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Pharmacological potency of crude extracts of *Costus Igneus*

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Abstract: *Costus igneus*, commonly known as the spiral flag, is a member of the *Costaceae* family and is primarily cultivated as an ornamental plant in India, it has gained recognition as the "insulin plant" as its ability to stimulate insulin production with overcoming demand for herbal remedies to address diabetes mellitus and the side effects associated with oral hypoglycemic agents, there is growing interest in exploring plant-based solutions. This review article explores the phytochemical properties of *Costus igneus*, its potential as an antidiabetic agent, its antibacterial activities, etc. Additionally, cytotoxic assays are discussed as part of the preliminary investigation into the plant's medicinal properties. The whole part of the plant is isolated with petroleum ether, chloroform, ethanol, water and the crude extracts have been tested for various pharmacological activities.

Keywords: Anti-diabetic agent *Costus igneus*, *Costaceae*, hypoglycemic, insulin plant.

Introduction

The botanical name *Costus igneus*, known as "katar kata" in Sanskrit, is commonly referred to as "Fiery *Costus*" or "Spiral Flag." This plant species is a member of the *Costaceae* family and can be found in torrid region of Africa, Asia, and Australia¹ and also in the coastal regions of India, such as the Uttar Kannada district in the state of Karnataka, *Costus igneus* is cultivated. Local tradition

involves consuming 2-3 leaves of this plant twice daily as a means to manage diabetes. Initial studies of plant extract have detected substantial of various compounds, including carbohydrates, proteins, steroids, alkaloids, tannins, glycosides, saponins, fixed oils, and flavonoids (as reported by Nandhakumar et al. in 2007). Research has also indicated that the flavonoids in the plant exhibit antioxidant properties²⁻³. Recent research on *Costus igneus* has shed light on the antimicrobial properties as well, which can be attributed to the secondary metabolites they produce⁴. These phytochemicals, with potent antibacterial effects, are crucial in combatting bacterial infections, especially in light of antibiotic resistance, and the entire *C. igneus* plant and has traditionally been utilized for its anti-diabetic properties, and its potential to secure the body from diseases, historically rhizome of plant has been used in variety of conditions, such as fever, rash, asthma, bronchitis, intestinal worm infestations, eye ailments, stomach issues, discomfort in the neck and jaw, tongue and mouth disorders, diabetes mellitus and the associated side effects of oral hypoglycemic agents¹. Additionally, it has been employed to address specific health concerns, including fever, edema, dyspnoea (wheezing), hemorrhoids, and spermaturia. In the Siddha medicine system, the root of *C. igneus* has been employed in various forms, including as a powder (chooranam), a decoction (kudineer), and an oil (thylam) and Chemical analysis has confirmed the resiatance of resinoids, essential oils, the alkaloid saussurine, inulin, and resin in *C. igneus*^{6,7}. The growing interest in exploring plant-based preparations from traditional medicinal systems. This insulin plant, originates near to southeast Asia but is now gaining interest of cultivation in India, because of its worldwide recognition as an ayurvedic herb. Furthermore, it is been initiated as ornamental plant, it also being used as dietary supplement in treatment of diabetes. Phytochemicals, bioactive compounds isolated from plants, hold great significance in herbal medicine, that plays a vital role in defining mechanisms and serve protection against several diseases^{8,9}. This research serves as primary investigation, with potential for addition support through more targets.

Plant Description

It is an ancient plant that has growth habitat, spreading and extending itself along the ground. It has simple leaves, oblong, alternate and evergreen with approximate length of 4-8 inches and showcasing parallel veins. It is tropical evergreen plant boats smooth large leaves arranged helically with dark green topsides and attract light purple undersides. These are the features that attract its look and it forms clusters with arching growth from its subterranean rootstocks. In summer days, it is found with pretty 1–5-inch orange flowers that appear in conical shape and clusters at branch tips. The rhizomatous shrub in plants propagates through underneath tuberous rhizomes that displays a

