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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS OF *COSTUS PICTUS* D DON

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ABSTRACT

The present study was carried out to investigate the phytochemical screening, antibacterial, antifungal activity and GC-MS analysis of *Costus pictus* leaf extracts. Antibacterial activity of *Costus pictus* leaf extracts was evaluated against *Escherichia coli, Salmonella typhi*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger, Aspergillus fumigatus, Aspergillus parasites, Candida* albicans and *M. Purpureus*. Three different solvents Ethanol, Petroleum ether and Aqueous were used for extraction. Phytochemical analysis revealed the presence of active ingredients such as Alkaloids, Steroids, Terpenoids, Glycosides, Tannins, Saponins, Phenols, Flavonoids and Proteins. GC-MS analysis of Petroleum ether and Ethanol extract indicated the presence of phytoconstituents such as Quercetin 7, 3, 4'-trimethoxy, Squalene, Hexadecanoic acid, Octadecanoic acid and Phytol. All the extracts exhibited different degrees of antimicrobial activity against selected microbes, among the extracts Ethanolic extract showed maximum zone of inhibition 18.017±0.076mm against *Salmonella typhi* and aqueous extract exhibited highest fungal activity 15.033±0.058mm against *M. Purpureus*. It is evident from the study that *C.pictus* has highest therapeutic potential due to the presence of rich amount of phytoconstituents along with antimicrobial activity.

INTRODUCTION

Nature has bestowed upon us a plenty of herbs and plants which are source of traditional medicines involved in the maintenance of human health since ancient times. In India, around 20,000 medicinal plants have been recorded, however only a small portion of botanical wealth are used in traditional medicines. Plant materials are invaluable sources in treatment of various diseases and research on certain plants has opened the way to development of various therapeutic agents (Suresh et al 2015). The basis of all currently available modern medicine remains rooted in traditional medicines and therapies. It is widely accepted that more than 80% of drug substances are either directly derived from natural products or developed from a natural compound (Marridass and Britto 2008). In general, plants with a long history in traditional medicinal system are potential candidates for drug discovery. For the last few decades, studies have been conducted for the development of drugs from plants that are relatively safer than synthetic medicines. Phytochemicals are non-nutritive chemicals naturally occurring in plants that have defense mechanism and protect from various ailments (Suresh *et al*, 2015).

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Plants contain hundreds of active ingredients and large number of plant species is yet to be screened for active compounds. Research to find out active ingredients from plants has been intensified. The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases (Chanda *et al* 2010). Even though there are numerous classes of drugs, pathogenic microorganisms are constantly developing resistance to these drugs (Al-Bari *et al* 2006) because of indiscriminate use of antibiotics. This has necessitated a search for new antimicrobial substances from other sources including plants. A large number of medicinal plants have been identified for its antimicrobial activities. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents (Costa *et al* 2008). The potential for developing antimicrobials from plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans *et al* 2002). Costus pictus is a perennial herb, introduced to India from Mexico. Costus pictus D Don, commonly known as spiral ginger or insulin plant is a member of Costaceae family. The plant is recognized by its yellow flowers with maroon striations. The methanolic extract of *C.pictus* leaves exhibited significant hypoglycemic activity in glucose fed mice (Shiny *et al* 2013).

The oral administration of aqueous extract of *Costus pictus* at a dose of 250mg/kg body weight signi cantly decreased the blood glucose with signi cant increase in plasma insulin level in diabetic rats at the end of 14 days treatment (Gireesh *et al* 2009). Scientific studies on *Costus pictus* have shown that they possess a range of pharmacological properties such as diuretic **(**Meléndez-Camargo et al 2006**)**, antispasmodic, anticancer (Malairaj Sathuvan *et al* 2012), anti-fungal (K Abirami *et al* 2014), antioxidant (Jayasri et al 2009**)** effects apart from its anti-diabetic activity.Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Gas chromatography-mass spectrometry is the best technique for the analysis of liposoluble constituents, especially volatile/semi-volatile compounds, and other biologically relevant molecules due to its high resolution, selectivity and sensitivity. Considering this, an attempt has been made to investigate the phytochemical screening and anti-microbial activity and GC-MS analysis of ethanol, petroleum ether and aqueous leaf extracts.

