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Phytochemical screening and cytotoxicity studies of rhizome extracts of *Cyperus pangorei Rottb.*

J.Fathima Benazir*, R.Suganthi, P.Ravichandran, M.Padmini Pooja

School of Biotechnology, Dr. G.R.Damodaran College of Science, Civil Aerodrome Post, Coimbatore-641014, Tamilnadu, (INDIA)

Ph: +91 422 2572719, +91 9442622723

E-mail: fathimabenazir@yahoo.co.in

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ABSTRACT

Drugs derived from plants are gaining new prospects as traditional antibiotics are becoming ineffective. This study was to screen the bioactive phytochemicals of *Cyperus pangorei* because biological screening of the plant was very limited and it was recently found to be used as a substitute to *C.rotundus* which is known to have many bioactive components. Quantitative analysis was by extracting and estimating the compounds by UV spectroscopy and other techniques and qualitative analysis was by thin layer chromatography. Since there was no available literature on the cytotoxic effects of the plant, the study also focused on cytotoxicity screening of the plant extracts. Vero cell lines and human lung carcinoma cell lines were used for this purpose. *C.pangorei* possessed several primary and secondary metabolites and the crude extract was not cytotoxic.

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KEYWORDS

Cyperus pangorei rottb.;
Rhizome;
Plant extract;
Primary metabolites;
Secondary metabolites;
Cytotoxicity.

INTRODUCTION

Cyperaceae is one of the largest monocotyledonous families, cosmopolitan in distribution and comprising over 5000 species in 120 genera[1]. This sedge family comprises of perennials and few annuals. They are more or less grass like plants generally occurring in marshy and wet areas[2]. *Cyperaceae* finds use mainly as food and fodder. Tuberous rhizomes of *C.esculentus* are used as food and species like *C.castaneus*, *C.elatus*, *C.haspan*, *C.iria* and *C.pilosus* are used as fodder reports Gupta[3].

Cyperus is one of the Largest genera in *Cyperaceae* with 650-700 species spread all over the world of which 80 species occur in India. Several species of *Cyperus* possess medicinal values. For example, *C.articulatus* and *C.prolixus* are used in birth control pills, induction of labor, hallucinogenic preparation and treatment of epilepsy, *C.incompletus* is an indigenous medicine for women's diseases[4] and *C.corymbosus Rottb.* is used as an abortifacient. These properties are conferred by bioactive phytochemicals. Plants have an almost limitless ability to synthesize these substances. Most are secondary metabolites of which atleast 12,000 have been isolated. Useful phytochemiclas are phenolics and polyphenols, quinines, flavones, flavonoids, tannins, coumarins, terpenoids and essential oils, alkaloids, lectins and polypeptides[5]. They can be analyzed quantitatively and qualitatively.

Cyperus pangorei Rottb. Is a perennial erect herb, rhizome decumbent, 3-7 mm thick, with brown scales and light brown roots 0.5-0.7mm thick. The culms of

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C.Pangorei provide material for mat making. Their rhizomes were found to be used as a substitute for *C.rotundus* in Gujarat[6]. *C.rotundus* was reported to have been widely used in ethno-medicine in China, Egypt, India, Java, Sudan, Turkey and south-east Asian countries[7,8]. It is in this context, the present study focused on biological screening of rhizome extracts and essential oils of *Cyperus pangorei Rottb.* As far as the literature survey could ascertain, there are no reports on the cytotoxic activities of C.*pangorei Rottb.* though there are some reports on its medicinal properties. Hence, the cytotoxic effects of the crude plant extract were assayed on Vero cell lines and Human lung carcinoma cell lines.

MATERIALS AND METHODS

Source of plant material

Cyperus pangorei Rottb. plants were collected along with rhizomes from streams near Alwarkurichi in Tirunelveli district, Tamilnadu.