soft, cylindrical form with fleshy consistency & a smooth, pale brown surface that remains within the temperature of approximately 30 to 41 °C. Its fragrance is noteworthy, that is pleasing indigenous to eastern Brazil, especially in Bahia and Santo Espirito, it's also observed in tropical Africa, Asia, Australia and north America. It then enrooted to India from America and was termed as herbal remedy for diabetes in India. *Costus* significantly acquires in regions of south to Kashmir. The spiral flag plant is adaptable to various light conditions, thriving equally well in both full sunlight and partial shade. It flourishes when planted in nutrient-rich soil with ample moisture, typically near a readily accessible water source. For propagation, it may be multiplied through techniques like splitting clumps, taking cuttings, or separating the offsets or miniature plantlets that emerge beneath the flower heads. Potential threats include pests such as mites and nematodes, particularly in sandy soil with low density. Encouragingly, as of now, there are no major diseases known to significantly hinder the growth of this plant¹⁰.



Figure-1 Insulin Plant



Figure-2: Stem of *Costus igneus*

MATERIALS AND METHODS

Chemicals and reagents

The solvent utilised for the extraction and HPLC profiling were commercially purchased from Sigma Aldrich Chemicals, Mumbai. Chemicals were also of analytical grade.

Collection and identification of plant material

The leaves of *Costus igneus* have been collected from the Sahyadri Science college, Shimoga, Karnataka. The plant was identified and authenticated (12-05-2018) at National Institute of Traditional Medicine, Belagavi and was preserved in herbarium (RMRC-1398). Whole plant was washed with water, shade dried at a room temperature and was made into fine powder.

Extraction

Dried powder(500g) was subjected for Soxhlet extraction in 2000 ml of petroleum ether for 40 syphons. The isolation was received in a r. b. flask and evaporated below pressure at 35 °C using rotavapor until all the solvent has been removed to get petroleum ether extract.

Phytochemical analysis

Phytochemical reports were performed to check the phytochemical substituents like, flavonoids, glycosides, alkaloids, phenols, tannins, saponins, steroids, carbohydrates by adapting the standard procedure of Harborne¹⁰

Antimicrobial activity

Two Gram-positive organisms namely *S. aureus* and *E. faecalis* and two Gram-negative organisms namely *E. coli* and *P. aeruginosa* and a fungal strain *Candida albicans* were used. These organisms were collected from Basic Science Research Centre, Belagavi, Karnataka India.⁴

Determination of MIC for bacteria and fungi

Dilution method was used to determine the minimum inhibitory concentration¹⁶. All the extracts of *Costus igneus* have been prepared in these concentrations i.e. 50mg/ml, 25mg/ml, 6.25mg/ml 3.12mg/ml and 1.56mg/ml. 0.1ml of each concentration is added to every 9ml nutrient broth containing bacterial cells and fungal spores. The above tubes were incubated aerobically at 37 °C for one complete day. Positive control was y set up by testing organisms without extract. The tubes with the minimal concentration of extract without growth after incubation were marked and recorded as the MIC¹⁷.

Cytotoxic activity

Cell lines namely Breast adenocarcinoma cell line (MCF-7), lung carcinoma cell line (A-549) and mouse embryo fibroblast (MEF-L929) were retained from the National Centre for Cell Sciences Pune. Cells were subculture in DMEM supplemented at 10% of FBS, 20µg/ml of penicillin and 100µg/ml streptomycin. Incubation was controlled at 37 °C with an atmosphere of 5% CO₂.

MTT assay

Anticancer activity of crude extract of *Costus igneus* on MCF-7 and A-549 cell lines were assessed by the MTT¹⁸⁻¹⁹. The cells were placed at density of 2×10^3 cell/well in a 96-well plate and maintained at 37 °C in 95% humidity and 5% CO₂ overnight. In these well plates, extract was seeded at concentrations of 600, 300, 150, 75, 37.50, 18.75 µg/ml. The plates were incubated for 48 hours. Each well was washed twice with phosphate buffer solution and 20µl of MTT staining solution was added. The plates were incubated for 4-6 hours in 5% of CO₂. After incubation, 100µl of DMSO was added. Presence of viable cells was visualized by the formation of purple colour due to development of formazan crystals. The optical density was recorded at 570nm using ELISA. Measurements were performed and the concentration required for a 50% inhibition of viability was determined using graph pad prism version 8. IC₅₀ value was calculated for all the extracts.

