International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 2 Issue 5 ǁ May 2013 ǁ PP.11-18

Phytochemical and hypoglycaemic activity investigation of *Costus pictus* plants from Kerala and Tamilnadu

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ABSTRACT*: Costus pictus family Costaceae, a recently introduced plant from Mexico has shown its potential as herbal drug for diabetes. In the present study 24 extracts were prepared from three materials (rhizome, stem and leaf) of two regions (Kerala and Tamilnadu) using different solvents (hexane, ethyl acetate, methanol and water) and they were subjected to phytochemical screening, and checked the hypoglycaemic activity in glucose fed albino mice. The preliminary phytochemical screening indicated very much similarity in the presence of chemical constituents in all 24 extracts of three samples of two regions and the methanol extract (200mg/kg, 500mg/kg b.w.) of leaf were exhibited significant hypoglycaemic activity in glucose fed mice. The study suggests the large scale cultivation of C. pictus at varied geographical locations as the phytochemical profile is quite stable with environmental variables.*

Keywords: *Costus pictus, hyperglycaemic, metformin, phytochemicals, TLC*

I. INTRODUCTION

Diabetes mellitus is a disorder very well known and widespread all over the world. Diabetes is a growing health concern worldwide and now emerging as an epidemic world over. The management of diabetes is still a major challenge. Thus there is great demand for research on natural products with anti-diabetic properties [1]. India has emerged as the diabetic capital of the world [2].

Different types of oral hypoglycemic agents such as insulin, suphonylurea etc. are used for the treatment of this disease, but they cause side effects on continued use. There is a growing interest in phytomedicine because of their effectiveness, fewer side effects and low costs [3]. Traditional anti-diabetic plants might provide new oral anti-diabetic compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing countries [4]. *Costus pictus* D. Don (Spiral ginger), commonly known as 'insulin plant' a member of Costaceae family and is used as a munching dietary supplement for the treatment of diabetes in Southern India [5]. The plant is a recent introduction from Mexico to Kerala.

India has a very great variation in geographical, topographical, climatological, edaphic conditions which leads to a great diversity in biological wealth. Variation in agronomic conditions (plant species, cultivar, developmental stage, plant organ, plant competition, fertilization, pH), season, climatic factors, water availability, light (intensity, quality, duration) and CO_2 are known to significantly affect the phytochemical profile of plant [6]. The large scale cultivation of *C. pictus* in various parts of India may provide a herbal remedy at low cost to the people affected with diabetes. The popularity of this plant is reaching all over India and diabetic people have experienced the amazing antidiabetic effect of *C. pictus* leaves. It is very essential to cultivate *C. pictus* in various parts of India to alleviate the complications of diabetes by promoting its miraculous effect. But before recommending for large scale cultivation, the multi location field trials for phytochemical constituents are essential. The present study is an initiative to evaluate the impact of geographical variables on phytochemicals and hypoglycaemic effect of the rhizome, stem and leaf samples cultivated at Kerala (Kannur) and Tamilnadu (Tuticorin).

II. MATERIALS AND METHODS

The plants were cultivated and identified by Dr. Santhosh Nampy, Department of Botany, St. Joseph's College, Devagiri, University of Calicut and herbarium specimen has been submitted to Botany Department (Ref. No. SJC/BOT/RES-EXT/1/2012). Leaf, stem and rhizome portions of one year old *C. pictus* plants were separately harvested, (from Kannur and Tuticorin) washed, chopped and dried at room temperature. The well dried materials were ground into fine powder and kept in air tighter zip bags separately.