Preparation of plant extract

The protocols of Bashir et al.,[9] and Erturk et al.,[10] were followed in preparing the plant extracts. Various alcoholic and ether extracts were prepared. After filtration through double layered Muslin cloth and filter paper the solvents were evaporated to dryness. The dried residues were weighed and used to prepare extracts pf different concentrations in 50% alcohol. The extracts were stored at 4°C for further bioassays.

Solvent extraction of essential oils

The rhizome (10g) was air dried and extracted in 100ml hexane, filtered using filter paper and evaporated to dryness at 40°C to obtain a concentrate. It was dissolved in ethanol and evaporated to get an absolute. The absolute with essential oil was weighed and dissolved in 50% ethanol, stored at 4°C for further assays.

Quantitative analysis of phyto active components

1. Primary metabolites

Estimation of starch according to the procedure of Sadasivam and Manickam[11] and cellulose, total soluble sugars and proteins according to Mahadevan[12] was done. RNA and DNA was then estimated[12]. This was followed by DNA analysis for which DNA was first isolated. It was then observed on Agarose gel stained with ethidium bromide. For the spectrometric analysis, the isolated DNA was diluted with Tris EDTA (TE) buffer and the absorbance was measured at 260nm. The concentration of DNA in μg/ml was calculated using the formula Optical Density (OD) value × 50μg/ml × dilution factor.

2. Secondary metabolites

Phenolics and bound phenols were first estimated[12]. Quinones were estimated by the reduction method. For estimating saponins, 0.5g of the sample was refluxed in 0.3ml of methanol for 2 hours. The methanol extract was treated with 1.5 ml hydrochloric acid (HCL) and re-extracted twice in water. It was dried under anhydrous Sodium sulfate (Na_2So_4). The weight of the extract was calculated as the percentage of the sample. Proanthocyanidin method and Vanillin HCL method were employed to measure condensed tannins and the Radial diffusion method for tannic acid.

Qualitative analysis of phyto active compounds

Thin layer chromatography (TLC) of phenols and phenolic acids and TLC of anthocyanins to detect anthocannins, chalcones, aurones, flavanols, flavones and biflavonyls was performed. According to Wilson[13], TLC of alkaloids was done. Finally was the two-dimensional TLC of hydrocinnamic acids and flavanoids.

Cytotoxicity screening

Cytotoxicity screening was done using Vero (Normal African Green monkey kidney cell line) and A-549 (Human lung carcinoma cell line). The cytotoxic property of the plant extract was confirmed by determination of mitochondrial synthesis by Microculture Tetrazolium (MTT) assay and total cell protein content by Sulphorhodamine B (SRB) assay respectively.

RESULTS AND DISCUSSION

Properties of rhizomes

The rhizome of *Cyperus pangorei* was brown and scaly with slight sweet and sour taste and aromatic odor.

Rhizome extract

Crude extracts were obtained from the rhizomes. They appeared reddish brown when extracted with

TABLE 1: Estimation of primary metabolites in rhizomes of *C.pangorei*

Primary metabolites	Concentration (mg/ g wt.)	Percentage (%)
Total Sugars	650	65
Starch	720	72
Cellulose	130	13
Glucose	800	80
Protein	600	60
DNA	9.8	0.98
RNA	5.5	0.55

TABLE 2: Estimation of secondary metabolites in rhizomes of *C.pangorei*

Secondary metabolites	Concentration	Percentage
Phenols (mg/ g wt.)	92.0	9.2
Bound Phenols (mg/ g wt.)	46.4	4.6
Quinones (mg/ g wt.)	53.3	5.4
Saponins (mg/ g wt.)	28.2	2.8
Condensed Tannins(mg/ ml)	18	-
Proanthocyanidins(mg/ ml)	36	-
Tannic Acid (mg/ ml)	8	-

methanol/ ethanol and brown with petroleum ether/ethyl acetate. The essential oil prepared by solvent extraction was brownish yellow with characteristic aromatic odor.

