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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, ANTIOXIDANT ANALYSIS AND ANTIPROLIFERATIVE EFFECT OF *COSTUS PICTUS* D. DON LEAF EXTRACTS

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ABSTRACT

This study was aimed to investigate the phytochemical constituents, antioxidant and anti cancer potential of *Costus pictus* D.Don leaf extracts. Three different solvents ethanol, petroleum ether and aqueous were used for extraction. Phytoconstituents of the extracts were analysed by standard methods. DPPH, FRAP and Total antoxidant activity are the methods used for investigation of antioxidant potential of different extracts of *C.pictus* leaves. Anti cancer potential of leaf extracts were studied against breast cancer cell lines (MCF-7). Phytochemical analysis revealed the presence of active ingredients such as Alkaloids, Steroids, Terpenoids, Glycosides, Tannins, Saponins, Phenols, Flavonoids and Proteins. The extracts had significant antioxidant and free radical scavenging activity. Treatment with ethanol, aqueous and petroleum ether extracts of *Costus pictus* leaf extracts decreased the growth rate and cell survival of MCF-7 cells. Our study suggests that bioactive compounds present in *Costus pictus* leaves could provide new leads to the development novel drugs for the management of oxidative stress related diseases.

INTRODUCTION

Cancer is one of the most prevalent diseases affecting individuals of different ages and sexes worldwide. It is a population of abnormal cells characterized by uncontrolled cellular proliferation, with the ability to invade or spread to other parts of body. More than 30% of cancers are caused by modifiable behavioural and environmental risk factors, including tobacco and alcohol use, dietary factors, insufficient regular fruit and vegetable intake, overweight and obesity, physical inactivity, chronic infections from Helicobacter pylori, hepatitis B virus (HBV), hepatitis C virus (HCV) and some types of human papilloma virus (HPV), environmental and occupational risks including exposure to ionizing and non - ionizing radiation (World health organization 2010). Breast cancer is one of the most common cancers in female worldwide (World cancer report 2014). Conventional treatment of cancer includes interventions such as psychosocial support, surgery, radiotherapy and chemotherapy (World health organization 2010). As these therapies failed to fulfill the benchmark for a successful cancer treatment due increasing rate of mortality associated with cancer and adverse side effects, nontoxic chemoprevention agents derived from plants were proposed for treatment which in turn is safe, cheap and convenient.

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Plants with known therapeutic potential were used for the treatment of various diseases and this forms the basis for all traditional systems of medicine. Over 50% of all modern clinical drugs are of natural product origin and the majority of standard anticancer drugs has been isolated or derived from natural sources, based on their use in traditional medicine (Cragg and Newman 2001).The bioactive compounds present in the plant are responsible for the medicinal properties of the plant (Rohit kumar Bargah 2015). This bioactive compounds or phytochemicals are naturally occurring in plants that have defense mechanism (Suresh *et al.* 2015) and are a source of pharmaceutical drugs. The utilization of the medicinal plants is more common in developing countries and experimental studies showed that the extracts of various plants can also protect against breast cancer (Farshori *et al.* 2013). Cancer cell lines derived from tumours are the most frequently used *in vitro* models for research purposes. It has proved to be a useful tool in genetic studies of breast cancer, and the characterization shows that they are good models for studying the biological mechanism involved in cancer (Louzada *et al.* 2012). The most commonly used breast cancer cell line is MCF-7 and the popularity is due to its exquisite hormone sensitivity through expression of oestrogens, making it an ideal model to study hormone response (Levenson and Jordan 1997). Among all *in vitro* methods MTT assay is most popular for estimating anticancer activity.

Costus pictus commonly known as spiral ginger or insulin plant belongs to Costaceae family. It is a perennial, spreading plant reaching about three feet tall.