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 of *Costus pictus* leaves

Alkaloids

Wagner's test

About 0.5ml of extract was treated with 2- drops of Wagner's reagent (solution of Iodine in potassium iodide) and the formation of reddish brown precipitate indicated the presence of alkaloids.

Steroids

2 ml of acetic anhydride was added to 0.5g of extract with 2ml sulphuric acid. The colour change from violet to blue or green in samples indicated the presence of steroids.

Terpenoids

Salkowski test

5ml of each extract was mixed in 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was found to show positive results for the presence of terpenoids.

Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated Sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer.

Tannins

About 0.5g of the powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Saponins

About 2g of the powdered sample was boiled in 2ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

Phenols

The 0.5g extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Flavonoids

5ml of dilute ammonia solution were added to a portion of the aqueous plant extract followed by addition of concentrated Sulphuric acid. A yellow colouration was observed in extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Proteins

Ninhydrin test

Each extract was treated with 2ml of 0.2% ninhydrin solution. Presence of violet colouration indicated amino acids and proteins.

ANTIMICROBIAL ACTIVITY

Microorganisms

Bacterial strain used in this work included gram-negative bacteria *Escherichia coli, Salmonella typhi*, *Pseudomonas aeruginosa* and gram- positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Fungal strains used are Aspergillus *niger, Aspergillus fumigatus, Aspergillus parasites, Candida* albicans and *M. Purpureus*. Microbial strains used were obtained from KMCH, Tamil Nadu, India.

Preparation and standardization of inoculums

All the bacterial and fungal strains were freshly sub cultured on Nutrient Agar and Potato Dextrose Agar for 24-48 hrs at 37 C

and 27 C respectively. The agar diffusion method was followed for antimicrobial susceptibility tests. Antibacterial assay (Bauer *et al* 1966). Nutrient agar medium was prepared and transferred into sterile petriplates. 25ml of the standardized bacterial inoculums was spread on agar medium using sterile cotton swab. The discs impregnated with extracts were placed on the inoculated agar medium. The standard antibiotic Ampicillin was also screened under similar conditions for comparison. All the petriplates were incubated at 37°C for 24 hours. After the incubation period, diameter of zone of inhibition was measured. These studies were performed in triplicate.

Antifungal assay

Potato dextrose medium was prepared and transferred into sterile petriplates. 200µl of the standardized fungal inoculums was spread on agar medium using sterile cotton swab. The discs impregnated in extracts were placed on the inoculated agar medium. Tetracycline (10µg/disc) was used as reference standard to determine the sensitivity of each microbial species tested. All the petriplates were incubated at 27°C for 72 hours. After the incubation period, diameter of zone of inhibition was measured. All the tests were performed in triplicate.

Statistical analysis

The data were statistically evaluated using one way ANOVA and expressed as mean ± standard deviation (n=3).

GC-MS

GC-MS was performed on a Thermo GC – Trace ultra Ver: 5.0, with a split injector and a Thermo MS DSQ, mass selective detector fused with silica capillary column having a dimension of 30 mts, 0.25 ID, and 0.25 µm thickness. The oven was programmed from an initial temperature 70 C raised to 260 C at 6 C/ min. Helium gas was used as the carrier gas at a constant flow rate of 1mL/min and volume of 1µl was injected at a split ratio 10:1. The identification of phyto-components was confirmed based on the peak area (%), molecular formula and retention time (RT).

RESULTS

Preliminary Phytochemical screening

The preliminary phytochemical screening of ethanol, petroleum ether and aqueous extracts of *Costus pictus* leaves are presentened in Table 1. Among the three extracts, ethanol extract was found to be rich in biological active compounds such as Alkaloids, Steroids, Terpenoids, Glycosides, Tannins, Phenol, Flavonoids and Proteins. Similarly, Petroleum Ether extract indicated the presence of Alkaloids, Steroids, Terpenoids, Glycosides, Phenols and Flavonoids. Phytochemical compounds like Alkaloids, Terpenoids, Glycosides, Phenols, Flavonoids, Saponin and Proteins were found in aqueous extract.

GC-MS analysis

The compounds present in the Petroleum ether and Ethanol extract of *Costus pictus* leaves were identified by GCMS.