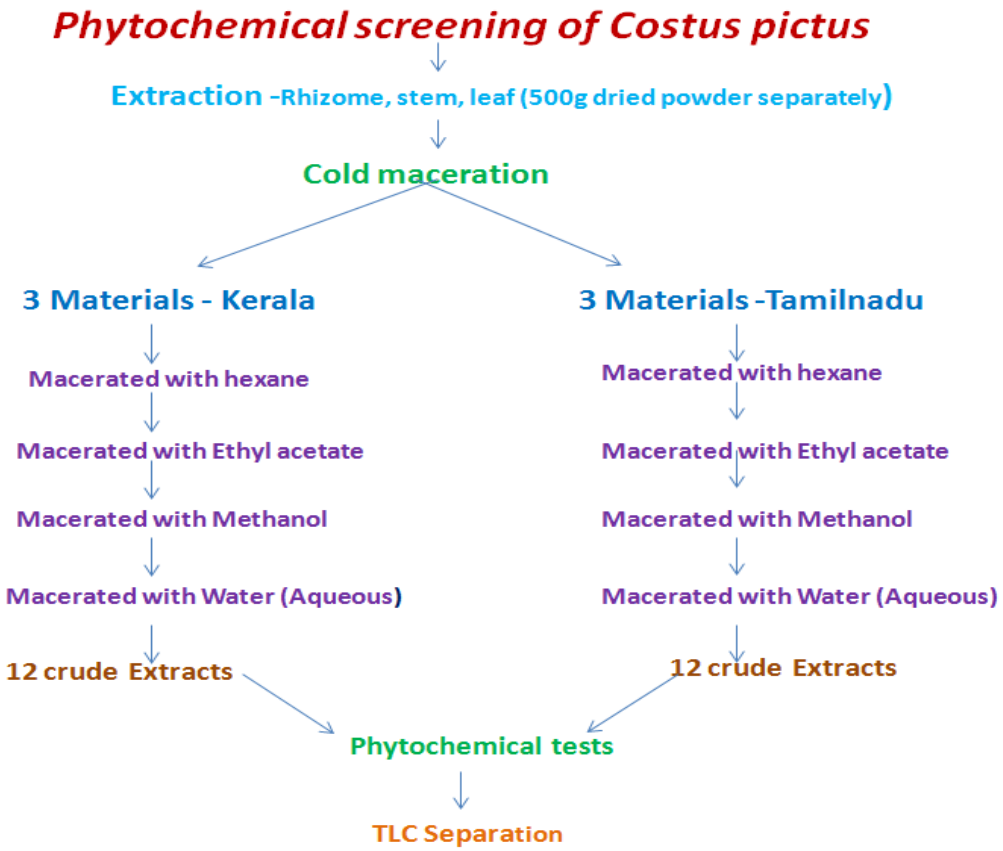
2.1. Phytochemical screening:

Phytochemical screening was performed using appropriate procedures [7 - 9] involving extraction and separation of secondary metabolites.

2.1.1. Extraction of the plant material by Cold Maceration:

500g of air dried, powdered material (rhizome, stem and leaves) was separately macerated with hexane, ethyl acetate, methanol and water (Aqueous) successively for 48 hours with occasional stirring [7]. The mixture was then filtered after 48 hours. The filtrates were evaporated to dryness using a rotary evaporator at 40^0C under reduced pressure.

Graphical presentation of the phytochemical analysis of *Costus pictus*



2.1.2. Separation of the major phytochemicals

2.1.2.1. Phytochemical tests:

1g of the plant extract was dissolved in 100 ml of the respective mother solvents to obtain a stock of 1% concentration (w/v). The extracts thus obtained were subjected to preliminary phytochemical tests. The qualitative phytochemical tests for steroids, triterpenoids, glycosides, phenols, alkaloids, quinones, coumarins, furanoids, flavonoids and tannins were performed on different extracts.

2.1.2.2. TLC (Thin Layer Chromatography) of extracts:

All the 24 extracts prepared through cold maceration were selected for TLC analysis. Pre-coated TLC plates (20 x 20 cm) were used as the stationary phase. Different mobile phases were tried and finally Chloroform: Ethyl Acetate: Methanol: Benzene in the ratio of 70: 4: 8: 24 was standardized as the best mobile phase to obtain clear spots. Freshly prepared p-anisaldehyde reagent was used as a spraying reagent. The samples (10, 20µl) were loaded on TLC plates at equal distance.

Rf values were calculated [10] after the scanning of the chromatograph. The result of phytochemical tests and TLC analyses indicated the presence of similar phytochemical profile in various extracts from three types of samples of two places. The methanol extracts of three samples (10, 20µl) of two locations were loaded for TLC also demonstrated similar mode of spots distribution. Keeping in view of these results, methanol extract of leaves was taken for hypoglycaemic activity through oral glucose tolerance test (OGTT) as this gave the maximum yield in the extraction and high concentration of phytochemicals in the phytochemical tests.