The long narrow leaves with lobed margin are spirally arranged on a red coloured stem. The yellow flowers with maroon striations grow intermittently throughout the year. *C.pictus* is valued for its tonic, stimulant and anti septic properties. Propagation of this plant is by stem cutting. Consumption of fresh raw leaves of *C.pictus* lowers the blood glucose level. Powdered leaves of *Costus pictus* possess therapeutic effect, when supplemented to streptozotocin induced diabetic rats, is found to reduce blood glucose (Devi and Urooj, 2008).

Scientific studies on *Costus pictus* have shown that they possess a range of pharmacological properties such as diuretic (Meléndez-Camargo et al 2006)**,** antispasmodic, anti fungal (Abirami *et al.,* 2014), antibacterial, and antioxidant (Malairaj Sathuvan *et al.,* 2012**)** effects apart from its anti-diabetic activity. Hence the present study has been undertaken to screen phytochemical compounds, investigate the antioxidant property and also to explore the possible anti-cancer potential of *Costus pictus* leaf extracts against breast cancer cell lines.

MATERIALS AND METHODS

Collection of plant material

Fresh and healthy leaves of *Costus pictus* were collected from in and around Palakkad (Kerala, India). Leaves were well washed, shade dried and powdered. The powdered sample is then stored in an airtight bottles for further analysis.

Preparation of Plant Extract

The powdered samples were extracted with petroleum ether, ethanol and water. 20gm of samples was dissolved separately in three different conical flasks with 150ml of petroleum ether, ethanol and water. The extraction was carried out for 48h in a rotary shaker at 150-160 rpm. The extracts were filtered using muslin cloth and residue is removed. The filtrate is then evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

Phytochemicals analysis in leaves of *Costus pictus*

The ethanol, aqueous and petroleum ether extracts were screened for the presence of Alkaloids, Steroids, Terpenoid, Glycoside, Tannins, Saponins, Phenols, Flavonoids and Proteins. Phytochemical analysis of the extracts was assayed by standard methods Harborne 1973, Trease and Evans 1989, Sfowara 1993).

ANTIOXIDANT ASSAY

DPPH Radical scavenging activity

One millilitre of 0.3 mM DPPH methanol solution was added to 1 mL of extracts (1000 µg/mL) at different concentration and allowed to react at room temperature (Rajesh *et al.* 2011). After 30minutes the absorbance values were measured at 517 nm. Methanol solution was used as a blank and DPPH solution (1.0 mL, 0.3 mM) with 1 mL methanol served as negative control. Ascorbic acid (1000 µg/mL) was taken as the positive control. The capability to scavenge the DPPH radical was calculated using the following equation

$$\% \text{ inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where '$A_{control}$' was the absorbance of the control reaction and 'A_{test}' was the absorbance in the presence of the extract/standard. The mean values were obtained from triplicate analysis. The antioxidant activity of the extract was expressed as IC_{50}.

Ferric reducing ability of plasma (FRAP)

FRAP agent was prepared by mixing 25 ml of acetate buffer (500 mM/l) with 2.5 ml of tripyridyltriazine (TPTZ) (10 mM/l) and 2.5ml of ferric chloride (20 mM/l) solution. The reaction mixture contained 300 µl of freshly prepared FRAP reagent warmed to 37**º** C, added to 10 µl of test along with 30 µl of water. Absorbance of this solution was taken at 593 nm, just after 4 min from the time of addition of FRAP reagent. An increase in absorbance indicated enhanced reducing potential of plasma (Iris *et al* 1996). Quantitative calculation for each sample was done using an equation obtained from the standard curve of Fe^{++}-TPTZ.

The equation used: *Absorbance = 0.274 x µM of* Fe^{++} *+ 0.114* [$R^2 = 0.974$].

Cell line

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were pass aged weekly, and the culture medium was changed twice a week.

Cell treatment

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h.

