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Research Article

PHYTOCHEMICAL AND CYTOTOXIC PROPERTIES OF WILD *SARCHOCLAMYS PULCHERRIMA* GOUD FROM ASSAM, NORTH EASTERN INDIA

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ABSTRACT

Objective: The present investigation was an attempt to evaluate the phytochemical constituents, free radical scavenging activities and anticancer properties of the leaf extracts of wild *Sarchoclamys pulcherrima* Goud, a wild vegetable plant used by Mishing tribe of Assam.

Materials and methods: The phytochemical analysis, free radical scavenging activities and cytotoxic properties of the plant was carried out in the ethanolic extract of leaves of *Sarchoclamys pulcherrima* Goud using DMSO as neutral solvent.

Results: The study indicated the presence of most of the major phytochemicals *viz.* alkaloids, saponins, flavonoids, tannins etc. in the leaf extract of the plant. The free radical scavenging activity of the sample against DPPH and ABTS was recorded as 84.87% and 76.9% respectively and found comparable to that of the ascorbic acid (88.2% and 83% respectively). In vitro cytotoxicity assay indicated a maximum cell damaging activity (60% Viability) at the exposure concentration (100 µg/ml leaf extract) and least activity (90% Viability) at the exposure concentration (20 µg/ml leaf extract).

Conclusion: The present investigation will help to evaluate the neutraceutical prospect of the plant species with scientific validation of a traditionally acclaimed medicinal food plant.

Keywords: *Sarchoclamys pulcherrima*; Antioxidants; Phytochemicals; Anti-cancer; Mishing tribe; Cytotoxicity.

INTRODUCTION

Cancer is considered as one of the most life threatening diseases with the record of 8.5 Lakhs new patients every year in India only and there is still ever increasing trends of cancer cases with increasing pressure of causative agents like change in food habits, food contaminant from pollutions and radiations etc [1]. The current global scenario indicates that breast cancer and colorectal cancer are the most prominent cancers in case of woman and man [2]. Study shows that there is higher incidence of cancer in entire North Eastern region of India in which the state Assam stood second after Mizoram [3]. The district wise distribution within the state Assam, indicated a highest incidence of cancer (1839 cases) in Kamrup followed by Dibrugarh (783 cases) and lowest in Silchar (242 cases) during the year 2005-06 [3]. However, community wise reports on cancer prevalence in these districts are still scanty.

In folklore system of medicine, plants are being extensively used in treatment of several ailments including cancers for centuries due to its availability, effectiveness, and cost free with fewer side effects [4]. That is why; the traditional knowledge of using plants in health care has been incorporated into the allopathic system of medicine [5]. By and large, in traditional system of food amongst tribes, there is sufficient dietary intake of essential molecules through total intake of phytochemicals in the plant materials. However, its absorption depends on the source, forms of compounds as well as physiological condition of the consumer [6]. Epidemiological studies have shown that the dietary intakes of fruits and green vegetables have strong correlation with the reduced risk of developing chronic diseases [1].

Plants contain various bioactive compounds such as alkaloids, tannins, saponins, flavonoids, phenols and other antioxidants as its secondary metabolites. These metabolites produce specific effects on the physiology of human being and other organisms [2]. The search for cancer drugs from natural sources began with the investigations conducted by Hartwell and his co-workers in the late 1960's using podophyllotoxin and its derivatives isolated from the plant *Podophyllum peltatum* [2]. That plant could be a source of novel cancer chemotherapeutic agent is proved by the development of anticancer agents from *Catharanthus roseus* (L.) G. Don [7]. It is estimated that over 74% out of 121 prescribed drugs for treatment of cancer are derived from plants that have folklore claims [8]. It is needless to mention that a huge resource of novel molecules with anticancer property are still lying un-trapped in the plant kingdom and for which the researchers all over the world are concentrating on the inventorization of herbal medicines to boost immune cells of the body against cancer [4]. Today, a good number of medicinal plants are investigated to understand their antitumor activity [5, 7]. In recent years, emphasis has been given on the screening and use of edible novel plant as total package of nutrient with herbal medicines [6].