2.2. Hypoglycaemic activity

2.2.1. Preparation of test and standard drug material:

The glucose solution was prepared by dissolving the Dextrose sugar (2g/kg b.w.) in distilled water for the oral administration in mice [11]. The methanol leaf extract (200mg, 500 mg/kg b.w.) were suspended in

0.4% w/v carboxy methyl cellulose (CMC) of high viscosity solution in order to get the test solutions. Metformin 250 mg/kg b.w. [12] was prepared by dissolving it in distilled water.

2.2.2. Test animals:

Healthy, Swiss albino mice (4 weeks of age) of 25–30 g (b.w.) were used as experimental subjects, maintained under controlled conditions (temperature 25 ± 2^0 C; relative humidity 50±5%; 12h light/dark cycle). The mice were kept with free access to certified pelleted rodent diet and water *ad libitum*. Institutional Animal Ethics Committee approved the animal experiments and the guidelines for Animal care were followed as recommended by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2.3. Dose selection for Oral glucose tolerance test (OGTT):

After checking the different doses, the 200 mg/kg and 500 mg/kg [13] doses of methanol extract were selected for the OGTT experiment in Swiss albino mice. The mice were randomly divided into different groups' namely normal control, metformin, and test groups, each consisting of five mice. Two doses (200, 500 mg/kg b.w.) of extract were administered to test animals. Metformin 250 mg/kg b.w. was administered to metformin group.

2.2.4. Evaluation of extract in oral glucose tolerance test (OGTT):

The oral glucose tolerance test [14] was performed in overnight fasted (18 h) normal mice. Mice were administered 0.2 ml oral glucose load followed by 0.2 ml of extracts and reference drug metformin orally by a cannula just after checking the fasting blood glucose. Blood was withdrawn from the tail vein by cutting the tip of the tail [15] at 0, 15, 30, 60, 90 and 120 minutes of glucose administration and glucose levels were estimated immediately using compatible blood glucose test strips of glucometer (Bayer's Glucometer Elite, Bayer Health Care, U.S.A.). The control group received only the glucose load.

III. RESULTS

3.1. Extraction of plant material

The 24 extracts yielded by cold maceration have shown considerable differences (Table 1). In the extraction, maximum yield was obtained from the leaves followed by stem and rhizome in both the samples collected from Kerala and Tamilnadu. The maximum extract yield was obtained with methanol solvent and it was followed by aqueous, ethyl acetate and hexane (least polar solvent).

Table 1: The colour, yield and yield percentage of 24 extracts of 3 samples of *Costus pictus* collected from Kannur (Kerala) and Tuticorin (Tamilnadu)

Solvent	Material	Samples from Kannur			Samples from Tuticorin		
		Colour	Yield(g)	% of yield	Colour	Yield(g)	% of yield
Hexane	Leaves	dark green	24.89	4.98	dark green	25.61	5.12
	Stem	dark green	1.73	0.35	dark green	2.23	0.45
	Rhizome	pale yellow	2.83	0.57	pale yellow	2.05	0.41
Ethyl Acetate	Leaves	dark green	25.81	5.16	dark green	24.76	4.95
	Stem	bright yellow	21.67	4.34	Yellow	22.54	4.51
	Rhizome	pale yellow	5.73	1.15	pale yellow	4.31	0.86
Methanol	Leaves	dark green	84.5	16.9	dark green	81.59	16.32
	Stem	bright yellow	40.34	6.07	Yellow	41.69	8.34
	Rhizome	pale yellow	7.91	1.60	pale yellow	8.47	1.69
Aqueous	Leaves	bright yellow	29.14	5.83	Yellow	26.46	5.29
	Stem	yellow	17.35	3.47	Yellow	17.38	3.48
	Rhizome	pale yellow	6.30	1.26	pale yellow	4.03	0.81

3.2. Phytochemical tests:

The preliminary phytochemical tests indicated the presence of steroids, triterpenoids, alkaloids, phenols, glycosides, quinones, coumarins and flavanoids in various extracts of leaf, stem and rhizome portions of *C. pictus*. All extracts furnished essentially same results. Among ten phytochemical tests conducted, eight tests gave positive results in respect of all 24 extracts of leaf, stem and rhizome. The furanoids and tannins were lacking in all the extracts. Phenols, quinones and coumarins were absent in rhizome extracts. All stem extracts also gave negative results for quinones. The presence of alkaloids, phenols, steroids, triterpenoids and glycosides was observed in all extracts (Table 2 & 3).

