarkers (Verma and Khosa, 2012). During HPTLC quantification, diosgenin was used as a standard marker and its concentration has been estimated in the ethanolic extract of the *Costus speciosus* (Verma and Khosa, 2012). A phytochemical evaluation study of *Costus speciosus* confirmed that the rhizome showed a special type of periderm called “storied cork” (Verma and Khosa, 2012). It consists of several, normally oblong, tangentially stretched, thin walled phellom cells (Verma and Khosa, 2012). There are numerous vascular bundles scattered throughout the cortical zone. Each bundle has a cluster of four or five narrow angular xylem elements and a smallest of phloem elements (Verma and Khosa, 2012). Calcium oxalate crystals of rhomboidal, cuboidal and cylindrical in shape are common in the ground parenchyma cells (Verma and Khosa, 2012). The results of analysis were validated in terms of accuracy and precision. The LOD and LOQ were found to be 5.69 ng and 17.25 ng/spot respectively. Therefore, the pharmacognostical and HPTLC profile of the rhizomes of *Costus speciosus* will assist in standardization for quality, purity and sample identification (Verma and Khosa, 2012).

6) Helminthiasis, or worm infestation (caused by various species of parasitic helminthes worms), is one of the most common disease in the developing countries and also considered as the most serious public health concern in the world (Srivastava et al., 2011a). According to the WHO study, half of the world's population particularly children are most at risk may be infected with gastrointestinal helminthes (Srivastava et al., 2011a). Almost 350 species of helminthes have been found in humans, and most colonise the gastrointestinal tract (Srivastava et al., 2011a). But others, such as schistosomiasis (bilharzia) and hookworm disease, can produce very serious morbidity (Srivastava et al., 2011b). Because of its prevalence, the problem of the treatment of helminthiasis is therefore one of very great practical therapeutic importance (Srivastava et al., 2011a). The anthelmintic activity of methanolic (25 mg/ml, 50 mg/ml and 100 mg/ml) and aqueous extracts (25 mg/ml, 50 mg/ml and 100 mg/ml) of the aerial parts of *Costus speciosus* was evaluated using Indian adult earthworms (*Pheretima posthuma*) as an experimental worms (Srivastava et al., 2011a). The aqueous extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of methanolic extract when compared with standard (Srivastava et al., 2011a). In case of the methanolic extract at 25 mg/ml, 50 mg/ml and 100 mg/ml concentrations, paralysis was observed at 8.10±0.37, 3.97±0.40 and 2.72±0.26 min respectively and death at 8.88±0.40, 4.78±0.32 and 3.70±0.45 min respectively (Srivastava et al., 2011a). The aqueous extract at dose of 25 mg/ml, 50 mg/ml and 100 mg/ml produced paralysis within 6.70±0.33, 3.62±0.30 and 2.55±0.27 min respectively while death was observed within 7.48±0.32, 4.48±0.31 and 3.62±0.29 min respectively (Srivastava et al., 2011a). The standard drug albendazole (20 mg/ml) showed paralysis at 11.65±0.51 min and death occurred after 13.67±0.36 min (Srivastava et al., 2011a). These experimental data showed significant anthelmintic activity of *Costus speciosus*, and hence it can be used as a promising anthelmintic agent (Srivastava et al., 2011a).

7) In another study, the antimicrobial activity and qualitative phytochemical evaluation of *Costus speciosus* has been reported (Arunprasath and Gomathinayagam, 2014). *In-vitro* antimicrobial activity of *Costus speciosus* was evaluated by disc diffusion method, and qualitative phytochemical constituents were analyzed by Harborne method (Arunprasath and Gomathinayagam, 2014). This study concluded that antimicrobial activity of Gram negative bacterial strain *Salmonella typhi* showed maximum inhibition at the concentration of 100 mg (24mm) in methanolic extract (Arunprasath and Gomathinayagam, 2014). Qualitative evaluation of *Costus speciosus* showed that maximum amount of phytochemical compounds in leaves by methanolic extract than the petroleum ether extract (Arunprasath and Gomathinayagam, 2014). In leaves, more amount of saponins were present (Arunprasath and Gomathinayagam, 2014). Therefore, these experimental results revealed that the leaves of *Costus speciosus* has high potent against Gram negative bacterial strain *Salmonella typhi* due to the presence of high degree concentration of saponins (Arunprasath and Gomathinayagam, 2014).