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

The active principle with their molecular weight (MW), retention time (RT), molecular formula and percentage composition in both the extracts are listed in Table-2, 3 and Fig: 1, 2. Major constituents identified in Petroleum ether extract of *C.pictus* leaves are Pentane, 3-ethyl-2,2-dimethyl (82.41), cis-Asarone (3.76), Tetratetracontane (3.42), 3,5-Bis(p-Dimethylaminostryl)-2,2-dimethyl-2H-pyr role 1 –Oxide (2.47) and Quercetin 7,3',4'-trimethoxy (1.65) whereas, Pentacosane (8.36), 13-Docosenamide (6.87%), 2-Pentadecanone, 6,10,14-trimethyl (6.86%), Octadecanoic acid, ethyl ester (6.15%), Linoleic acid ethyl ester(6.15%), Octacosane (6.11%), Hexadecanoic acid, ethyl ester (5.43%), Docosane (5.32%), Heptacosane (5.18%), Neophytadiene (4.79%), (S)-4-Hydroxymethyl-2-phenyloxazoline (4.40%), Tricosane (3.29%) and Squalene (2.38%) were the main compounds indentified in ethanolic extract of *Costus pictus* leaves.

Antibacterial activity

The Ethanol, Petroleum ether and Aqueous leaf extract of *Costus pictus* were tested for their antibacterial activity *against Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa*. The activity was determined by measuring the diameter of zone of inhibition. All the extracts have exhibited different degrees of antibacterial activity. The Zones of inhibitions of different extracts in millimeters of Bacterial strains are summarized in Table- 4 and graphical representation of ethanol extract is shown in Fig.3.

Ethanol extract of *C.pictus* leaves recorded highest inhibition zone (18.017±0.076 mm) against *Salmonella typhi* while less inhibition zone (10.033±0.058mm) was seen against *Bacillus subtilis*. *C.pictus* leaves showed activity (11.617±0.104mm) against *Escherichia coli*, whereas, failed to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Aqueous extract of *C.pictus* leaf exhibited antibacterial activity against all the tested pathogenic bacteria. Maximum activity (14.100±0.100mm) is showed against *Bacillus subtilis* and the minimum being (8.867±0.058mm) recorded against *Pseudomonas aeruginosa*. Aqueous extract showed moderate antibacterial activity (11.033±0.058mm), (11.000±0.100mm), and (10.033±0.058mm) against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* respectively. Petroleum ether extract of *C.pictus* leaf exhibited activity against all the tested pathogenic bacteria. Highest activity (10.167±0.058mm) observed against *Bacillus subtilis* and the least activity (9.083±0.076mm) was recorded against *Staphylococcus aureus*.

Table 2. GC-MS analysis of ethanol extract of *Costus pictus* leaves

S.no	RT	Compound name	Molecular formula	Molecular weight	Peak area (%)
1.	6.84	1-Dodecene	C12H24	168	2.28
2.	8.22	Tridecane	C13H28	184	0.82
3.	9.69	1-Hexadecene	C16H32	224	1.68
4.	17.12	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	C11H16O2	180	1.43
5.	17.64	10-Heneicosene	C21H42	294	0.88
6.	18.65	Neophytadiene	C20H38	278	4.79
7	19.27	Phytol, acetate	C22H42O2	338	2.05
8.	19.89	2-Pentadecanone, 6,10,14-trimethyl	C18H36O	268	6.86
9.	22.91	Hexadecanoic acid, ethyl ester	C18H36O2	284	5.43
10.	25.31	Docosane	C22H46	310	5.32
11.	26.69	Octadecanoic acid, ethyl ester	C20H40O2	312	6.15
12.	26.69	Linoleic acid ethyl ester	C20H36O2	308	6.15
13.	27.27	Tricosane	C23H48	324	3.29
14.	28.04	(S)-4-Hydroxymethyl-2-phenyloxazoline	C10H11NO2	177	4.40
15.	29.61	Butyl 9.cis.,11.trans.-octadecadienoate	C22H40O2	336	1.88
16.	30.02	Pentacosane	C25H52	352	8.36
17.	30.67	Nonacosane	C29H60	408	0.84
18.	31.20	Octacosane	C28H58	394	6.11
19.	32.55	Heptacosane	C27H56	380	5.18
20.	35.23	Benzenamine, 4,4',4"-methylidynetris[N,N-dimethyl	C25H31N3	373	1.12
21.	35.66	Ethyl tetracosanoate	C26H52O2	396	1.21
22.	36.59	Squalene	C30H50	410	2.38
23.	39.06	13-Docosenamide	C22H43NO	337	6.87