The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100
% Cell inhibition = 100 - [A] Test / [A]control x 100

RESULTS

Phytochemical analysis

In the present study ethanol, aqueous and petroleum ether extract of *Costus pictus* leaves were screened for its phytochemical constituents (Table-1). The ethanol extract of *C.pictus* leaves showed chemical constituents like Alkaloids, Steroids, Terpenoids, Glycosides, Tannins, Phenols, Flavonoids and Proteins. Petroleum Ether extract indicated the presence of Alkaloids, Steroids, Terpenoids, Glycosides, Phenols and Flavonoids. Biological active compounds such as Alkaloids, Terpenoids, Glycosides, Phenols, Flavonoids, Saponins, and Proteins were found in aqueous extract.

Table 1. Qualitative Phytochemical analysis of *Costus pictus* leaf extracts

Phytochemicals	Solvents		
	Aqueous	Ethanol	Petroleum ether
Alkaloids	+	+	+
Steroids	-	+	+
Terpenoids	+	+	+
Glycosides	+	+	+
Tannins	-	+	-
Saponins	+	-	-
Phenols	+	+	+
Flavonoids	+	+	+
Proteins	+	+	-

+ = Presence of constituents; - = Absence of constituents

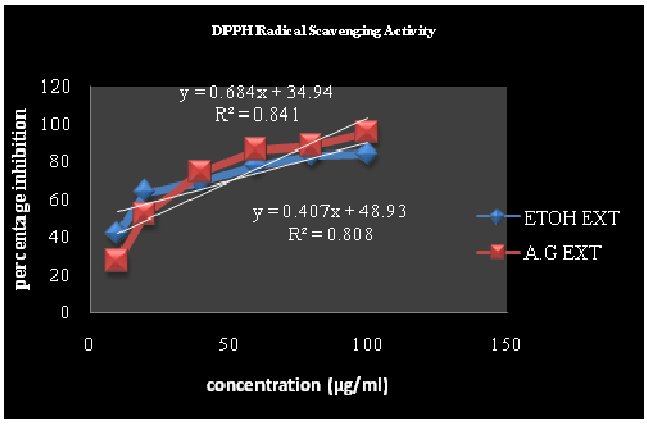

Fig 2.1. Graphical representation of % inhibition of DPPH radical scavenging activity of Petroleum ether, Ethanol, Aqueous extracts and standard (ascorbic acid)

Antioxidant activity

The antioxidant activities of the medicinal plant is generally studied with respect to their free radical scavenging assay as they may be responsible for various bioactivities (Farhat *et al* 2013; Nava-lopez *et al* 2014; Iqbal *et al* 2015). In the present study ethanol, petroleum ether and aqueous extracts of *Costus pictus* leaves were evaluated for its free radical scavenging activities. Three methods DPPH, FRAP and Total antioxidant activity were used for investigation of antioxidant activity of different extracts of *C.pictus* D.Don. DPPH free radical method has been widely used to determine the antioxidant activity of plant extracts based on the reduction of DPPH, a stable free radical. DPPH scavenging ability of *Costus pictus* were determined in ethanol, petroleum ether and aqueous extracts as shown in Table-2 and graphically represented in Fig.2.1. All the results were compared with the standard ascorbic acid. IC 50 value was determined for each extracts as well as for standard ascorbic acid. The study showed that IC 50 of aqueous (22µg/ml) and ethanol (26µg/ml) extracts were significantly lower than the petroleum ether extract (50µg/ml).

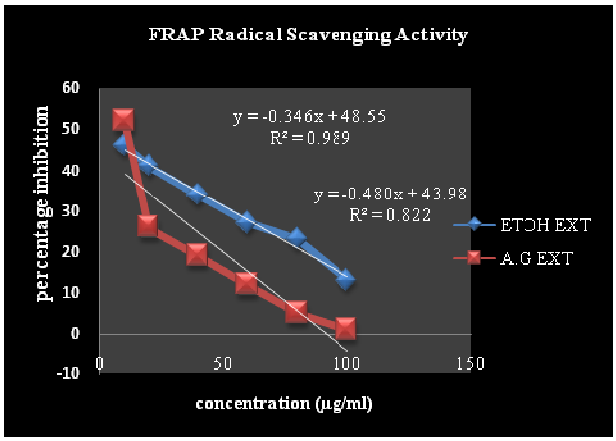

Fig 3.1. Graphical representation of % inhibition of FRAP radical scavenging activity of extracts of *Costus pictus* leaves