8) Furthermore, another experimental study which also supported the claim by traditional healers that the extract of methanolic and ethyl acetate extracts of *Costus speciosus* roots have anticancer activities (Baskar et al., 2012). Anticancer activity has been partially validated by identifying the extracts in the plants and their potent proapoptotic activity (Baskar et al., 2012). Therefore, *Costus speciosus* plant extracts has the potential sources for anticancer drug discovery (Baskar et al., 2012).

9) A study was conducted to investigate the genotoxic and/or antigenotoxic effect of aqueous extract of the medicinal plant *Costus speciosus* which is used frequently for the treatment of various disorders in Saudi Arabia (Qari, 2010). In this study, root tip meristems of *Allium cepa* were treated with elevated concentrations of *Costus speciosus* (Qari, 2010). Samples were taken at 48 hrs of each concentration (2, 5, 10, 20 µg/mL) and subjected to cytogenetic and molecular genetics assays (DNA-RAPD fingerprinting) (Qari, 2010). It was found that the extract has no clastogenic or mutagenic activities, mitotic index decreased mildly compared with the control (Qari, 2010). Frequency of chromosomal aberrations showed no significance in all concentrations at time of exposure (Qari, 2010). Most aberrations of the dividing cells were disturbance of chromosomes (Qari, 2010). The RAPD results demonstrated that monomorphic numbers of genetic bands mostly, which were the electrophoretic products of PCR for all treatments compared with the control (Qari, 2010). Also the results exhibited the ability of *Costus speciosus* as antigenotoxic and anticytotoxic potential against EMS induced DNA damage, cytotoxic and clastogenic effects in *Allium cepa* cells (Qari, 2010). Therefore, these experimental data showed that *Costus speciosus* extract could not induce significantly genotoxic effect on *Allium cepa* cells (Qari, 2010). Furthermore, this study implies

that combined treatment of *Costus speciosus* has a strong inhibitory role against the genotoxic action of EMS (Qari, 2010). Hence this study strongly concluded that the extract of *Costus speciosus* is not genotoxic, or cytotoxic but might be anti-genotoxic agent (Qari, 2010).

10) In addition to this, *Costus speciosus* alkaloids have been shown to possess both *in vitro* and *in vivo* anticholinesterase activity (Srivastava et al., 2011a). The ethanolic extract of the rhizome of *Costus speciosus* possesses anti-inflammatory and antipyretic properties (Binny et al., 2010; Srivastava et al., 2011a). Anti-inflammatory property was studied in carrageenan induced paw edema and cotton pellet induced granuloma formation (Binny et al., 2010; Srivastava et al., 2011a). Significant anti-inflammatory effect was found against carrageenan induced edema formation in rats at a dose of 800 mg/kg and against cotton pellet granuloma formation in rats at doses of 400 mg/kg and 800 mg/kg (Binny et al., 2010; Srivastava et al., 2011a). The antipyretic property was studied in yeast-induced pyrexia in rats (Binny et al., 2010; Srivastava et al., 2011a).

11) A mixture of saponin isolated from the rhizomes of *Costus speciosus* effectively protected against pregnancy in rats, when fed at 5-500 μg/100 g body wt. for 15 days (Tewari et al., 1973; Srivastava et al., 2011a). Therefore, saponin mixture showed antifertility activity in rats (Tewari et al., 1973; Srivastava et al., 2011a). It is concluded that *Costus speciosus* root extract possesses anti-hyperglycemic, antihyperlipemic and antioxidative effects, which might prove to be of clinical importance in the management of diabetes and its complications (Bavarva and Narasimhacharya, 2008; Srivastava et al., 2011a).

