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Studies On Antibacterial Activity Of Root Extract Of *Costus Igneus*

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Abstract: Antibacterial activity of *Costus igneus* was conducted. The plant *C. igneus* was well known for its anti-diabetic property. Traditionally the plant was used to treat fever, rash, asthma and intestinal worms. Two growth regulator IAA (Indole 3-acetic acid) and IBA (Indole butyric acid) in combinations were applied to MS medium for direct root induction. MS medium supplemented with 0.5mg/ml IBA, induced maximum amount of root culture (fresh weight was 1.566 g and dry weight 0.102 g) after 30days. In vitro raised roots were subjected to its antimicrobial activity. Acetone, chloroform and methanol were used as solvents to extract plant materials from IBA and IAA derived roots and the extracts were subjected to antimicrobial activity against four gram negative bacteria namely *Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella sp, Proteus vulgaris*. In which *Klebsiella pneumonia* was found to be most susceptible to both IBA and IAA derived roots. Whose zone of clearance was found to be 25 mm, which was almost equal to that of commercially available antibiotic gentamycin
Keywords: *Costus igneus*, Indole 3-acetic acid, Indole butyric acid, Gentamycin.

Introduction

Innumerable biologically active compounds that are found in plants[1] possess antibacterial properties[3]. Plant produced compounds are of interest as sources of safer or more effective substitutes for synthetically produced antimicrobial agents[2]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population[5].

Costus igneus (*C. igneus*) commonly known as insulin plant in India, belongs to the *Costaceae* family. The whole plant *C. igneus* were used for it anti-diabetic property and prevents the body from disease, protects mind and which prolongs the longevity of life. The rhizome has been used to treat fever, rash, asthma, bronchitis, intestinal worms, ailments of eyes, stomach, neck, jaws, tongue, mouth and also be used for curing fever, edema, wheezing (dyspnoea), haemorrhoids, spermaturia. In siddha medicine system *C. igneus* root has been used as in the form of powder (chooranam), decoction (kudineer) and oil (thylam). Until now, *C. igneus* has been reported to contain resinoids, essential oil, and alkaloid named saussurine, inulin and resin[6].

Adventitious root culture is the unique technique which renders the secondary metabolites in huge amount and it fulfils the global demand in field of medicine, agriculture, drug production, pigment production, dye production and so on. Root cultures can be used in many ways including studies of carbohydrate metabolism, mineral nutrients requirements, essential need for of vitamins, growth regulators, differentiation of the root apex and gravitropism. The advantage of using root cultures is that they grow rapidly, relatively easy to prepare and maintain, show a low level of variability and can be easily cloned to produce a large supply of experimental tissues. However, there is no previous report for the adventitious root culture for *C. igneus*. Hence the present study is aimed for the induction and culture of adventitious roots of *C. igneus* and the analysis of the induced *C. igneus* root for its antimicrobial activity in different microorganisms.

The use of herbal medicine is now wide spread for the treatment of various diseases and disorders, it is redundant[3]. The use of pharmaceuticals has led to unforeseen side effects such as genetic alterations, biomagnifications and even death. Unforeseen side effects often appear after a drug has been on the market for years and is taken by many. Drug testing does not find these effects, as the number of patients in trials is not generally high enough. Also, trials are controlled by the company that wants the medicine approved, they are slanted to find efficacy and safety Austin *et al.* (1994)[7]. On the other hand, the use of herbal medicines has several advantages. One advantage is its wide availability and simple in preparation. Plants can contain sugars, minerals, proteins and other chemicals that interact with the active chemical in a variety of ways viz. they may concentrate or intensify its effect, they may make it easier to digest or absorb, or they may lessen its harsh or toxic side effects Ivorra *et al.* (1989)[9].

Medicinal plants used to treat hypoglycemic and hyperglycemic conditions are of considerable interest to ethnobotanical community as the plants contain valuable medicinal properties in its different parts. In traditional medicine, diabetes mellitus is treated with diet, physical exercise and medicinal plants. Even though, more than 1200 plants were used in the control of diabetes mellitus, approximately 30% of the antidiabetic plants were pharmacologically and chemically investigated Chen *et al.* (1990)[8]. On the other hand, potential hypoglycemic agents have also been detected in more than 100 plants which were used for antidiabetic therapy.