Anti-inflammatory activity

Murine macrophage cell line (RAW 264.7) was obtained from National Centre for Cell Science Pune. The cells are subculture and maintained in DMEM with 2 Mm L-glutamine containing 10% HI-FBS, 100IU/ml penicillin, 100µg/ml streptomycin in a humidified atmosphere at 37 °C within 5% CO₂.

Assay for NO production by activated macrophages

The cell toxicity was performed on 96 well plates. Cells were incubated with 1µg/ml of LPS, for 24 hours. 200M AG plus 1µg/ml LPS served as control for the removal of NO production¹¹. Cell viability of the macrophages was determined by MTT assay. After incubation, the cells were treated with methanolic extract and incubated. Cells were washed once before adding 50µl of PBS-free medium containing 5mg/ml of MTT solution. After 4 hours of incubation at 37 °C. The medium was discarded. Therefore, the formazan blue that formed within the cell was dissolved in 100µl of DMSO. The optical density (ELISA reader) was measured at 540nm¹²⁻¹³.

Nitric oxide determination

The nitrile gathered in culture medium is measured as a pointer of NO production supported Griess reaction. Briefly 100µl of cell culture medium was mixed with 100µl of Griess reagent incubation at room temperature for 10minute then absorbance at 540 nm is measured in a micro plate reader. Fresh culture medium was utilised as the blank in the experiment¹⁴.

Results and Discussion:

Table: 1 phytochemical investigation of crude extracts.

Sl. No	Test		Petroleum ether extract	Ethyl acetate	Ethanollic extract	DCM
01	Alkaloids	Mayer's test	+ve	+ve	+ve	+ve
		Wagner's test	-ve	+ve	+ve	-ve
		Hager's test	+ve	+ve	+ve	+ve
		Dragendroff's test	+ve	-ve	+ve	+ve
02	Carbohydrates	Molisch's test	-ve	-ve	+ve	-ve
		Reducing sugar test	-ve	-ve	+ve	-ve
		Monosaccharide test	-ve	-ve	+ve	-ve
		Pentose sugar test	-ve	-ve	+ve	-ve
		Hexose sugar test	-ve	-ve	+ve	-ve
03	Steroids and Triterpenoids	Salkowski's test	+ve	+ve	+ve	-ve
		Liebermann sterol test	+ve	-ve	+ve	-ve
04	Tannins and Phenols	Ferric chloride test	-ve	-ve	+ve	+ve
		Lead acetate test	-ve	-ve	+ve	-ve
05	Glycosides	Legal test	+ve	+ve	+ve	+ve
		Killer-killini test	+ve	+ve	+ve	+ve
		Borntegers test	+ve	+ve	+ve	+ve
06	Saponins	Foam test	+ve	-ve	+ve	+ve
		Haemolysis test	+ve	-ve	+ve	+ve
07	Proteins	Biuret's test	-ve	-ve	+ve	+ve
		Millon's test	-ve	-ve	+ve	+ve
		Ninhydrin test	-ve	-ve	+ve	+ve
		Xanthoprotein test	-ve	-ve	+ve	+ve
08	Flavonoids	Shinoda test	+ve	+ve	+ve	-ve

+ve = constituent present, -ve = constituent absent

The phytochemical or secondary metabolites play an important role towards the biological activity of medicinal plants^{20,27}. In the present study, the qualitative results of ethanolic extract of *Costus*

igneus unveiled the presence of various phytochemicals like alkaloids, tannins, steroids and flavonoids. The above phytochemicals are well known for their pharmacological activities^{21,28}. Tannins and flavonoids are well known for their anti-inflammatory, antimicrobial, and anticancer activities. Standardization and characterization of herbal drugs is an area of continuous scientific interest in herbal drug production as shown in **table 1**.