12) The effect of freeze-dried rhizome juice of *Costus speciosus* on body weight, liver and kidneys of normal and STZ-induced diabetic rats were studied (Bavarva and Narasimhacharya, 2008; Srivastava et al., 2011a). Plant derived antioxidants play a very important role in alleviating problems related to oxidative stress (Girgis et al., 2015). Therefore, protective effect of the medicinal plant *Costus speciosus* against genotoxicity and histopathological alterations of streptozotocin (STZ)-induced diabetes mellitus in male rats (Girgis et al., 2015). The results of this experimental study revealed that the treatment with STZ induced high frequency of micronuclei, DNA damage, chromosomal aberrations and histopathological changes (Girgis et al., 2015). However, costus or nanocostus treatment reduced all these parameters especially with 50 mg/kg between nanocostus (Girgis et al., 2015). This study proved that treatment with *Costus speciosus* has a strong inhibitory effect against the genotoxicity and histopathologic alterations induced by STZ. In conclusion, these results strongly suggested that the extract of *Costus speciosus* might be anti-genotoxic, and anti-histopathologic agent indicating the protective nature of these compounds (Girgis et al., 2015).

13) A recent experimental study concluded that the essential oil from the rhizome of *Costus speciosus* from Kerala, India were obtained by steam distillation and analyzed by GC-FID and GC-MS showed considerable variation in composition to those belonging to other global regions (Thambi and Shafi, 2015). A total of twenty six compounds were identified from the oil accounting for 97.82% of its contents (Thambi and Shafi, 2015). The major components of the oil are α-humulene and zerumbone which were already reported for their potential anticancer properties (Thambi and Shafi, 2015). The oil also possesses considerable *in vitro* anti-bacterial activity against gram positive bacteria and gram negative bacteria (Thambi and Shafi, 2015). Medicinal use of the plant could be related with the biological properties of the individual compounds present in the oils (Thambi and Shafi, 2015).

14) The rhizome extract of three *Costus species* (*Costus speciosus, Costus pictus* and *Costus igneus*) were studied for their antibacterial activity against gram positive bacteria *Bacillus subtilis* (ATCC 633), *Staphylococcus aureus* (ATCC 9144) and gram negative bacteria *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC25619) (Sulakshana et al., 2013). The rhizome extract of three species inhibited the growth of all the test bacteria (Sulakshana et al., 2013). The zone of inhibition increased with increase in concentration of the test solution (Sulakshana et al., 2013). High inhibition zone was observed in *Costus pictus* against *Staphylococcus aureus* (1.26 cm) followed by *Pseudomonas aeruginosa* (1.21 cm) followed by *Escherichia coli* (1.18cm). *Bacillus subtilis* showed low inhibition zone (1.12 cm). In *Costus speciosus*, the zone of inhibition ranged from 1.25 cm for *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* (1.21cm), *Escherichia coli* (1.15 cm) and *Bacillus subtilis* (1.10 cm) (Sulakshana et al., 2013). Higher antibacterial activity of *Costus igneus* rhizome extract was found against *Staphylococcus aureus* (1.20 cm), followed by *Pseudomonas aeruginosa* (1.18cm), *Escherichia coli* (1.11 cm) and *Bacillus subtilis* (1.0 cm) (Sulakshana et al., 2013).