Table 2 Preliminary phytochemical tests of different extracts of leaves, stem and rhizome of *Costus pictus* collected from Kannur (Kerala)

Extracts		Phytochemicals/ Secondary Metabolites									
		Steroid	Triterpenoid	Alkaloid	Phenol	Glycoside	Quinones	Coumarins	Flavanoid	Furanoid	Tannin
Hex	L	+	+	+	+	+	+	+	+	-	-
	S	+	+	+	+	+	-	+	+	-	-
	R	+	+	+	-	+	-	-	+	-	-
EA	L	+	++	+	+	++	+	+	+	-	-
	S	+	++	+	+	++	-	+	+	-	-
	R	+	++	+	-	++	-	-	+	-	-
Meth	L	+	++	++	++	++	+	+	++	-	-
	S	+	++	++	++	++	-	+	++	-	-
	R	+	++	++	-	++	-	-	++	-	-
Water	L	+	+	+	+	+	+	+	+	-	-
	S	+	+	+	+	+	-	+	+	-	-
	R	+	+	+	-	+	-	-	+	-	-

Presence (+), high concentration (++), Absence (-) of phytochemicals L-Leaf, S-Stem, R-Rhizome

Table 3: Preliminary phytochemical tests of different extracts of leaves, stem and rhizome of *Costus pictus* collected from Tuticorin (Tamilnadu)

Extracts		Phytochemicals/ Secondary Metabolites									
		Steroid	Triterpenoid	Alkaloid	Phenol	Glycoside	Quinones	Coumarins	Flavanoid	Furanoid	Tannin
Hex	L	+	+	+	+	+	+	+	+	-	-
	S	+	+	+	+	+	-	+	+	-	-
	R	+	+	+	-	+	-	-	+	-	-
EA	L	+	++	+	+	++	+	+	+	-	-
	S	+	++	+	+	++	-	+	+	-	-
	R	+	++	+	-	++	-	-	+	-	-
Meth	L	+	++	++	++	++	+	+	++	-	-
	S	+	++	++	++	++	-	+	++	-	-
	R	+	++	++	-	++	-	-	++	-	-
Water	L	+	+	+	+	+	+	+	+	-	-
	S	+	+	+	+	+	-	+	+	-	-
	R	+	+	+	-	+	-	-	+	-	-

Presence (+), high concentration (++), Absence (-) of phytochemicals L-Leaf, S-Stem, R-Rhizome

3.3. TLC (Thin Layer Chromatography) of extracts:

The chromatography on TLC plates, of various extracts of three samples of two places gave similar pattern of spots (Fig. 1 & 2). Results of TLC showed that 9 spots were clearly detectable and other spots were not so apparent. The major spots found in all extracts gave similar Rf values (Table 4 & 5). Various extracts of leaf, stem and rhizome portions gave similar profiles of separation by way of different spots, except for first two spots present in leaf and stem extracts that were absent in extracts of rhizome.

The results of phytochemical tests (Table 2 & 3) and TLC (Table 4 & 5) have also shown similarity by way of major chemical constituents in all the extracts of three types of samples of two locations. Hence for further hypoglycaemic activity, only one type of extract and one sample was used. The methanolic extract of leaf (collected from two places) as shown in Table 1 gave maximum yield (16.9%, 16.32%), and higher concentration of phytochemicals (Table 2 & 3), and it was selected for evaluation of hypoglycaemic activity.

Table 4: Rf values of 9 spots of TLC of 12 crude extracts of *Costus pictus* collected from Kannur (Kerala)

Spots	Types of Extracts											
	HL	H S	HR	EaL	EaS	EaR	ML	MS	MR	AqL	AqS	AqR
1	0.0882	0.0873	0.0883	0.0885	0.0875	0.0878	0.0879	0.0884	0.0881	0.0882	0.0873	0.0868
2	0.1588	0.1467	0.1571	0.1566	0.1455	0.1464	0.1512	0.1563	0.1567	0.1581	0.1536	0.1586
3	0.3235	0.3342	0.3353	0.3242	0.3521	0.3532	0.3351	0.3461	0.3205	0.3156	0.3222	0.3240
4	0.4706	0.4657	0.4745	0.4651	0.4712	0.4723	0.4733	0.4710	0.4722	0.4731	0.4724	0.4712
5	0.6765	0.6544	0.6621	0.6546	0.6645	0.6761	0.6753	0.6771	0.6683	0.6739	0.6698	0.6727
6	0.7059	0.7132	0. 7121	0.7061	0.7044	0.7071	0.7017	0.7063	0.7055	0.7063	0.7057	0.7048
7	0.7648	0.7712	0.7646	0.7655	0.7743	0.7723	0.7664	0.7711	0.7710	0.7646	0.7657	0.7658
8	0.8235	0.8331	0.8324	0.8238	0.8321	0.8322	0.8256	0.8251	0.8254	0.8326	0.8250	0.8241
9	0.8529	0.8621	0.8630	0.8541	0.8516	0.8611	0.8567	0.8548	0.8577	0.8542	0.8613	0.8545