Table 2: Results for antimicrobial activity against tested organisms

Extracts	Microorganisms								
		<i>S. aureus</i>	<i>S. mutans</i>	<i>E. faecalis</i>	<i>E. Coli</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>		
CIP	MIC	6.25	3.12	6.25	6.25	3.12	6.25	MIC	1.56
	MBC	12.5	6.25	12.5	12.5	6.25	6.25	MFC	6.25
CIC	MIC	12.5	25	6.25	25	6.25	6.25	MIC	6.25
	MBC	25	50	25	50	12.5	12.5	MFC	12.5
CIE	MIC	6.25	3.12	1.56	6.25	1.56	3.12	MIC	1.56
	MBC	12.5	6.5	3.12	12.5	6.25	6.25	MFC	3.12
CIH	MIC	1.56	3.12	6.25	6.25	3.12	6.25	MIC	3.12
	MBC	25	25	50	12.5	25	25	MFC	6.25
Std. µg/ ml	MIC / MBC	2	2	2	1	< 4	1	MIC/ MFC	16

ethanolic extract of *Costus igneus* was evaluated for its antibacterial activity and found active against Gram positive and Gram-negative microorganisms by assay for MIC and MBC. The results are represented in table 2 For antifungal activity (*Candida albicans*) strain was used. Standard Ciprofloxacin and Fluconazole were studied.

Table 3. Cytotoxic activity of leaves extracts of *Costus igneus*

Extracts	Cancer Cells
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	Conc. in $\mu\text{g/ml}$	MCF-7		A-549	
		% inhibition	IC ₅₀ value	% inhibition	IC ₅₀ value
CIP	1000	36.62	260.900	34.09	230.300
	500	45.07		46.29	
	250	65.14		59.75	
	125	79.04		78.39	
	62	87.79		87.45	
	31.5	95.92		95.61	
CIC	1000	34.37	320.800	44.46	297.400
	500	50.00		51.60	
	250	63.01		60.41	
	125	79.71		69.41	
	62	93.59		80.24	
	31.5	96.21		91.40	
CIE	1000	34.86	200.600	31.25	195.100
	500	41.60		39.08	
	250	59.23		44.66	
	125	72.86		53.85	
	62	82.30		70.89	
	31.5	94.78		90.77	
CIH	1000	35.44	350.800	45.15	320.200
	500	43.79		56.71	
	250	56.41		67.54	
	125	78.06		78.99	
	62	88.16		88.22	
	31.5	95.44		94.21	
Standard Paclitaxel		0.35 μM		0.32 μM	

The MCF-7, A-549 were performed. Cell viability was understood by the MTT assay. The extract induced cell cytotoxicity in a concentration dependent manner, as illustrated. The results are represented in table 3. The extract would inhibit the proliferation of the cancer cells. The results of ethanolic extract were found to have a greater cytotoxic effect on MCF-7 cells with a 50% inhibitory concentration (IC₅₀) value of 200.6±2.2 $\mu\text{g/ml}$ and cytotoxic effect on A-549 cells with IC₅₀ value of 195.1±1.3 $\mu\text{g/ml}$. The effect induced by the extract is also similar to standard chemotherapeutic drugs paclitaxel which is commonly used in the treatment of breast and lung cancer (IC₅₀ 0.35/ml μM and IC₅₀ 0.32/ml μM) respectively. On the other hand, biocompatibility study was also performed using normal mouse embryo fibroblast L929 cells and study findings displayed more than 90 percent cell viability on exposure to test extract. This finding suggests the safety of selected test extract on normal mouse fibroblast cells.

Table-4: IC₅₀ Values of plant extracts on RAW264.7 cells

Extracts	Conc. in µg/ml	% of NO inhibition	IC ₅₀ (µg/ml)
CIP	800.00	42.48	450.700
	400.00	56.29	
	200.00	69.30	
	100.00	78.86	
	50.00	89.62	
	25.00	92.39	
CIC	800.00	50.44	650.00
	400.00	68.97	
	200.00	75.55	
	100.00	86.68	
	50.00	92.61	
	25.00	96.65	
CIE	800.00	35.12	356.800
	400.00	49.14	
	200.00	68.36	
	100.00	86.25	
	50.00	93.26	
	25.00	96.89	
CIH	800.00	52.96	733.40
	400.00	69.78	
	200.00	73.36	
	100.00	84.88	
	50.00	86.36	
	25.00	94.83	

The anti-inflammatory activity of ethanolic extract was determined using RAW 264.7 murine macrophage cells. ethanolic extract of *Costus igneus* have been taken in six different concentrations. The result shows a concentration range of 25-800µg/ml in the presence of LPS (1µg/ml) which affected cell viability. Inhibitory results of ethanolic extract on the nitrite in RAW 264.7 cells are

shown in **table 4**. To study the effect of ethanolic extract during a co-incubation period of 24 hours was examined by using nitric oxide (NO) production.