15) In another report, Nair et al. (2014) indicated that phytochemicals of leaves of *Costus speciosus* showed potentiality for natural therapeutic product development for human hepatocellular carcinoma (Nair et al., 2014). The human hepatocellular carcinoma (HepG2) cells treated with 100 µg/mL methanol leaf extract of *Costus speciosus* for 24 hrs displayed a significant reduction in cell viability ($p \leq 0.05$) (Nair et al., 2014). The methanol extract perturbed cell cycle progression, modulated cell cycle and regulated, signal molecules were involved in induction of apoptosis in human hepatocellular carcinoma (HepG2) cells (Nair et al., 2014). This is the first report which demonstrated *in vitro* anticancer activity of leaf extract of *Costus speciosus* in relation to liver cancer (Nair et al., 2014). Leaves of *Costus speciosus* were used for Ayurvedic treatment regimes in malignancies and mental illness (Nair et al., 2014). Rhizome extract from the *Costus speciosus* plant is used to treat malignancies, pneumonia, urinary disorders, jaundice, rheumatism and diabetes (Nair et al., 2014).

The major chemical constituents of *Costus speciosus* are diosgenin, curcumin and curcuminoids (Rani et al., 2012). The rhizome and roots also contain saponins, 5α-stigmasten- 3b-ol, sitosterol-β-D-glucoside, dioscin, prosapogenins A and B of dioscin, gracillin, and quinines (Rani et al., 2012). Diosgenin is reported to induce apoptosis in human leukemia cells (Liu et al., 2005). Curcumin and curcuminoids are well known for their chemotherapeutic property on solid cancer as well as leukemia (Liu et al., 2005; Rani et al., 2012; Nair et al., 2014). Hence experimental evidences reported by Nair et al. (2014) were valuable for identifying plant-based chemotherapeutic drugs as currently used liver cancer drug sorafenib causes many side effects. Furthermore, this study was the first report to identify the remarkable anticancer activity of methanolic extract of *Costus speciosus* leaves in liver cancer (Nair et al., 2014).

16) Another similar study was conducted to investigate the consequences of long-term use of *Costus speciosus* leaf aqueous extract (CSlwex) with respect to hepatic and renal functions of Wistar rats (Subasinghe et al., 2015). In this experimental study, Wistar rats (170-250 g) were divided into 9 groups (n=5 each) and labelled as A to I (Subasinghe et al., 2015). Insulin resistance was induced in six groups D to I. *Costus speciosus* leaf aqueous extract oral treatment was conducted for 12 weeks as given next (Subasinghe et al., 2015). After the therapy, serum ALT, AST and creatinine were analyzed. Histopathology of liver and kidney were assessed for toxicity. No significant increase in ALT (34.77±6.19 IU/L, p=0.304) or AST (137.55±9.83 IU/L, p=0.928) were found in insulin resistant rats (Subasinghe et al., 2015). Also, *Costus speciosus* leaf aqueous extract did not change ALT and AST significantly in healthy rats (Subasinghe et al., 2015). Serum creatinine of either insulin resistant or healthy rats treated with CSlwex did not increase significantly compared to the respective controls (insulin resistant- 34.53±1.38 µmol/L; healthy- 42.56±3.27 µmol/L) (Subasinghe et al., 2015). No features of liver or renal toxicity were observed histopathologically in CSlwex treated rats or controls (Subasinghe et al., 2015). Therefore, this experimental study concluded that 1500-3000 mg/kg doses of *Costus speciosus* leaf aqueous extract does not create or initiate hepatic or renal toxicity after 12 weeks continuous therapy (Subasinghe et al., 2015). Furthermore, the leaf extract of *Costus speciosus* can be recommended for daily use up to 12 weeks in both normal and IR-induced rats (Subasinghe et al., 2015).

17) *Costus speciosus* rhizome is effective in lowering lipid profile (Eliza et al., 2009a; Bavarva and Narasimhacharya, 2008; Subasinghe et al., 2014). Hexane extract of *Costus speciosus* rhizome had elevated the high density lipoprotein cholesterol (HDL-C) while reducing the low density lipoprotein cholesterol (LDL-C) levels in diabetic rats (Eliza et al., 2009a; Bavarva and Narasimhacharya, 2008; Subasinghe et al., 2014). In addition to this, 450 mg/kg *Costus speciosus* rhizome ethanolic extract being the best dose, showed a reduction in both plasma, and hepatic total lipids, as well as total cholesterol and triglycerides (Eliza et al., 2009a; Bavarva and Narasimhacharya, 2008; Subasinghe et al., 2014). Furthermore, two compounds such as costunolide and eremanthin had individually elevated serum HDL-C and decreased total cholesterol, triglyceride and LDL-C in diabetic rats (Eliza et al., 2009a; Bavarva and Narasimhacharya, 2008; Subasinghe et al., 2014).