S. pulcherrima Goud is a perennial semi evergreen medium sized tree found in the forests of upper Assam. The tender leaves of this plant are basically used by Mishing tribe for culinary purposes especially for cooking of pork meat. The Mishing tribe of Assam, India has the typical food habit of consuming more animal protein, homemade alcoholic beverages and large variety of leafy vegetables along with rice as their staple food [9, 10, 19]. The boiled leaf extract of *S. pulcherrima* is also widely used by the community for treatment of chronic dysentery. However, no scientific report was found with respect to the phytochemicals as well as the antitumor activities of this traditionally used vegetable plant. Therefore, the present study aims the qualitative and quantitative estimation of phytochemicals present in the leaf extract of *S. pulcherrima* Goud and in vitro assessment of its anticancer activity against Murine Dalton's Lymphoma Ascites (DLA) cell line.

Fig. 1: A twig of *S. pulcherrima* Goud

MATERIALS AND METHODS

Chemicals and reagents:

All the chemicals used in the study were of analytical grade and procured from Merck India Pvt. Ltd.

Maintenance of Murine DLA cell line:

The DLA cell line used in the present experiment is being maintained *in vivo* in 10–12 weeks old mice by serial intraperitoneal (i.p.) transplantation of approximately $1x10^6$ viable cells per animal (0.25 ml phosphate buffered saline, PBS, pH 7.4). DLA-transplanted hosts usually survive for 20–21 days. The ethical evaluation of experimental protocols were reviewed and approved by the Institutional Animal Use and Care Committee of North Eastern Hill University, Shillong, India. The care, welfare and use of the animals were in accordance with the National Institutes of Health (NIH) guidelines.

Collection and processing of plant material:

Leaves of *S. pulcherrima* (Goud) (Figure 1) were collected from Dibrugarh during pre monsoon season, shade dried and then powdered. The powdered leaf was macerated with ethanol, for 48 h and filtered using Whatman filter paper No. 1. The filtrate was then evaporated at a constant temperature of 50°C until a semi dried powder/sticky mass of plant extract was obtained which is kept in refrigerator for further use. These crude extract were dissolved separately in distilled water and Dimethyl sulphoxide (DMSO) as neutral solvent for biochemical analysis and for anti-tumor activity of the plant respectively.

Qualitative and quantitative estimation of phytochemicals and antioxidants:

Phytochemical analysis was done by biochemical methods prescribed by Sadasivam and Manickam, 2005 [11]. The DPPH radical scavenging activity was carried out following the method of Stanojevic et al., 2009 [12], and ABTS assay by Re, et al. 1999 [13].

The antioxidant present in the plant material was estimated by DPPH and ABTS radical scavenging assay [12, 13].

The capacity of scavenging free radicals was calculated as scavenging activity (%) =

$$\frac{\text{Absorbance in control} - \text{Absorbance in sample}}{\text{Absorbance in control}} \times 100$$

Where,

Absorbance in control = absorbance of DPPH/ABTS radical + methanol

Absorbance in sample = absorbance of DPPH/ABTS radical + sample extract/standard for both the above mentioned equations.

In vitro determination of cytotoxicity of the plant extract against Murine Dalton's lymphoma Ascites (DLA) cell lines:

Assay for short term cytotoxicity: The in vitro short term cytotoxicity of ethanol extract of *S. pulcherrima* Goud was assayed using Murine Dalton's lymphoma Ascites (DLA) cell lines. Briefly 1×10^6 ml^{-1} viable cells of the cell line suspended in 0.1 ml of phosphate buffered saline (PBS) (0.2 M, pH 7.4) was impregnated with various concentrations of the extract (20 µg/ml to 100 µg/ml) and the final mixture volume made to 1.0 ml using PBS. The preparations were incubated at 37°C for 6 h. After incubation, the viability of the cells was determined by trypan blue exclusion method [14]. Percent cytotoxicity was calculated after comparing with the untreated control using cisplatin as standard drug.

Apoptosis assay using fluorescence microscopy:
Fluorescence based apoptosis was determined by using acridine orange and ethidium bromide (AO/EtBr) staining method. Cells (approximately 1×10^6 ml^{-1}) were seeded in 24-well plate along with 100µg/ml of plant extract and then incubated at 37°C for different time period (0 to 6 hrs.). The cells were washed separately with PBS and treated with AO/EtBr (100 µg/ml of each dye). The cells were observed under fluorescent microscopy using a blue filter, and photographed (ASA-400 Kodak); nonviable cells were observed in red–orange color due to damaged membrane which allows EtBr to enter into the cell whereas, viable cells were green in color.

Statistical analysis

All the experiments were performed in triplicate and the results were expressed in mean ± S.D. Student's *t*- test was performed.