H-hexane, Ea-Ethyl acetate, M-Methanol, Aq-Aqueous, L-Leaf, S-Stem, R-Rhizome

Table 5: Rf values of 9 spots of TLC of 12 crude extracts of *Costus pictus* collected from Tuticorin (Tamilnadu)

Spots	Types of Extracts											
	HL	H S	HR	EaL	EaS	EaR	ML	MS	MR	AqL	AqS	AqR
1	0.0890	0.0885	0.0879	0.0883	0.0875	0.0873	0.0882	0.0881	0.0879	0.0884	0.0879	0.0886
2	0.1584	0.1567	0.1581	0.1561	0.1495	0.1564	0.1502	0.1560	0.1566	0.1573	0.1546	0.1583
3	0.3243	0.3342	0.3353	0.3242	0.3521	0.3532	0.3351	0.3461	0.3205	0.3156	0.3222	0.3240
4	0.4705	0.4677	0.4715	0.4631	0.4722	0.4703	0.4740	0.4720	0.4725	0.4732	0.4723	0.4732
5	0.6754	0.6564	0.6634	0.6543	0.6625	0.6763	0.6773	0.6731	0.6724	0.6729	0.6699	0.6757
6	0.7122	0.7021	0. 7111	0.7082	0.7044	0.7068	0.7097	0.7089	0.7132	0.7088	0.7090	0.7118
7	0.7651	0.7710	0.7648	0.7681	0.7714	0.7731	0.7697	0.7701	0.7730	0.7647	0.7687	0.7694
8	0.8230	0.8321	0.8324	0.8231	0.8221	0.8312	0.8216	0.8230	0.8214	0.8320	0.8240	0.8236
9	0.8531	0.8634	0.8631	0.8532	0.8516	0.8510	0.8537	0.8546	0.8570	0.8582	0.8527	0.8571

H-hexane, Ea-Ethyl acetate, M-Methanol, Aq-Aqueous, L-Leaf, S-Stem, R-Rhizome

3.4 Hypoglycaemic activity:

The effect of leaf methanol extracts of *C. pictus* (200 mg/kg and 500 mg/kg b.w.) on glucose tolerance test has been shown in table 6 and figure 5. The administration of methanol extracts (200 mg/kg and 500 mg/kg b.w.) of *C. pictus* leaves improved the glucose tolerance in fasted normal mice. However the elevated glucose level (225.2mg/dL) was reduced considerably (54.4 mg/dL) in fasted glucose fed mice on methanol extract (500mg/kg b.w.) administration. After the administration of extracts, blood glucose level was lowered significantly (P <0.001) at 15, 30, 60, 90, and120 minutes. The reduction was dose and time dependant in mice.

Table 6: Hypoglycaemic activity (OGTT) of Methanol leaf extract

Groups	Fasting	After 15 min	After 30 min	After 60 min	After 90 min	After 120m
Control	76.0±3.8	301.0±4.9	170.2±4.3	124.6±3.6	115.6±3.1	83.4±3.9
Met-250	85.8±3.8	279.0±1.1***	139.2±1.4***	62.4±3.2***	40.2±2.5***	46.2±2.4***
MLE-200	71.4±2.3	269.8±1.3***	156.2±1.1**	94.2±1.6***	78.4±0.9***	61.6±0.8***
MLE-500	76.2±0.9	225.2±2.4***	123.8±1.5***	78.0±1.8***	61.2±2.4***	54.4±0.8***

MLE-Methanol leaf extract, Met- Metformin
Values are mean±S.E.M. of 5 mice in each group

***-p<0.001 when compared to normal control group
**-p<0.01 when compared to normal control group
*-p>0.05 when compared to normal control group