18) Another study demonstrated for the first time, the efficiencies of *in vitro* derived plants and callus cultures of *Costus speciosus* extract could be used in the rapid biosynthesis of stable silver nanoparticles (Malabadi et al., 2012b). Synthesis of silver nanoparticles may be influenced directly or indirectly by phytochemicals in plants such as phenolics, flavonoids, and diosgenin compounds. This study also highlighted a cost effective and environment friendly technique for green synthesis of silver nanoparticles (Malabadi et al., 2012a, 2012b, 2012c, 2012d). These silver nanoparticles were found to be highly toxic against different multi drug resistant clinical samples such as gram-positive bacteria *Bacillus*

subtilis and *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* (Malabadi, 2005; Malabadi and Vijayakumar, 2005; Malabadi et al., 2012b). This has provided the evidence for developing a large scale commercial production of value-added products for biomedical / nanotechnology based industries, which is an important step in the field of application of nanotechnology (Malabadi et al., 2012a, 2012b, 2012c, 2012d). In another similar study, Malabadi (2005) also concluded that the hexane, methanol and aqueous extracts of leaf and rhizomes of *Costus speciosus* were used by Indian traditional healers for treating skin diseases, diabetes, jaundice, snake bites and/or anti-inflammatory properties and was screened for *in vitro* antibacterial activities against pathogens isolated from infected burn patients (*Shigella*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas*, *Bacillus subtilis* and *Salmonella*) (Malabadi, 2005). No antibacterial activity was recorded with water extracts (Malabadi, 2005). The disc-diffusion method showed a significant zone of lysis against all the pathogens studied (Malabadi, 2005; Malabadi and Vijayakumar, 2005, 2007, 2008; Malabadi et al., 2005b; Malabadi et al., 2007; Malabadi et al., 2010).

Conclusion

This review paper justifies and updated pharmaceutical, phytochemical and ethnobotanical properties of medicinal plant *Costus speciosus*. The plant is widely used in traditional system of medicine such as Ayurveda for the treatment of many health disorders including diabetes. Micropropagation of *Costus speciosus* via *in vitro* shoot multiplication played an important role at a commercial scale would help to meet current market demands of herbal medicine supply. There are many ongoing efforts and reports from various research laboratories throughout India working on herbal medicine preparations, characterization, documentation and preservation and conservation of different species of *Costus* for the future generation. This has opened a new ray of hope and justification of the age old traditional Indian herbal medicine resources. However, in a novel drug research discovery, a separate study of toxicity level of optimum dose, lethal dose should also be reported for every herbal medicine including *Costus speciosus* since herbal medicines contains many phytochemicals which affects liver and kidney functions. In general, consumption of herbal medicines has no side effects but higher concentration (lethal dose) and period of consumption might affect body functions. Till today, there are no reports of *Costus speciosus* herbal preparations or any *Costus speciosus* isolated compounds triggering the activity of human dendritic cells (DEC25) increasing the immunity towards viral fevers. Therefore, a future study should also be conducted to demonstrate and conclude the effect of *Costus* species and its compounds in controlling and increasing immunity against deadly viral fevers such as dengue, chikungunya, Ebola and Zika, and and mosquito-borne infectious plasmodium type disease Malaria too. Finally, we hereby conclude that insulin plant, *Costus speciosus* is not only a valuable but an important cheap herbal medicine with multiple pharmacological activities controlling many health disorders.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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