CONCLUSION

The result showed potential antimicrobial, anti-inflammatory and anticancer activities of leaf extract of *Costus igneus*. The antimicrobial activity was carried out by broth dilution method and it shows good inhibition against tested bacterial and fungal strain. The anti-inflammatory activity was done by using the RAW 264.7 murine macrophage cell line. The ethanolic extract showed good results. The cytotoxic studies revealed that the ethanolic extract inhibits the proliferation of cancer cells. More comprehensive studies related to ethanolic extract will enhance pharmaceutical exploration in the field of carcinogenic disease prevention.

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CONFLICT OF INTEREST

Conflicts of interest declared none.

References

1. Meti, R. (2018). Standardization, value addition and sensory evaluation of products prepared from insulin plant leaves (*Costus igneus*). *International Journal of Advanced Educational Research*, 3, 374-376.

2. Urooj, A. (2010). Nutrient profile and antioxidant components of *Costus speciosus* Sm. and *Costus igneus* Nak.
3. Shivaprakash, G., Thottacherry, E. D., Rai, S., Nandini, M., and Kumarachandra, R. (2014). Evaluation of Antioxidant potential of *Costus igneus* in ethanol induced peroxidative damage in albino rats. *Journal of Applied Pharmaceutical Science*, 4(8), 052-055.
4. Pawar, R., Rane, A., and Dhamnaskar, R. (2020). AGONIST STUDY OF PHYTOLIGANDS OF *C. IGNEUS* TARGETED AGAINST ENZYMES OF ANTIOXIDANT DEFENCE SYSTEM.
5. Kamalakkanan, N., Rajadurai, M., and Prince, P. S. M. (2003). Effect of *Aegle marmelos* fruits on normal and streptozotocin-diabetic Wistar rats. *Journal of medicinal food*, 6(2), 93-98.
6. Saravanan, A., Karunakaran, S., Vivek, P., and Dhanasekaran, S. (2014). Studies on antibacterial activity of root extract of *costus igneus*. *Intern J Chem Tech Res [Internet]*, 6(9).
7. Kala, S. (2014). Antimicrobial Activity of *Coleus For skohlii* (Wild) Briq and *Costus igneus* NE Br. *Journal of Pharmacy and Biological Science*, 9, 01-06.
8. Jose, B., and Reddy, L. J. (2010). Analysis of the essential oils of the stems, leaves and rhizomes of the medicinal plant *Costus pictus* from southern India. *Int J Pharmacy Pharm Sci*, 2(Suppl 2), 100-1.
9. Benny, M. (2004). Insulin plant in gardens.
10. R. Selvaraj, S. Thamizharasan, S. Umamaheswari, *In Vitro* Anti-Diabetic Activity of *Costus igneus* roots, *Indian journal of research*, 2017, 6.
11. Urooj, A. (2010). Nutrient profile and antioxidant components of *Costus speciosus* Sm. and *Costus igneus* Nak.
12. Hegde, P. L., Harini, A., Kumar, K. S., and Rao, P. N. (2016). Macro-microscopy and TLC atlas of leaves of *Costus igneus* Nak. *Journal of Ayurveda Medical Sciences*, 1(1).
13. Mathew, F., and Varghese, B. (2019). A review on medicinal exploration of *Costus igneus*: the insulin plant. *Int J Pharm Sci Rev Res*, 54(2), 51-57.
14. Mathew, F., and Varghese, B. (2019). A review on medicinal exploration of *Costus igneus*: the insulin plant. *Int J Pharm Sci Rev Res*, 54(2), 51-57.