Primary metabolites

The rhizome was examined for primary metabolites. One gram fresh weight of the rhizome had 65% of total soluble sugars, 72% of starch, 13% cellulose, 80% glucose, 60% proteins, 0.98% of DNA and 0.55% RNA (TABLE 1). The plant genomic DNA was isolated from the rhizome. Clear fluorescent bands were observed on Agarose gel stained with ethidium bromide. The molecular weight of the band was 21.1 kb approximately when compared with a marker (λ Hind III, EcoRI double digest). The concentration of the isolated genomic DNA was 23.2μg/ g of the tissue.

High concentration of primary metabolites like polysaccharides in the rhizome act as biologically active agents[14]. Monosaccharide like glucose inhibited pathogenic bacteria, acting as analogue to substrate[5]. A high concentration of total soluble sugars and glucose cause osmotic imbalance by rupturing cell wall of microorganisms. Polypeptides have also been reported to have antimicrobial activity. Ibrahim et al.,[15] have reported that proteins and polysaccharides of certain plants remarkably reduce the number of viable Ehrlich Ascites tumor cells, as well as the DNA, RNA and protein synthesis in cells.

Secondary metabolites

1. Quantitative analysis

Plant secondary metabolites like phenols (9.2%), bound phenols (4.64%), quinones(5.4%), saponins (2.8%), condensed tannins (18mg/ml), proantho cyanidins (36 mg/ml) and tannic acid (9mg/mg) were present in fresh rhizomes and rhizome extracts. Maximum tannic acids were extracted in case of methanolic and ethanolic extracts than petroleum ether and ethyl acetate extracts (TABLE 2).

Phenols and bound phenols are toxic to microorganisms[5]. They damage membranes, inhibit enzymes, deprive substrate and disrupt biochemical processes[16]. Phenolics that are constitutively present in plants and that are believed to confer disease resistance include simple compounds such as hydroxystilbenes, isoflavanoids, flavones and hordatines. Phenolic compounds possessing a carbon side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and are cited as antimicrobial. Essentially, all phenolics are antibiotic.

Quinones are responsible for the browning reaction in cut or injured fruits and vegetables. They are an intermediate in the melanin synthesis pathway in human skin. The individual redox potential of a particular quinone- hydro quinone pair plays an important role in many biological systems. In addition to providing a source of stable free radicals, they are known to complex irreversibly with nucleophilic amino acids in proteins often leading to inactivation of proteins and loss of function. For this reason quinone antimicrobial effects are great. Vitamin K, a complex naphtha quinone is known for its anti-hemorrhagic activity and oxidation in body tissues.

Many human physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and a wide range of anti-infective actions, have been assigned to tannins. Condensed tannins, proantho cyanidins, hydrolysable tannins and quinones are generally protein poisons remarks Cowan[5].

Saponins might contribute to antimicrobial activity by altering the permeability of cell membranes and by interacting non-specifically with proteins and exerting general toxicity[16]. Animal studies have demonstrated that tannic acid exhibits anti-carcinogenic activity in chemically induced cancers. Recently it has been re-

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TABLE 3 : Thin layer chromatography to screen for secondary metabolites of *C.pangorei*

Components	One dimensional TLC	Two dimensional TLC	Rf value
Flavanoids	-	Red band	0.32
Alkaloids	UV fluorescence	-	0.73
Phenols and phenolic acids	Blue spots	-	0.95
Hydrocinnamic acid	-	UV blue fluorescence	0.05
Anthocyanins	Yellow spots	-	0.35
Chalcones and aurones	Red coloured spots	-	0.38
Flavanols	UV bright yellow fluorescence	-	0.33
Flavones	UV yellow green fluorescence	-	0.24
Biflavonyls	Dull brown spots	-	0.13

