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Research Article

PHYTOCHEMICAL, ANTIOXIDANT, AND FREE RADICAL SCAVENGING ACTIVITIES OF HYDRO-ETHANOLIC EXTRACT OF AERIAL PARTS OF *POTHOS SCANDENS* L.

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ABSTRACT

Objective: The present study was carried out to evaluate the phytochemical, antioxidant, and free radical scavenging activity of 50% hydro-ethanolic extract of *Pothos scandens* L.

Methods: To evaluate the total phenolics, alkaloid, and flavonoid content of 50% hydro-ethanolic extract of *P. scandens* and also to assess the antioxidant and free radical (2, 2 diphenyl-1-picryl hydrazine radicals, hydroxyl radical, superoxide radical, and nitric oxide radical) scavenging activity, inhibition of lipid peroxidation (LPO), and total antioxidant activity of the plant extract. All experiments were performed in triplicate (n=3), and the results were expressed as mean±stanadard deviation.

Results: The result indicated that the plant contained more amounts of total phenolic content, followed by alkaloid and flavonoid content. The plant extracts showed effective radical scavenging activity and showed pronounced inhibitory action against LPO.

Conclusion: The study concluded that 50% ethanolic extract of *P. scandens* L. has showed the significant antioxidant property. This extract can therefore be used to screen its potential activities against many diseases which are caused by free radicals.

Keywords: *Pothos scandens* L., 2, 2 diphenyl-1-picryl hydrazine, Total phenolics.

INTRODUCTION

For thousands of years, natural products especially plants have been used for maintaining human health as well as for the treatment of various diseases [1,2]. Plants possess a wide range of activities due to the presence of various phytoconstituents such as lipids, steroids, alkaloids, tannins, flavonoids, and phenolic compounds [3,4].

A free radical is defined as any atom or molecule possessing unpaired electrons - superoxide anion, hydroxyl, hydroperoxyl, peroxyl, alkoxyl, nitric oxide, peroxynitrite, etc [5]. These free radicals play a cardinal role in diseases which include cancer, aging, cardiovascular diseases, and inflammatory diseases [6]. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them [7]. A wide range of antioxidants from both natural and synthetic origin has been used for treating various human diseases [8]. However, due to increasing safety concerns about synthetic antioxidants, search for natural antioxidants is gaining importance. Although herbal medicine is effective in the treatment of various ailments, only a few of them have been scientifically explored [9,10].

Pothos scandens, in Malayalam (Anaparuva, Paruvakodi); Tamil (Anaparuga); Kannada (Adkebiluballi) a medicinal aroid, which belongs to the family Araceae is a climbing shrub with aerial roots growing on trees and rocks like ivy [11]. The bruised root of the plant is applied to promote healing of abscesses, after being fried in oil. The Indian people use an infusion of the leaves of this plant as a bath for curing convulsions and epilepsy. It has been also reported that the whole plant is used against various health problems and disorders such as diarrhea [12]. According to the ethnobotanical data collected during the field surveys made on several visits between 2004 and 2006 to three Akha communities in Chiang Rai in northern Thailand, it was found that the traditional healers use whole aerial parts of *P. scandens* to treat cancer [13]. Recently, the ethanolic extract of *P. scandens* has been reported to be effective in wound healing [14]. The extracts of the aerial part of *P. scandens* have been shown to inhibit mast cell derived immediate-type allergic reactions and mast cell degranulation [15]. The methanolic extract of leaves of *P. scandens* has been reported to have cytotoxic as well as thrombolytic potential [16].

After thorough literature survey, it was found that no study has so far been carried out to evaluate scientifically the potency of *P. scandens* to fight cancer. Since many free radicals are known to play an important role in diseases such as cancer, this study was designed as a preliminary attempt to understand the highly acclaimed properties of *P. scandens* by evaluating its phytochemical constituents and its antioxidant potential through the use of various *in vitro* assays.

METHODS

Chemicals and reagents

All the chemicals used were of analytical grade and purchased from Himedia, Merck, or Sigma.