Fig. 1 & 2 TLC of 24 extracts of of 3 samples (10µl &20µl) collected from Kannur (Kerala) Tuticorin (Tamilnadu)

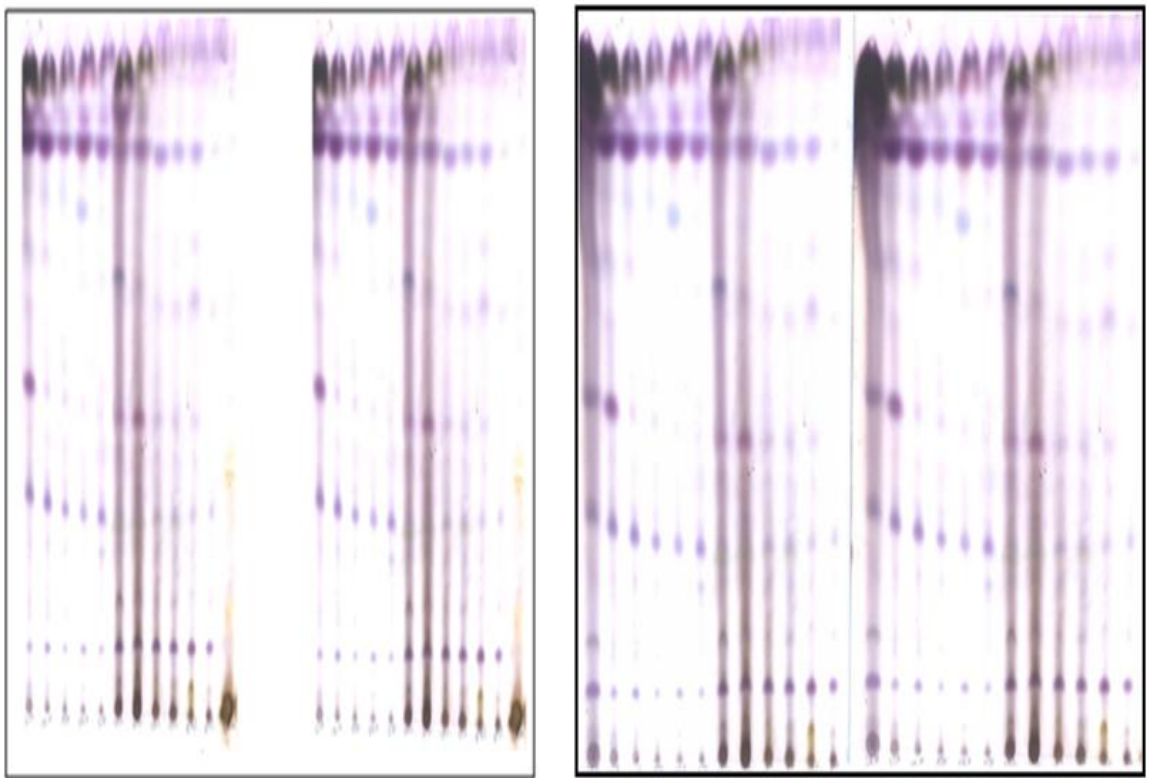


Fig. 3 & 4 TLC of methanol crude extracts of leaf, stem and rhizome (Kerala, Tamilnadu) in different concentrations ((10µl &20µl)