Diabetes Mellitus

Motivation and overview of diabetic research

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. The World Health Organization (WHO) warns that the deaths due to diabetes will increase all over the world by 80% in some regions, over the next ten years. Among these, India host to the largest diabetes population in the world with an estimated 35 million people, amounting to 8% of the adult population. WHO also predicts that the diabetes currently affects almost two hundred million people worldwide. Only 5% of the diabetes in the world is type 1 (IDDM). The remaining 95% is type 2 (NIDDM).

Diabetes epidemic in India

The first national study on the prevalence of type 2 diabetes in India was done between 1972 and 1975 by the Indian Council of Medical Research (ICMR, New Delhi). Screening was done in about 35,000 individuals above 14 year of age, using 50 g glucose load. Capillary blood glucose level >170 mg/dl was used to diagnose diabetes. The prevalence was 2.1 % in urban population and 1.5% in the rural population while in those above 40 year of age, the prevalence was 5% in urban and 2.8% in rural areas. Subsequent studies showed a rising trend in the prevalence of diabetes across different parts of India. In 1988, a study done in a small township in south India reported a prevalence of 5% Jhonson *et al.* (1990)[4].This study also revealed that the prevalence in the southern part of India to be higher-13.5 % in Chennai, 12.4 % in Bangalore and 16.6 % Hyderabad; compared to eastern India (Kolkatta), 11.7 %; northern India (New Delhi), 11.6 %; and western India (Mumbai) 9.3 % Arun *et al.* (2011)[10].

Definition of diabetes

Diabetes mellitus is a syndrome characterised by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from low levels of the hormone insulin with or without abnormal resistance to insulin's effects.

Types of Diabetic mellitus

The World Health Organisation recognises three main forms of diabetes: Type 1, Type 2 and gestational diabetes (occurring during pregnancy), which have different causes and population distributions. Type 1 is usually due to autoimmune destruction of the pancreatic beta cells. Type 2 is characterised by insulin resistance in target tissues. This causes a need or abnormally high amounts of insulin and diabetes when the beta cells cannot meet its demands Babu *et al.*(2003)[11].

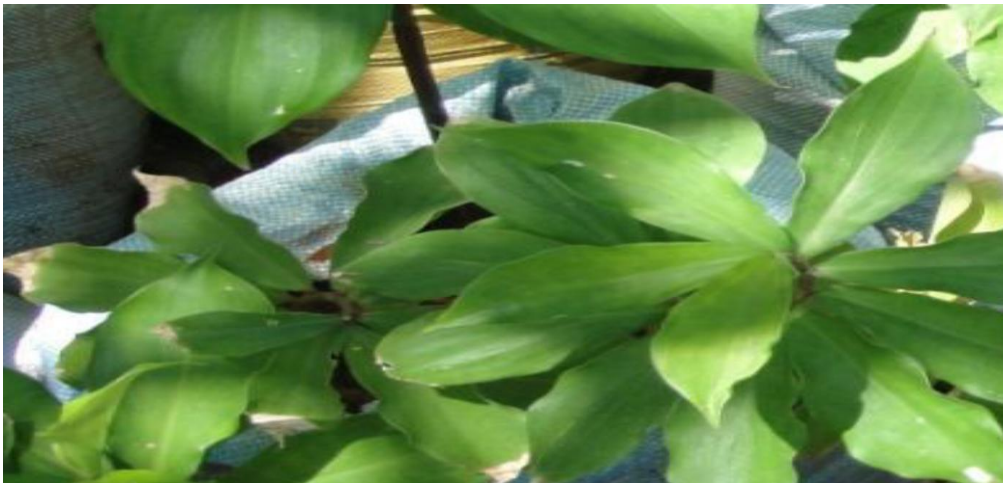
Diabetic complication

Diabetes can cause many complications. Acute complications (hypoglycemia, ketoacidosis or non ketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long term complications include cardio vascular disease, chronic renal failure, retinal damage, nerve damage, and micro vascular damage, which may cause erecting dysfunction and poor healing Hatwal *et al.* (2004)[13].