Table 3. GC-MS analysis of petroleum ether extract of *Costus pictus* leaves

S.no	RT	Compound name	Molecular formula	Molecular weight	Peak area (%)
1.	3.08	Pentane, 3-ethyl-2,2-dimethyl-	C9H20	128	82.41
2.	4.81	Tetradecane,1-chloro-	C14H29Cl	232	0.15
3.	11.25	9-Octadecen-12-ynoic acid, methyl ester	C19H3202	292	0.11
4.	11.25	d-Nerolidol	C15H26O	222	0.11
5.	11.91	Stearic acid, 3-(octadecyloxy)propyl ester	C39H78O3	594	0.12
6.	12.46	Docosane	C22H46	310	0.12
7	13.35	á –sesquiphellandrene	C15H24	204	0.17
8.	15.19	cis-Asarone	C12H16O3	208	3.76
9.	16.47	Tumerone	C15H22O	218	0.42
10.	17.16	2-Acetyl-3-(2-cinnamido)ethyl-7-methoxyindole	C22H22N2O3	362	0.09
11.	19.99	2-Pentadecanone,6,10,14-trimethyl	C18H36O	268	0.40
12.	21.42	(E,E)-FARNESYLACETONE	C18H30O	262	0.25
13.	21.68	Hexadecanoic acid,methyl ester	C17H34O2	270	0.16
14.	25.17	Phytol	C20H40O	296	0.64
15.	25.94	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	C29H48O	412	0.60
16.	30.59	QUERCETIN 7,3',4'-TRIMETHOXY	C18H16O7	344	1.65
17.	31.00	Cyclohexane, (1-hexadecylheptadecyl)-	C39H78	546	0.13
18.	33.81	Nonacosane	C29H60	408	0.45
19.	34.34	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	C12H10FN5	243	0.19
20.	34.89	2-Iodo-3 ,4 ,4,5-tetramethoxybiphenyl	C16H17IO4	400	0.31
21.	36.99	Tetratetracontane	C44H90	618	3.42
22.	39.13	3,5-Bis(p-Dimethylaminostryl)-2,2-dimethyl-2H-pyr role 1-Oxide	C26H33N3O	403	2.47

C.pictus leaves exhibited moderate acivity (10.083±0.076mm), (10.117±0.104mm) and (10.033±0.058mm) against *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* respectively.

Antifungal activity

Antifungal acitivity of *Costus pictus* leaves were tested for their fungal activity against *Aspergillus niger, Aspergillus fumigatus, Aspergillus parasites, Candida* albicans and M*onascus Purpureus*.

The Zones of inhibitions of different extracts are recorded in millimeters. The results obtained in the evaluation of the antifungal activity of *Costus pictus* leaf extracts against selected fungi are listed in the table- 5; graphical representation of aqueous extract is shown in Fig.4. All the three extracts have exhibited different degrees of antifungal activity. Ethanolic extract of *C.pictus* leaves recorded maximum activity (10.033±0.058mm) against *Aspergillus niger* minimum activity (8.283±0.029mm) was seen against *Candida albicans*.

The extract also showed moderate antifungal activity (8.550±0.050mm), (9.167±0.058mm), and (9.700±0.100mm) against *Aspergillus fumigatus*, *Aspergillus parasite* and *M. purpureus* respectively. Aqueous extract of *C.pictus* leaves recorded highest inhibition zone (15.033±0.058mm) against *M. purpureus* while less inhibition zone (11.083±0.076mm) and (11.100±0.100mm) was noted against Aspergillus *niger* and *Aspergillus parasites* respectively. Moderate activity (14.067±0.058mm) and (12.083±0.076mm) was noticed against *Aspergillus fumigatus* and *Candida albicans* respectively.