Plant collection and identification

P. scandens were collected from in and around Palai, Kottayam, Kerala. They were identified and certified by the Taxonomist, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (Plant identification no: BSI/SRC/5/23/2013-14/Tech/685).

Preparation of the plant extract

The aerial parts of *P. scandens* were shade dried and ground to coarse powder. The coarse powder was extracted using 50% ethanol using Soxhlet apparatus. Air dried and pulverized plant material was weighed out and packed into a thimble, which was in turn placed in a Soxhlet extractor. The extraction was allowed to continue until the solvent was clear. The extracts were condensed to dryness using rotary evaporator.

Estimation of total alkaloid and phenolic content

Total alkaloid content was determined according to the method described by Shamsa *et al.* [17]. The total phenolic content was determined according to the method described by Siddhuraju and Becker [18].

Estimation of total flavonoid content

The flavonoid content was determined by the use of a slightly modified colorimetry method described previously by Zhishen *et al* [19]. 0.5 ml aliquot of appropriately (5 mg/ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate, and the results were expressed as rutin equivalent.

Free radical scavenging activity

The free radical scavenging activity of 50% ethanolic extract of *P. scandens* was evaluated by checking its ability to scavenge various radicals such as 2, 2 diphenyl-1-picryl hydrazine (DPPH) radical (by method of Blois [20]), hydroxyl radical (by method of Klein *et al.* [21]), Superoxide radical (by method of Beauchamp and Fridovich [22]) and Nitric oxide radical (by method of Sreejayan and Rao [23]). The percentage radical scavenging activity of the sample was calculated as follows:

$$\% \text{ radical scavenging activity} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100 \quad (1)$$

Lipid peroxidation (LPO) inhibiting activity

The LPO inhibition ability of the sample was carried out using a modified procedure of Ohkawa *et al.* [24]. Goat liver was washed thoroughly in cold phosphate buffer saline (pH 7.4) and homogenized to give a 10% homogenate. The homogenate was filtered and centrifuged at 10000 rpm for 10 minutes, and the supernatant was used to carry out the assay. To 0.5 ml of 10% homogenate, 0.5 ml of the sample (100-500 µg) was added. To this, 0.05 ml of 0.07 M ferrous sulfate was added and incubated at room temperature for 30 minutes. To the incubated solution, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% trichloroacetic acid (in 1% SDS) were added. The tubes were incubated at 100°C for 1 hr and cooled to room temperature. About 5 ml of butanol was added and centrifuged at 3000 rpm for 10 minutes. The upper layer was used to read the absorbance at 532 nm. The percentage inhibition was calculated using equation (1).

All the above-mentioned analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Phosphomolybdenum reduction activity

The total antioxidant activity of the sample was evaluated by the phosphomolybdenum method according to the method of Prieto *et al.* [25]. An aliquot of 0.1 ml of sample solution was combined in a 4 ml vial with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. The results reported are mean values expressed as milligrams of ascorbic acid equivalents per gram sample.

Statistical analysis

All experiments were performed in triplicate (n=3) and the results were expressed as mean±SD. Statistical analysis was carried out using SPSS 16.0.

RESULTS

Total alkaloid, phenolic, and flavonoid content

In the current study, the total alkaloid, phenolic, and flavonoid were estimated. The result indicated that the extract contained more amounts of total phenolic components followed by total alkaloids and total flavonoids Table 1.

Free radical scavenging activity

The results of the study confirm that the extract has scavenging effect against all of the radicals analyzed (Table 2). It was also evident from the study that the free radical scavenging activity of the plant extract was concentration dependent. The plant extract showed almost similar range of inhibitory effect against DPPH radical, hydroxyl radical, and nitric oxide radical, but the inhibitory effect against superoxide radicals was much less with respect to others.

LPO inhibiting activity

The ability of the plant extract to inhibit LPO is given in Table 2. The result indicates that plant extract was having an effective inhibitory action against LPO with an IC_{50} of 169±3.00 µg/ml.