Description of *Costus igneus*

The large, smooth, dark green leaves of this tropical evergreen plant have light purple undersides and are spirally arranged around stems, forming attractive, arching clumps, arising from underground root stocks (Fig .1.1). Plants reach to about two feet tall, with the tallest stems falling over and lying on the ground. Beautiful, 1.5 inch diameter, orange flowers are produced in the warm months, appearing on cone-like heads at the tip of branches.

Fig.1.1 *Costus igneus*



Plant Details

Scientific Name: *Costus igneus*, Common name(s) : Spiral-Flag, Fiery costus
Family: Costaceae, Plant type: perennial, Soil tolerances: occasionally wet; slightly alkaline; Drought tolerance: moderate.

Materials And Methods

Mother plant

The mother plant were grown in plastic pots. The plant materials were watered twice a day using sprinkler system to maintain condition of the soil. The explants were collected from two months old, healthy and diseases free plant. Juvenile leaf segments were used as explants.

Preparation of culture medium

The basal medium for the culture of *C.igneus* was MS medium enriched with 3%(w/v) sucrose and 0.2%(w/v) phytagel. Before autoclaving at 121C for 15 minutes the pH of MS medium was adjusted to 5.8 by adding 0.1 N NaOH (Sodium hydroxide) or HCL (Hydrochloric acid). For induction of adventitious root, growth regulators were supplemented with the culture medium.

Surface sterilized protocols

Explants were collected from healthy and actively growing mother plant .Young leaf segments were used as explants.The explants were rinsed under running tap for 10 minutes to remove visual contaminants.Then the explants were subjected to ethanol, sodium hypochlorite and mercuric chloride treatments to obtain aseptic culture. In treatment 1, the explants rinsed with 70% ethanol for 0.5 minutes followed by 3% sodium hypochlorite for 6 minutes. Whereas in treatment 2, the explants were treated with 70% ethanol for 0.5 minutes and followed by 3% sodium hypochlorite for 1 minutes and 0.1% mercuric chloride for 3 minutes. In treatment 3, explants were rinsed in 70% ethanol for 1.5 minutes and followed by 3% sodium chloride for 1.5 minutes and 0.1% mercuric chloride for 4 minutes. Following each treatments explants were washed with sterile distilled water. All the 3 treatments were repeated thrice (3 replicates, with 30 explants / replicate). The surface sterilized explants were inoculated aseptically.

Culturing of explants

The edges of surface sterilized explants were trimmed and dried in sterile tissue paper before transferring onto the culture medium. The explants were separately inoculated in culture tubes containing 10ml MS medium to avoid cross contamination. The tubes were capped and sealed with parafilm to avoid contamination risk.

The cultures were observed daily to identify any contamination, growth of explants and mortality of explants. Contamination-free cultures were used for sub-culturing on medium containing growth hormones (auxins). Sub culturing was done every one weeks onto the same medium composition. The growth and incidence of adventitious root was noted after 15 days of culturing. All cultures were incubated in a culture room under dark condition.

Adventitious Root Culture

The sterilized explants (0.5 – 1 cm) were inoculated on MS solid medium containing 3.0 % (w/v) sucrose, 0.2 % phytagel (w/v) along with various concentration of auxin such as IAA and IBA.

Effect of IBA and IAA on Adventitious root culture

The explants were inoculated on various concentrations of auxin such as IAA and IBA in the range of (0.05 – 2.0 mg/L) and incubated under total darkness at 24ºC±2 ºC for adventitious root formation. The cultures were monitored at 7days interval and results were assessed at the end of 15th day. After 15 days of inoculation the roots produced from the cut end of the leaves, were separated from the explants and the growth in different auxin regimes was assessed in terms of fresh and dry weight. Fresh weight of roots was determined by blotting the harvested root on filter paper after the gentle wash in distilled water. Dry weight was obtained after drying them at room temperature for 48hrs. The roots were powdered using mortar and pestle and about 100mg of root powder was used for studying its antimicrobial activity.