Petroleum ether extract of *C.pictus* leaf exhibited activity against all the tested fungi. Highest activity (12.083±0.076mm) observed against *Aspergillus niger* and the least activity (10.100±0.100mm) were recorded against *Aspergillus fumigatus*. Moderate activity 11.033±0.058mm, 11.117±0.104mm and (10.467±0.058mm) observed against *Aspergillus parasites, Candida albicans* and *M. purpureus* respectively.

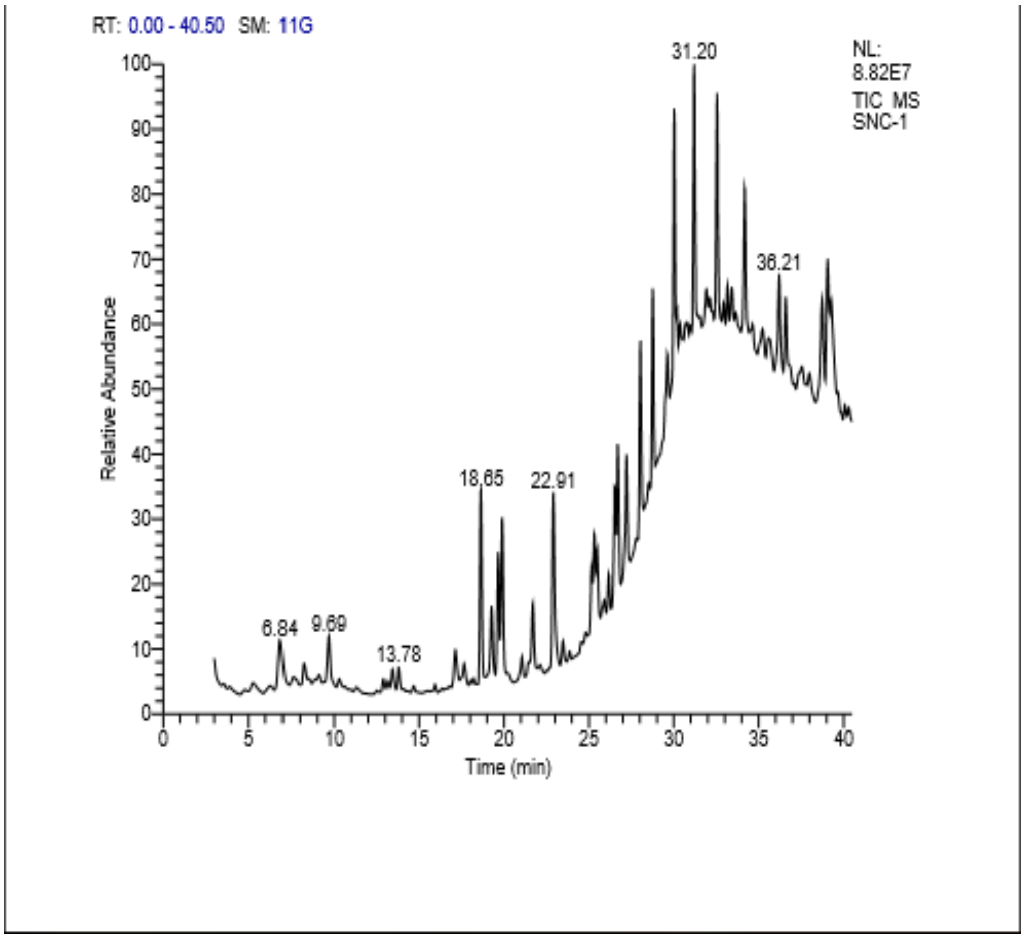

Fig.1. GC-MS analysis of Ethanol Extract of *Costus pictus* leaves

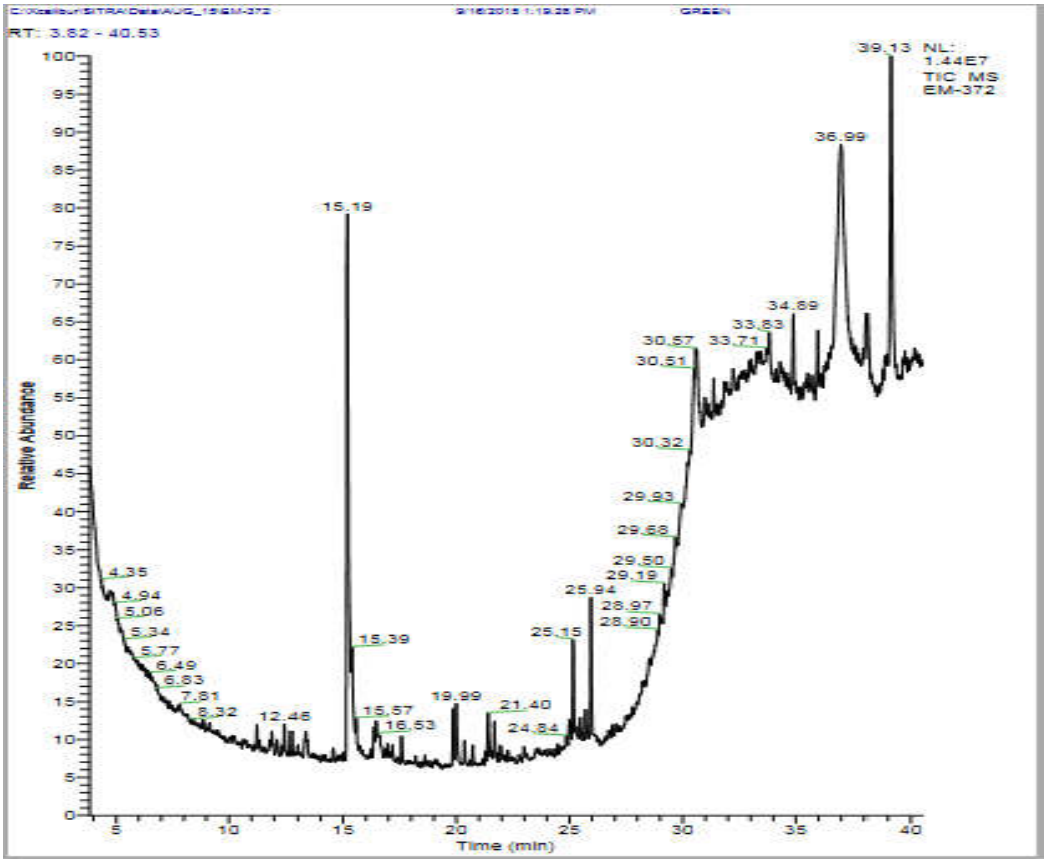

Fig.2. GC-MS analysis of Petroleum ether Extract of *Costus pictus* leaves

Table 4. Antibacterial activity of Ethanol, Aqueous and Petroleum ether extracts of *C.pictus* leaves against selected microbes

Bacteria	Ethanol				
	Concentration(mg/L)	40	60	80	100
E.coli	Zone Diameter (mm)	8.033±0.058	10.033±0.058	11.150±0.050	11.617±0.104
B. subtilis		8.450±0.050	9.100±0.100	10.033±0.058	10.033±0.058