TABLE 4 : Cytotoxic assay of crude rhizome extracts of *C.pangorei*

Determination of CTC_{50} in Vero cell line			
Assay	**MTT assay**	**SRB assay**	**Average CTC_{50}**
Alcoholic extract of *C.pangorei*	973.48µg	898.27µg	935.87µg
Determination of CTC_{50} in human lung carcinoma cell line			
Assay	**MTT assay**	**SRB assay**	**Average CTC_{50}**
Alcoholic extract of *C.pangorei*	973.48µg	>1000µg	986.74µg

ported that tannic acid and ester-bond containing green tea polyphenols are potent proteosome inhibitors *in vitro* and *in vivo*[17]. Coumarins are antithrombic, anti-inflammatory, vasodilatory and antimicrobial activities.

2. Qualitative analysis

The presence of other secondary metabolites like flavonoids, alkaloids, phenols and phenolic acids, hydrocinnamic acids, anthocyanins, chalcones, aurones, flavonols, flavones and biflavonyls were analyzed using one dimensional and two dimensional TLC(TABLE 3). Thin layer chromatography is the easiest and cheapest. Advanced techniques such as Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) can be carried out for the detection of unknown compounds in the sample. HPLC coupled with UV photodiode array detector (LC/ UV) has been widely used for the analysis of crude plant extracts.

Flavanoids were detected by the presence of red bands and phenols and phenolic acids with blue spot. Green fluorescence indicated alkaloids and bright blue fluorescent spot showed hydrocinnamic acid. Anthocyanins were detected by the presence of yellow spots, chalcones and aurones by red spots, flavonols by bright yellow fluorescence and flavones by yellow green fluorescence and biflavonyls by brown spots respectively. The Rf values were 0.32, 0.73, 0.95, 0.05, 0.35, 0.38, 0.33, 0.24 and 0.13 respectively.

Flavonoids have been documented to be effective against HIV and a variety of other microorganisms. The rhizomes of *C.esculentus* have considerable amounts of water-soluble flavonoid glycosides, which are antioxidants with anticancer properties which could possibly contribute to reducing oxidative damage cells and tissues as is the case of HIV. The enhanced antimicrobial activity of flavonoids and flavonols is due to their binding in the extra cellular and soluble proteins and may disrupt microbial cell walls. Alkaloids are highly reactive substances with biological activity in low doses. They are found to have antimicrobial activities. Their action is attributed to their ability to intercalate with DNA. Previous studies have shown that hydrocinnamic acids are inhibitory to Gram positive bacteria.

Cytotoxicity screening

They crude plant extract exhibited cytotoxic activity against Vero cell lines and Human lung cancer cell lines only at a very high concentration of 935.87 and 986.7µg/ml respectively (TABLE 4). This can be considered negligible. This lack of cytotoxicity of the crude extract, though antitumor properties have been reported in case of *C.rotundus* and *C.esculentus*. This might be attributed to the crude method of preparation of the extract. Antitumor properties of *Cyperus species* have been reported only in case of abdominal and cervical cancers, which is mainly by the Human Papiloma Virus. So the activity might be against the virus and not to the cells. Lack of cytotoxicity is beneficial in making crude preparations used as drugs in Ayurveda and Siddha. Currently, applied radiation therapy and other standard chemotherapeutic drugs kill some tumor cells through induction of apoptosis. Unfortunately, a majority of human cancers are resistant to these therapies. Thus, plant derivatives have great potential to be developed into anticancer drugs because of their multiple mechanisms and low side effects.

CONCLUSION

To conclude with, this study has thus established the presence of bioactive components in the rhizomes

of *Cyperus pangorei* and the non-cytotoxic properties of the crude extract. Further investigations are recommended to incorporate the useful and harmless *C.pangorei* into the modern health care system. Further, the attempt to isolate DNA from the hard recalcitrant rhizomes will find use in studying the genes involved in expressing these phyto active components. It would also form a preliminary base for drug discovery and formulation of new drugs through genetic alterations and cloning.

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