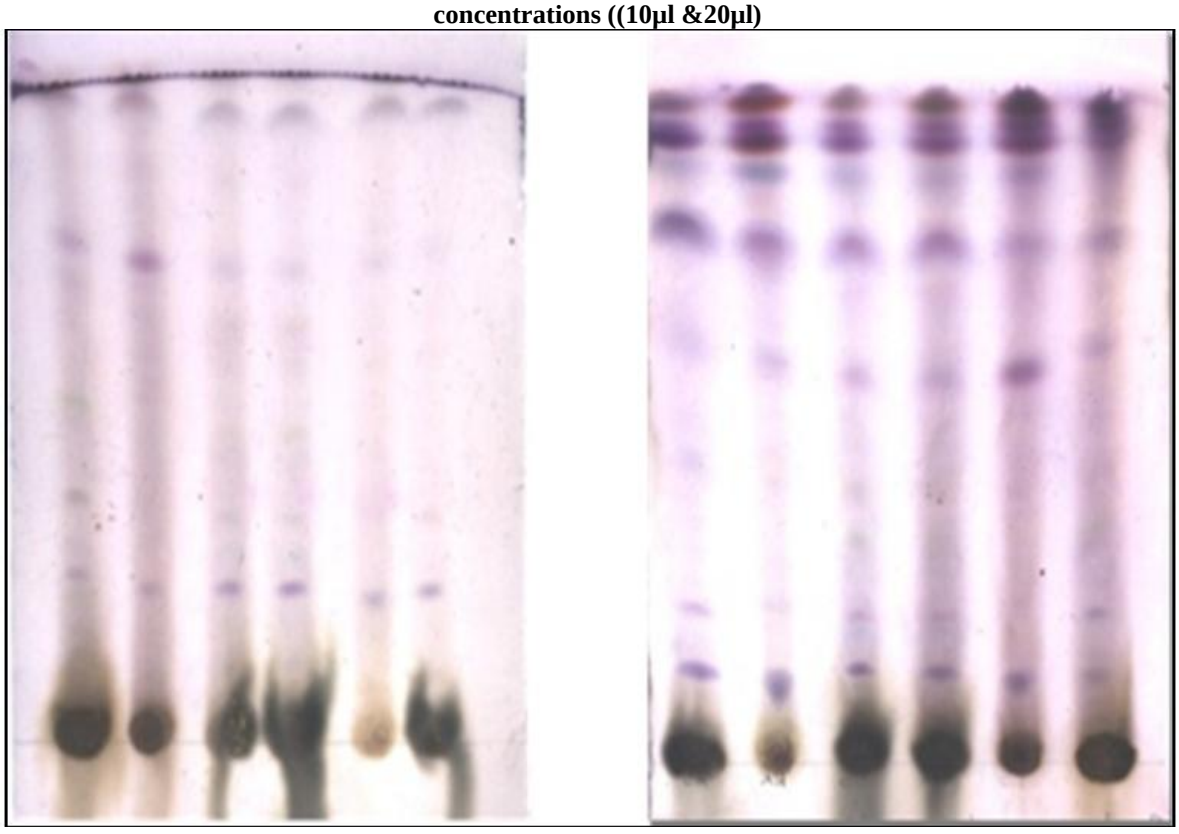
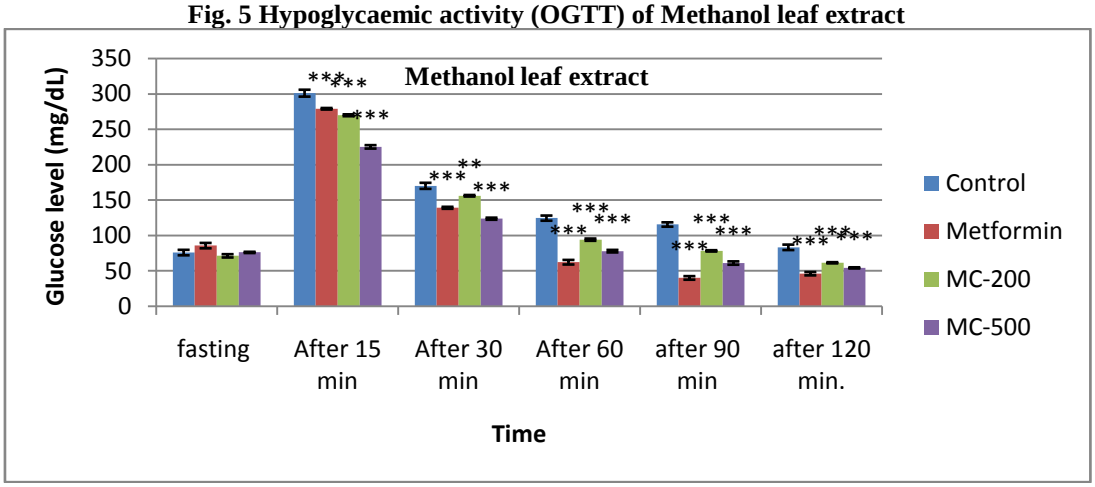


Fig. 5 Hypoglycaemic activity (OGTT) of Methanol leaf extract



IV. DISCUSSION

The findings of this study should pave the way to further explore the antidiabetic potential of *C. pictus* for world-wide use, and especially in India. Even though a few peripheral studies have been reported on phytochemical constituents [16] of *C. pictus* and investigations are also in progress in different laboratories none of them is much informative. This report may be the first information on the comparative phytochemical investigation of *C. pictus* collected from different places even though peripheral information available on its phytochemistry.