S.aureus		-	-	-	-
S.typhi		10.083±0.076	12.083±0.076	15.100±0.100	18.017±0.076
P.aeruginosa		-	-	-	-
	Aqueous				
E.coli	Zone Diameter (mm)	9.083±0.076	9.483±0.029	10.067±0.058	11.033±0.058
B. subtilis		10.067±0.058	11.083±0.076	13.100±0.100	14.100±0.100
S.aureus		8.100±0.100	9.150±0.304	9.500±0.100	11.000±0.100
S.typhi		7.117±0.104	8.033±0.058	8.483±0.029	10.033±0.058
P.aeruginosa		7.067±0.058	7.783±0.029	8.083±0.076	8.867±0.058
	Petroleum ether				
E.coli	Zone Diameter (mm)	8.083±0.076	8.583±0.029	9.117±0.029	10.083±0.076
B. subtilis		7.100±0.100	8.033±0.058	9.000±0.100	10.167±0.058
S.aureus		7.200±0.050	7.750±0.050	8.033±0.058	9.083±0.076
S.typhi		8.033±0.058	8.400±0.100	9.150±0.050	10.117±0.104
P.aeruginosa		8.117±0.104	8.550±0.050	9.067±0.058	10.033±0.058
	Standard				
E.coli	Zone Diameter (mm)	11.133±0.153			
B. subtilis		20.100±0.100			
S.aureus		18.183±0.161			
S.typhi		9.033±0.058			
P.aeruginosa		25.950±0.050			