Antibacterial Activity

Materials and media preparation

Gram negative Bacterial cultures such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella sp* were used in the present study to determine the antibacterial activity of the *C. igneus*, *in vitro* raised root extracts. Above mentioned bacterial strains are laboratory stains. Nutrient Agar medium is one of the most commonly used medium for several route lines of bacteriological purposes.

Root sample preparation

The shade dried and powdered IBA and IAA derived root materials were used for the sample preparation. About 10 grams of the IBA and IAA derived root materials was extracted with 5ml of acetone, chloroform and methanol using soxhlet apparatus at 800°C. Further, the solvent was evaporated using a rotary vacuum evaporator. The residues were dissolved with dimethyl sulfoxide (DMSO) and used for antimicrobial activity.

Preparation of inoculums

About 100 µl of overnight culture of *P. aeruginosa*, *Salmonella spp*, were taken. These cultures were spread plated over the petriplates containing agar medium.

Disc diffusion method

The anti-bacterial activities of the test samples were carried out by disc diffusion method. Size of disc in the present study is 0.5 mm. *Gentamycin* were used as positive control and the concentration of each disc was 10 µg. Two different concentrations of samples (40 and 60µg/ml) of both IBA and IAA derived roots extracts were loaded on discs (Prepared from whatman filter paper No.1) and placed on the plates. Solvent (DMSO) is maintained as negative control. Later plates were incubated at 37°C for 24hours. Results were later observed as inhibition zones and were expressed in millimetres.

Results and Discussion

Sterilization of explants

The sterilization results of the leaf explants using 3 different treatments are presented in Table 1.

Table 1: Sterilization of leaf explants of *Costus igneus* with various treatments

Treatment	Sterilants and Concentration	Duration (min)	% of contamination
1.	70% Ethanol 3% NaOCl	0.5 6.0	100
2.	70% Ethanol 3% NaOCl 0.1% $HgCl^2$	0.5 1.0 3.0	40
3.	70% Ethanol 3% NaOCl 0.1% $HgCl^2$	1.5 1.5 4.0	10

Treatment 3 was found to be the best; it reduces the contamination level up to 10%.

Plant tissue culture work requires strict maintenance of asepsis in all the operations. In the present study, among three types of treatments, treatment 1 & 2 were found to have 100% and 40% percentage fungal contaminations in the cultured vessels respectively while treatment 3 (70% alcohol- 1.5 minutes, 3% sodium hypochlorite- 1.5 minutes, 0.1% mercuric chloride 4 minutes) was found to have only 10% of contamination in the cultured vessels. Hence, it was found to be the best when compare to others and chosen for further studies.

Adventitious roots induced by *in vitro* methods showed high rate of proliferation and active secondary metabolism [8, 19]. Adventitious roots are natural, grow vigorously in phytohormone supplemented medium and have shown tremendous potentialities of accumulation of valuable secondary metabolites.

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the words population. There are about 45,000 plant species in India with capacity to produce a large number or organic

Chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity [18, 11]. In the present work different extract of *C. igneus* showed higher activity to *P. aeruginosa*, *K. pneumonia*, *Salmonella sp*, *P. vulgaris*. The results confirmed that antimicrobial potential in plant material (root and stem) shows more potential against in all the organisms tested as the zone of inhibition is more when compare to other organism tested. Gothandam [7] established the antimicrobial activity of *C. igneus* with good zone of inhibition in all the organisms tested. The zone of inhibition of the present study goes in accordance with the results of Gothandam [7]. However, the extracts of the present study were prepared from the IBA and IAA induced roots.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [9]. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy.

However, the present study of *in vitro* antimicrobial evaluation of the IBA and IAA induced roots of *C. igneus* forms a primary platform for further phytochemical and pharmacological studies. In the present study, we have found that IBA and IAA induced roots of *C. igneus* possess anti microbial activity which may be due to the presence of phytochemicals. Further studies are required to isolate the active components from the IBA and IAA induced roots of *C. igneus.*

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