Phosphomolybdenum reduction activity

Phosphomolybdenum reduction is a method to quantify the total antioxidant activity of the plant extract, and it was found to be 137.08±1.65 mg ascorbic acid equivalents per gram extract.

DISCUSSION

Medicinal plants play an important role in the development of potent therapeutic agents. Plant-derived drugs came into use in the modern medicine through the uses of plant material as an indigenous cure in folklore or traditional systems of medicine [26]. Free radicals in the form of reactive oxygen and nitrogen species are an integral part of normal physiology, over production of which due to an imbalance in bodily antioxidant defense system may lead to chronic diseases [27].

In the present study, as it is revealed from the phytochemical analysis that the plant contains a significant amount of alkaloids, phenolics, and flavonoids which attribute to the antioxidant capability of the plant.

The DPPH radical is considered to be a model of lipophilic radical. DPPH test provides information on the reactivity of test compounds with a stable free radical. When the odd electrons are paired off in the presence of free radical scavenging, the absorption reduces and the DPPH solution decolorizes as the color change from deep violet to light yellow [28]. The plant extract was able to inhibit the DPPH radical though its activity was less than that of the standard used. The most reactive free radical formed in the biological system and the quick initiator of LPO process is hydroxyl radical [29]. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity [30]. The extract showed much lesser IC_{50} values for inhibiting hydroxyl radical which shows the capability of the plant as a potent antioxidant.

Nitric oxide in excess concentration is known to cause several diseases [31]. Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals and gets converted to other harmful reactive oxygen species such as hydrogen peroxide and

Table 1: Total alkaloid, phenolic, and flavonoid content of 50% ethanolic extract of *P. scandens*

Sample	Total alkaloids (mg AE/g extract)	Total phenolics (mg TAE/g extract)	Flavonoids (mg RE/g extract)
P. scandens	23.63±0.70	37.49±0.51	1.62±0.08

P. scandens: Pothos scandens, AE: Atropine equivalent, TAE: Tannic acid equivalent, RE: Rutin equivalent, SD: Standard deviation. Values are expressed as mean±SD (n=3)

Table 2: Antioxidant activities of 50% ethanolic extract of *P. scandens*

Sample	IC_{50} (µg/ml)				
	DPPH$^{\bullet}$	OH$^{\bullet}$	$O_2^{\bullet}$	NO$^{\bullet}$	LPO inhibition
P. scandens	310.58±3.36	240.44±4.62	1278.79±5.65	304.90±3.24	169.97±3.00
Standard	3.76±0.07*	21.15±0.15$^{\#}$	39.81±0.22*	49.06±0.18*	38.69±2.48*

P. scandens: Pothos scandens, BHT: Butylated hydroxytoluene, LPO: Lipid peroxidation, SD: Standard deviation. *Ascorbic acid, $^{\#}$BHT values are expressed as mean±SD (n=3)

hydroxyl radical, damaging biomolecules which results in chronic diseases [32]. From our study, it was found that the plant extract had the ability to inhibit nitric oxide radical to a moderate level but showed poor inhibitory action against superoxide radical. Further, the plant extract was very effective in inhibiting LPO to a great extent which may be due to its ability to scavenge hydroxyl radical [33].

The presence of alkaloid, phenolic, and flavonoid can be attributed to the antioxidant and free radical scavenging ability of the plant, as previous studies with plant possessing these components showed significant antioxidant activity [34-36]. The total antioxidant capacity of the plant also points toward its use as a potent source of antioxidant. The therapeutic potentials of medicinal plants as natural antioxidants in reducing free radical induced tissue damage [37] and in the maintenance of health and protection from some disorders such as cancer [38] have been reported. This finding provides scientific evidence to support the medicinal uses of *P. scandens.*

CONCLUSION

The present study concluded that 50% ethanolic extract of *P. scandens* L. has shown significant antioxidant property. This extract can therefore be used to screen for potential activities against many diseases such as cancer which are triggered by free radicals. Further studies are underway to identify the active compounds responsible for the activities exhibited by the plant and to evaluate the anticancer efficacy of the plant against cancer cell lines and animal models.

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