15. Kalailingam, P., Sekar, A. D., Samuel, J. S. C., Gandhirajan, P., Govindaraju, Y., Kesavan, M., ... and Tamilmani, E. (2011). The efficacy of *Costus igneus* rhizome on carbohydrate metabolic, hepatoprotective and antioxidative enzymes in streptozotocin-induced diabetic rats. *Journal of Health Science*, 57(1), 37-46.
16. Yuvarani, T. H. A. N. I. K. A. S. A. L. A. M., Manjula, K. E. S. A. V. A. N., and Gopu, P. A. (2017). Growth and characterization of calcium hydrogen phosphate Dihydrate crystals influenced by *Costus igneus* aqueous extract. *Int J Pharm Pharm Sci*, 9(5), 173-8.
17. Ramya Urs, S. K., and Chauhan, J. B. (2015). Phytochemical screening, Antimicrobial activity and Anti-oxidant activity of *Costus igneus*. *European J Molecular Bio and Biochemistry*, 2(2), 93-96.
18. Chetty, S., Adiga, S., and Reddy, S. (2014). Evaluation of the effect of *Costus igneus* on learning and memory in normal and diabetic rats using passive avoidance task. *Int J Pharm Pharm Sciences [Internet]*, 6(2).
19. Mani, P., Kanivalan, N., and Rajakumar, R. (2014). Anti-Diabetic and Hypolipidemic Effects of *Costus igneus* Leaves Extracts Against Streptozotocin Induced Diabetic Albino Rats. *Acta Biomedica Scientia*, 1, 74-79.
20. Mani, P., Kanivalan, N., and Rajakumar, R. (2014). Anti-Diabetic and Hypolipidemic Effects of *Costus igneus* Leaves Extracts Against Streptozotocin Induced Diabetic Albino Rats. *Acta Biomedica Scientia*, 1, 74-79.
21. Krishnan, K., Mathew, L. E., Vijayalakshmi, N. R., and Helen, A. (2014). Anti-inflammatory potential of β -amyrin, a triterpenoid isolated from *Costus igneus*. *Inflammopharmacology*, 22, 373-385.
22. Palanivel, V., Mohamed Jihad, E. V., and Senthil Kumar, K. L. (2013). Evaluation of hypoglycemic activity of *Costus igneus* extract (whole plant) on alloxan induced diabetic rats. *International journal of advanced pharmaceutical genuine research*, 1(2), 9-19.
23. Radha, A., Balasubramanian, K., Shruti, B. S., and Nandhini, S. R. (2015). Studies on Optimization of Medium in Induction and Regeneration of Callus and Shoot from *Costus igneus* and its Phytochemical Profile. *Journal of Academia and Industrial Research (JAIR)*, 4(2), 75.
24. Dhanasekaran, S., Akshaya, M., and Preethi, S. (2014). In Vitro anti-proliferative potential of leaves of *Costus igneus*. *International Journal of Innovations in Engineering and Technology*, 4, 277-283.

25. Nagarajan, A., Arivalagan, U., and Rajagurua, P. (2011). In vitro root induction and studies on antibacterial activity of root extract of *Costus igneus* on clinically important human pathogens. *Journal of Microbiology and Biotechnology Research*, 1(4), 67-76.
26. Meléndez-Camargo, M. E., Castillo-Nájera, R., Silva-Torres, R., and Campos-Aldrete, M. E. (2006). Evaluation of the diuretic effect of the aqueous extract of *Costus pictus* D. Don in rat. In *Proceedings of the Western Pharmacology Society* (Vol. 49, pp. 72-74).
27. Remya, R., and Daniel, M. (2012). Phytochemical and pharmacognostic investigation of antidiabetic *Costus pictus*. D. Don. *Int J Pharm Biomed Res*, 3(1), 30-39.
28. Bhat, V., Asuti, N., Kamat, A., Sikarwar, M. S., and Patil, M. B. (2010). Antidiabetic activity of insulin plant (*Costus igneus*) leaf extract in diabetic rats. *Journal of Pharmacy Research*, 3(3), 608-611.
29. Krishnan, K., Vijayalakshmi, N. R., and Helen, A. (2011). Beneficial effects of *Costus igneus* and dose response studies in streptozotocin induced diabetic rats. *Int J Curr Pharm Res*, 3(3), 42-6.