The scavenging activity increased with increasing concentration of extracts. The ferrous ion chelating abilities of samples are shown in Table -3 and graphical representation in Fig-3.1. IC50 value of each extract was compared with that of standard ascorbic acid. Among the three extracts, aqueous extracts had a higher Fe^{2+} chelating effect 12.54µg/ml whereas, ethanol extract of *C.pictus* leaves showed IC50 41.90µg/ml antioxidant activity. Petroleum ether extract exhibited IC50 value 62.50µg/ml.

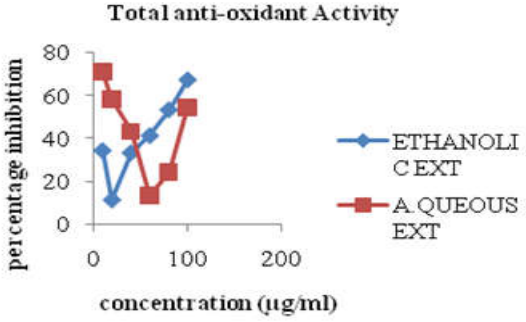

Fig 4.1. Graphical representation of Total anti-oxidant Activity activity of *Costus pictus* leaf extracts

Total antioxidant activity of aqueous, petroleum ether and ethanol extracts of *Costus pictus* leaves are summarized in Table- 4 and graphical representation in Fig.4.1.

For cytotoxicity, different concentrations of ethanol, aqueous and petroleum ether extracts of *Costus pictus* leaves were tested against MCF-7 cell line Table- 5. MTT assay was carried out in 96 well plates.

Table 2. % inhibition of DPPH radical scavenging activity of Petroleum ether, Ethanol, Aqueous extracts and standard (ascorbic acid)

S.no	Concentration (µg/ml)	% inhibition			
		Aqueous	Ethanol	Petroleum ether	Standard
1.	10	27	42	50	20
2.	20	52	64	51	44
3.	40	74	69	67	66
4.	60	86	78	78	86
5.	80	88	83	79	96
6.	100	95	84	80	98
	IC_{50} Value (µg/ml)	22	26.28	50	39.66

Values are expressed as mean± SD (n=3)

Table 3. % inhibition of FRAP radical scavenging activity of Petroleum ether, Ethanol and Aqueous extracts of *Costus pictus* leaves

S.no	Concentration (µg/ml)	% inhibition			
		Aqueous	Ethanol	Petroleum ether	Standard (ascorbic acid)
1.	10	52	46	49	68
2.	20	26	41	41	41
3.	40	19	34	36	20
4.	60	12	27	33	15
5.	80	5	23	27	3
6.	100	1	13	16	1
	IC_{50} Value (µg/ml)	12.54	41.90	62.50	14.13

Values are expressed as mean± SD (n=3)

Table 4. Total anti-oxidant activity of *Costus pictus* leaf extracts

S.no	Concentration (µg/ml)	% inhibition			
		Aqueous	Ethanol	Petroleum ether	Standard (ascorbic acid)
1.	10	71	34	42	40
2.	20	58	11	23	10
3.	40	43	33	10	32
4.	60	13	41	24	41
5.	80	24	53	42	47
6.	100	54	67	70	62
	IC_{50} Value (µg/ml)	31.89	72.75	92.84	44.31

Values are expressed as mean± SD (n=3)

Table 5. Anti cancer activity of Costus pictus leaf extracts

S.no	Concentration (µg/ml)	% inhibition		
		Aqueous	Ethanol	Petroleum ether
1.	18.75	9.238058	10.14607	13.34386
2.	37.5	20.52902	24.27951	23.05567
3.	75	28.10896	33.8334	32.13581
4.	150	37.34702	44.37426	42.9925
5.	300	50.53296	56.02053	52.98066
	IC_{50} Value (µg/ml)	293.94	222.46	255.24

Total antioxidant activity of ethanol extract showed IC50 (72.75µg/ml) and petroleum ether extracts (92.84µg/ml) whereas, aqueous extract showed significantly lower IC50 value (31.89µg/ml). Antioxidant capacity of Quceretin has been used as reference standard.