RESULTS

The qualitative estimation of phytochemicals and antioxidants present in the plant sample was carried out and presented in Table 1 and 2 respectively. The extract recorded 84.87% and 76.9% free radical scavenging effect against DPPH and ABTS respectively. From the present study it is found that the *S. pulcherrima* has potent free radical scavenging activity and it is comparable with that of ascorbic acid.

Table 1: Qualitative test of phytochemicals present in the leaf extract of *S. pulcherrima*

Phytochemicals	Alkaloids							
	Dragendorf's test	Mayer's test	Hager's test	Wagner's test	Saponins	Flavonoid	Phenol	Tannin
S. pulcherrima leave extract	+	+	+	+	+	+	+	+

Table 2: Antioxidant activities of *S. pulcherrima* leave extract

Sample	Antioxidant activity (% inhibition in mg/ml)	
	DPPH radical scavenging activity	ABTS radical scavenging activity
S. pulcherrima	84.87	76.9
Ascorbic acid	88.2	83

Figure 2 shows the results of in vitro (% Viability of the cells) test for short term cytotoxicity of different doses of crude ethanolic extract of *S. pulcherima* leaves against the Murine Dalton's lymphoma Ascites cell line. The figure indicated a decreasing trend in Viability of the cells exposed to the plant extract from 90 to 62% while the concentration of the extract was increased from 20 to 100 (µg/ml) respectively. However, Cisplatin still maintains maximum activity against the cell line with minimum Viability (27%). In this respect it could be presume that the cytotoxicity effect of the given sample is a dose dependent and higher activity is expected from the purified compound.

Figure 3 shows that the plant extract have damaging effect on the DLA cell line. The result also indicated a proportionate increase in the number of damaged cells with increase in the doses of extract applied against the cell line. Maximum cell damage was observed in the cell line culture impregnated with 100 µg of plant extract while little or no damage was recorded in the culture that was incubated with 20 µg of the plant extract.

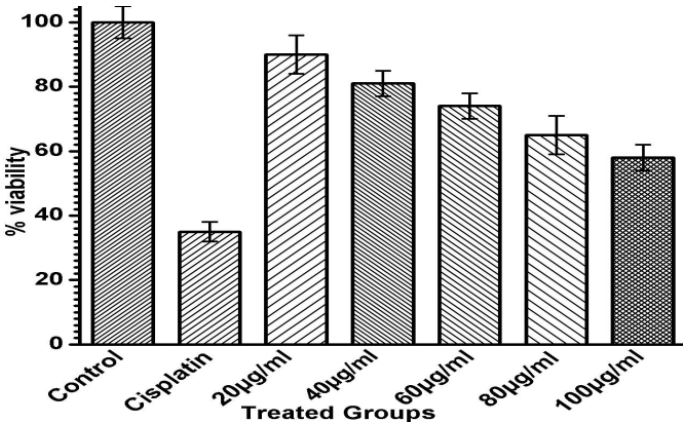

Fig. 2: In-vitro test for short term cytotoxicity of different doses of crude ethanol extract of *S. pulcherrima* leaves against the Murine Dalton's Ascites lymphoma cell line.