Each value represents mean ±SD of three replicated experiments; - = no inhibition

Table 5. Antifungal activity of Ethanol, Aqueous and petroleum ether extracts of *C.pictus* leaves against selected microbes

Fungi	Ethanol				
	Concentration (mg/L)	40	60	80	100
A.niger	Zone Diameter (mm)	8.033±0.058	8.183±0.076	9.083±0.076	10.033±0.058
A.fumigatus		7.117±0.104	7.400±0.100	8.183±0.076	8.550±0.050
A.parasitus		7.200±0.100	8.033±0.058	8.600±0.100	9.167±0.058
C.albicans		7.200±0.050	7.650±0.050	8.033±0.058	8.283±0.029
M.purpureus		8.067±0.058	9.033±0.058	9.267±0.058	9.700±0.100
	Aqueous				
A.niger	Zone Diameter (mm)	9.067±0.058	9.533±0.058	10.100±0.100	11.083±0.076
A.fumigatus		10.100±0.100	11.083±0.076	12.183±0.076	14.067±0.058
A.parasitus		8.783±0.076	9.450±0.050	10.033±0.058	11.100±0.100
C.albicans		9.083±0.076	9.367±0.058	10.067±0.115	12.083±0.076
M.purpureus		11.033±0.058	12.400±0.100	13.083±0.076	15.033±0.058
	Petroleum ether				
A.niger	Zone Diameter (mm)	9.100±0.100	9.817±0.076	11.083±0.076	12.083±0.076
A.fumigatus		8.033±0.058	8.240±0.053	9.033±0.058	10.100±0.100
A.parasitus		8.117±0.104	9.033±0.058	10.150±0.050	11.033±0.058
C.albicans		8.067±0.115	8.450±0.050	9.067±0.058	11.117±0.104
M.purpureus		8.200±0.100	9.100±0.100	10.100±0.100	10.467±0.058
	Standard				
A.niger	Zone Diameter (mm)	14.083±0.076			
A.fumigatus		15.043±0.075			
A.parasitus		25.017±0.076			
C.albicans		10.100±0.100			
M.purpureus		12.083±0.076			

Each value represents mean ±SD of three replicated experiments

DISCUSSION

Phytochemicals are naturally occurring in medicinal plants that have defense mechanism. The bioactive compounds present in the plant are responsible for the medicinal properties of the plant (Akhilja *et al* 2010). In the present study Ethanol, Aqueous and Petroleum ether extract of *Costus pictus* leaves were screened for its phytochemical constituents. 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 represent a class which affects the central nervous system, reduces appetite and behaves as diuretic (United state department 2010). Steroids help in regulating the immune response (Shah *et al* 2009). Terpenoids also possess antimicrobial, antiparasitic, antiviral, antiallergic, antispasmodic, antihyperglycemic, anti-inflammatory and

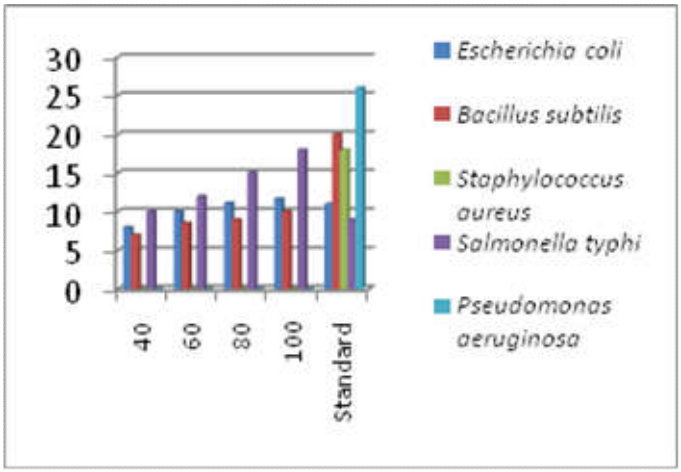

Fig.3. Graphical representation of the Zones of inhibitions of Ethanol extracts in millimeters of Bacterial strain