The presence of phytochemicals and their concentration in various extracts of the same plant samples of two places did not show any major difference (Table 2 & 3) in the phytochemical analyses. The results of phytochemical tests and TLC (Table 2 & 3, Fig. 1 & 2) of 24 extracts also showed similarity in the chemical constituents.

Methanolic leaf extract of *C. pictus* showed maximum number as well as concentration of phytochemicals. This result is matching with the findings of previous studies [16-18]. The methanolic leaf extract was also shown to exhibit maximum and pronounced antidiabetic activity, and the same is reported by the author. Previous studies have also reported similar finding [16, 18- 21]. The other species of Costus have also been reported to have similar effects with methanolic extract [22]. The samples collected from two places have demonstrated same result in the phytochemical analysis and they produced similar profile of spots in thin layer chromatography. This result indicates that environment does not have any influence in the production of phytochemical content and its distribution in various parts of the *C. pictus*.

C. pictus is well known for the antidiabetogenic activities [23]. The earlier studies of the plant extracts on diabetic rats have shown prevention of diabetes and related complications [20]. The results of oral glucose tolerance test revealed the potency of *C. pictus* leaf extracts in the reduction of elevated blood glucose to normal level in fasted animals. The results of OGTT demonstrate that the improvement of glucose tolerance started within 15 minutes of oral administration of the extract. Although the gradual reduction of the glucose level was observed, the maximum reduction was recorded only after 120 minutes of extract administration (Table 6 and Fig.5).

Furthermore, it is also reported that the plant extract is effective in the blood glucose reduction in normal non-fasted animals. Reduction in the fasting and the postprandial blood sugar levels in the dexamethasone induced hyperglycaemic rats with leaves of insulin plant have been reported by Shetty *et al.*, [24]. The methanolic crude extract at 500 mg/kg b.w. have shown the most pronounced effect ($p< 0.001$) comparable to that of control group in the oral glucose tolerance test. The effect of the extracts was dose dependant and time related. The same result was found in the dose response study of methanol extract (100 mg/kg b.w.) of *Costus igneus* in streptozotocin induced rats [25, 26]. The significant reduction ($p< 0.001$) of glucose level was found after 120 minutes of extract administration in dose selection test.

The mice did not show any abnormalities of toxicity during the experiment, and thereafter. In the investigation of toxicity study of *C. pictus*, it was found that the oral administration of methanol extract up to 500 mg/kg b.w. on mice and ethyl acetate extract upto 1g/kg b.w. on rats did not produce any toxic effect [20]. The results of the oral glucose tolerance test of methanol extracts (200 and 500 mg/kg b.w.) of *C. pictus* leaves the 500mg/kg b.w. dose was exhibited immediate reduction in the elevated glucose level than 200mg/kg b.w. in glucose fed mice.

The present study clearly indicates that the administration of *C. pictus* leaf extract to glucose fed mice normalizes blood glucose level. Recent studies have revealed that the administration of methanol extract of *C. pictus* at a dose of 120 mg/kg b.w. to alloxan-induced diabetic rats caused anti-diabetic effects [16].

Histopathological studies of the liver, kidney, and pancreas showed that methanolic extract of *C. pictus* leaf extract was also non-toxic [16]. Even though the hypoglycaemic effect of *C. pictus* leaf extracts were studied scientifically, none of the investigations reported comparative evaluation of phytochemical analysis of different samples of two places and the glucose lowering effect of the leaf methanolic extract as it possess maximum phytoconstituents.

V. CONCLUSION

In spite of the climatic changes prevailing in Kerala and Tamilnadu, the plants collected from these places did not show any difference in the phytochemicals and in their potent hypoglycaemic activity. From this study it is understood that the environmental factors do not have any impact on the production of phytochemical content and profile in the *C. pictus* plant. Therefore *C. pictus* can cultivate widely to find the availability throughout India as an antidiabetic herbal remedy.

ACKNOWLEDGEMENTS

The facility and support provided by the Principal of the College and faculty members of Botany Department at St. Mary's College, Tuticorin is gratefully acknowledged.

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