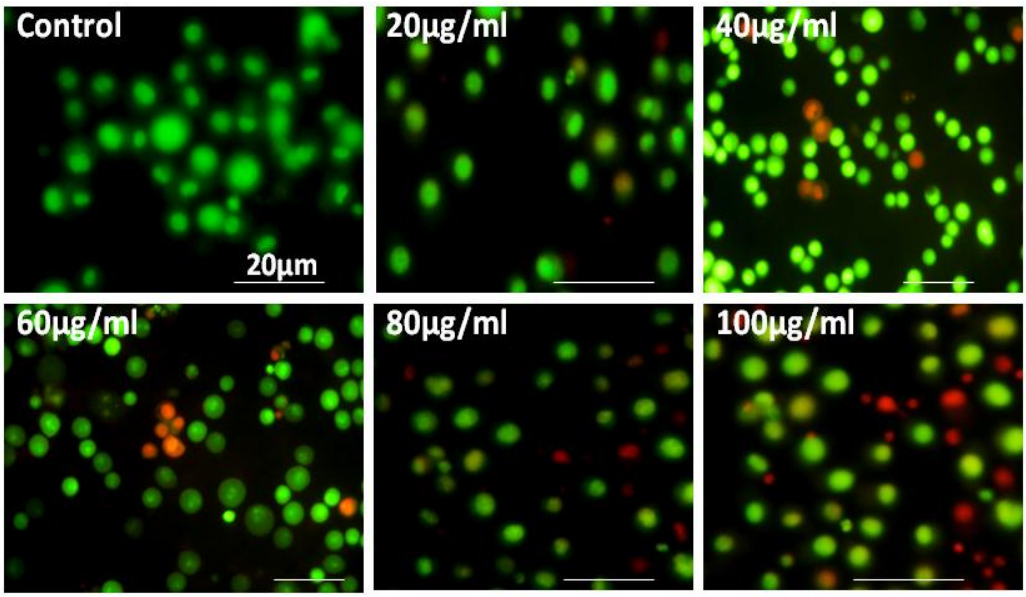

Fig. 3: Floor picture of Apoptosis study using fluorescence microscope-(the damaged cells { are in orange/ red color and viable DLA cells are shown in green color).

DISCUSSION

As reported elsewhere in earlier studies, the phytochemicals play vital role in curing different types of diseases including cancer and therefore plants having these phytochemicals are still used both in modern as well as traditional system of medicine [15, 20, 21, 22, 23]. In North Eastern part of India, quite a large number of plants are used for treatment of various ailments including cancer based on traditional knowledge system practiced by various communities [16]. As plants are considered huge reservoir of phytochemicals such as flavonoids, alkaloids, tannins and other polyphenolic compounds, hence it is believed that the plant diet are useful to keep away most of the problems associated with diseases [6]. By virtue of these phytochemicals, some plants also exhibit anticancer property by contributing to the prevention of free radical generated disease like cancer [5]. Besides these, the free radical scavenging property of certain plant makes it a food with versatile health benefit [6, 17].

In the present investigation, qualitative analysis (table 1) shows the presence of all important phytochemicals such as alkaloids, flavonoids, saponin, tannins and phenols in the sample. Flavonoids, phenols and alkaloids of plant origin are directly correlated to the antioxidant power [17]. In the same way, the total antioxidant is also directly correlated to the antiproliferative property of the plant [6]. In the present study we recorded the presence of all these compounds in the sample. Alkaloids which were detected in the sample are also reported to be bound to tubuline and inhibit cell proliferation [4]. The tannin a heterogeneous group of compound with reported pharmacological significance was also detected in the crude sample under study [5]. As could be seen in the table 2, the free radical scavenging power of the crude sample was recorded as 84.87% and 76.9% against DPPH and ABTS respectively which was roughly comparable to that of the ascorbic acid. These phytochemicals together serve either as inhibitor of reactions forming free radicals or by scavenging the free radicals from the body [5].

To our expectation, the crude extract of *S. pulcherrima* Goud also exhibited a dose dependant antitumor activity as observed in both apoptosis as well as short term cytotoxicity test (Figure 2 and 3). The maximum antitumor activity (62% Viability) was recorded in the cell line culture impregnated with 100 µg/ml crude extract. On the other hand percentage of Viability was decreased from 90% to 76% while extract doses were increased from 20 to 80 µg/ml respectively. This observation of our study was corroborating the results of previous studies by Islam, et al., 2009 [7]. The damaging activity of the plant extract was noticed at the membrane structure (Figure 3). Similar studies indicates that different phytochemicals

causes cell damage in different ways *viz.* alkaloid isolated from *Catharanthus roseus* (Hypoglycemic folklore medicine) binds to the microtubule and prevents mitotic cell proliferation [4]. Study also shows that flavonoids, tannins and other phytochemicals all combinely produce cytotoxic effect on tumor (DLA) cells line [5, 18]. It is also suggested that herbal drugs play anticancer role by enhancing immune system as well as body's detoxification function, inhibit angiogenesis and also cell differentiation [1, 4, 8]. Whatever may be the mechanism, the promising result displayed by the plat extract in the short term cytotoxicity test accompanied by the apoptosis test and free radical scavenging assay justified the efficacy of the plant as traditional vegetable with potent antioxidant and antitumor properties.

CONCLUSION

Although several new approaches are coming up for drug discovery in recent years, none of them could replace the natural products derived from plant and animal origin [20]. Wholesome consumption of plant materials in traditional food habit amongst different tribes may provide important clue to the discovery of potent plant principles for combating the dreaded diseases like cancer. The findings of the present study is encouraging for nutraceutical and pharmacological evaluation of the plant under study which is exclusively used by the ethnic tribe with high incidence of tobacco consumption, extensive use of red meat protein and alcohol in their diet. The present work is a kind of first hand report on a wild vegetable plant with potential antitumor property. However, an elaborate research is needed to isolate and study the active principle of the plant in vivo.

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