In vitro cytotoxic effect of plant extracts

Plant extracts contain unlimited compounds and have the capacity to produce cytotoxicity that fascinates researchers in the quest for new and novel therapeutic drugs (Jain and Jain 2011).

The result revealed that all the extract decreased the growth rate and cell survival of breast cancer cell lines. The maximum concentration used in the study was 300µg/ml; at this concentration aqueous, ethanol and petroleum ether extract showed IC50 value 293.94µg/ml, 222.46µg/ml and 255.24µg/ml respectively.

DISCUSSION

In the present study biochemical constituents, free radical scavenging activity and anticancer activity of *Costus pictus* leaves were evaluated.

The result revealed *Costus pictus* leaf extracts are rich in phytochemical compounds such as Alkaloids, Steroids, Terpenoids, Glycosides, Tannins, Saponins, Phenols, Flavonoids and Proteins. Alkaloids derived from medicinal plants show biological activities like antimicrobial, antioxidant, cytotoxicity (Singh *et al* 2016), antispasmodic and pharmacological effects(Thite *et al* 2013), anti in-flammatory (Augusto *et al* 2011) antimalarial (Dua *et al* 2013). According to research Tannins are known to possess antitumor activity and antiviral activities (Kumari and Jain 2012). In vitro studies have shown flavonoids possess anticancer activity (Priya Batra *et al* 2013). DPPH is frequently used to determine radical scavenging activity of natural compounds as it is a stable radical (Harini *et al* 2012). In DPPH free radical scavenging activity aqueous and ethanol extract showed significant lower IC50 Value as compared to that of the standard. This shows the potent radical scavenging activity of *Costus pictus* leaves. Aqueous extracts had a higher Fe^{2+} chelating effect than ethanol and petroleum ether extracts of *C.pictus* leaves as compared to the standards.

This reveals the high Fe^{2+} chelating effect of *Costus pictus* leaves. Metal ion chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalyzing transition metal (Amin *et al* 2013). Total antioxidant activity is used for the analysis of fat- soluble and water – soluble anti oxidants. In this study, aqueous extracts showed remarkable total antioxidant activity than ethanol and petroleum ether extracts. Antioxidant studies revealed that the tested plant have moderate to significant antioxidant and free radical scavenging activity. Among the three extracts Aqueous extract displayed significant antioxidant activity followed by ethanol extract as measured by DPPH, FRAP and Total antioxidant activity. MCF-7 cell lines has proved to be a useful tool in genetic studies of breast cancer, and the characterization shows that they are good models for studying the biological mechanism involved in cancer. Among all *in vitro* methods MTT assay is most popular for estimating anticancer activity. In the present study, *Costus pictus* leaf extract showed inhibition against MCF-7 cell lines. Previous studies also reported significant cytotoxic activities of various plant extracts on MCF-7 cell lines ((Rubalakshmi and Karmegam 2011; Sivaprabha *et al* 2015).The cytotoxic properties of the plant may be due to the presence of phytochemicals.

Conclusion

The present investigation showed more positive for the presence of phytochemicals in *C.pictus* leaves. The result of the present investigation showed that the selected plant has antioxidant and anticancer activity and this is contributed by the phytochemicals present in them. It was concluded that the phytoconstituents present in *C.pictus* leaves could provide bioactive compounds for the development of new leads to the treatment of oxidative stress related diseases. However, further experiments both *in vivo* and *in vitro* will obtain scientific support for the treatment of cancer.

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