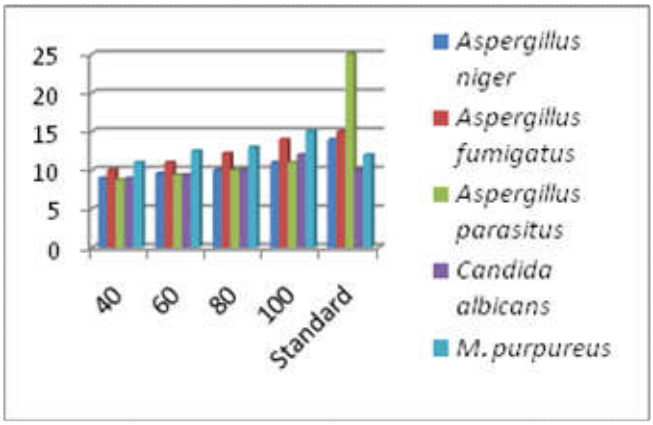

Fig.4. Graphical representation of the Zones of inhibitions of Aqueous extracts in millimeters of fungal strains

immunomodulatory properties (Rabi and Bishayee 2009). *In vitro* studies have shown flavonoids possess a wide range of biological and pharmacological activities such as antibacterial (Cushine and Lamb 2011; Manner *et al* 2013), antifungal, (Chepkirui *et al* 2014), anti-allergic, anticancer (Priya Batra *et al* 2013; Woo and Kim 2013). GC-MS analysis of Ethanol and Petroleum ether extracts of *C.pictus* leaves indicated the presence of 23 and 22 chemical compounds respectively. This reveals *C.pictus* leaves possess compounds that have potent medicinal characteristics. Hexadecanoic acid (palmitic acid), Octadecanoic acid, Quercetin, Squalene and Phytol are the main compounds identified. Among the phytochemicals identified, Hexadecanoic acid has antioxidant property (Sudha *et al* 2012). Quercetin is a member of flavonoid family used for treating atherosclerosis, diabetes, osteoporosis and for reducing risk of cancer. Phytol, a diterpene constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and chronic inflammatory diseases (Ogunlesi *et al* 2009). Phytol is also used as precursor for the production of Vitamin K1 and Vitamin E. Squalene, a triterpene acts as antibacterial, antitumor and cancer preventive agents (Sermakkani and Thangapandian, 2012). Octadecanoic acid is used to bring out a pearly effect in soaps, shampoos and other cosmetic products. These findings bestow importance of plants, which could be of considerable interest to the development of new drugs.

Aqueous and Petroleum ether extract of *C.pictus* leaf exhibited antibacterial activity against all the tested pathogenic bacteria. This indicated that the Aqueous and Petroleum ether extracts of *Costus pictus* leaves has a broad-spectrum of antibacterial activity against both gram positive- *Staphylococcus aureus* and *Bacillus subtilis* and gram negative bacteria- *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The activity shown by *C.pictus* leaf extracts against *E.coli* and *salmonella typhi* were high as compared to that of standard. This shows the disease resistance ability of *Costus pictus*, which may be due to the presence of phenolics and alkaloid substances (Ariharan *et al* 2012). Aqueous extract of *C.pictus* showed highest fungal activity against *M. Purpureus* and is high when compared to the activity shown by the standards. The activity shown by *C.pictus* leaf extracts against *Aspergillus niger* and *Aspergillus fumigatus* was very close result as compared to the standard. The factors responsible for this high susceptibility of microbes to the extracts are not exactly known but may be attributed to the presence of phytochemical compound. Thus, the study ascertains the value of the plants, which can be used as a source of phytomedicine to act against microbes.

Conclusion

The presence of rich amount of phytochemical compounds along with antibacterial and antifungal activity justifies the use of plants in traditional and modern health care practices. However, extensive research in the area of isolation and identification of biological activity of the individual phytoconstituents will give fruitful results. From the research findings it can be concluded that *Costus pictus* leaves possess a wide range of biological and pharmacological properties. So it is suggested as the plant of pharmaceutical importance.

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