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Research Article

EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *ACONITUM HETEROPHYLLUM*

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ABSTRACT

The roots of the plant *Aconitum heterophyllum* (*A. heterophyllum*) are traditionally used for curing hysteria, throat infection, dyspepsia, abdominal pain, diabetes and diarrhea. Therefore, the aim of the present study was to evaluate qualitative and quantitative phytochemical constituents and *in vitro* antioxidant activities of roots of *A. heterophyllum* collected from Bhopal region of Madhya Pradesh. Antioxidant activity was carried out by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay. The phytochemical screening of root of *A. heterophyllum* revealed the presence of flavonoids, phenol and saponinsin the hydroalcohol extracts. The percentage yield of hydroalcohol extract of the root of *A. heterophyllum* was 6.6% (W/W). Quantitative analysis showed that total flavonoids content of roots of hydroalcoholic extract was 1.731mg/100mg. The results of DPPH scavenging activity for root hydroalcohol showed IC_{50} value 297.05 when compared to Ascorbic acid (standard) which was 18.69. It indicates the plant has the potency of scavenging free radicals and it may provide leads in the ongoing search for natural antioxidants from various medicinal plants to be used in treating diseases related to free radical reactions.

Keywords: *Aconitum Heterophyllum,* Hydroalcohol extract, Phytochemical constituents, Antioxidant activity.

1. INTRODUCTION

Use of plants as medicinal substances is as old as human civilization and mankind continues to rely on them for healthcare [1]. At present, around 80% population residing in the developing or underdeveloped countries still use plant-based medicines to combat their ailments [2]. Naturally-derived compounds have significantly contributed in the discovery of new chemical entities. The process of drug discovery from nature involves multi-disciplinary approach and is interconnected with many disciplines like ethnobotany, phytochemistry, biology and various chemical separation processes along with combinatorial synthetic techniques. It is currently estimated that around 87% of drugs are derived directly or indirectly from nature. Approximately, 420 000 plant species occur in nature [3]. Oxidative stress is considered as the principal cause of human ailments. Oxidation of lipids, proteins, and DNA is related to several life-threatening diseases like cancer [4], atherosclerosis [5], heart disease [6], diabetes [7], preeclampsia [8], and neurodegenerative diseases like Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease, celiac disease [9-12] and Parkinson's disease [13]. Several free radicals are produced throughout metabolic process; however, the body balances oxidation and antioxidation using its multiple defense mechanisms [14-16]. Aging process is directly linked to systemic oxidative stress. Declined nutritional antioxidants availability and accumulation of oxidation products have been recognized as main contributors in human aging [17]. According to the Denham Hartman's free radical theory of aging, it is believed that consequences of building-up of bio-molecules, spoiled through free radicals leads to aging [18,19]. Antioxidants are substances that are accountable for the prevention of reactive oxygen species formation or scavenge them [20]. Most of the dietary antioxidants are derived from plants. Moreover, antioxidants, obtained from medicinal plants, have attracted the researchers' attention due to the risks, associated with several available synthetic antioxidants including butylated hydroxyanisole and/or butylated hydroxytoluene [21]. *A. heterophyllum* Wall commonly known as Atis or Patis belonging to family

Ranunculaceae is a perennial herb distributed over temperate parts of western Himalaya extending from Kashmir to Kumaon [22]. Studies on traditional system of medicine showed that the plant is used in curing hysteria, throat infection, dyspepsia, abdominal pain, diabetes and is considered as a valuable febrifuge nervine tonic especially combating debility after malaria and in hemoplageia [23]. The plant has shown to contain alkaloids heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isoatisinehetidine, hetsinone, benzoylheteratisine [24]. Aconitum has also shown to exhibit antipyretic, analgesic, anti-fungal, anti-bacterial, insecticidal, brime shrimp cytotoxic activities and is used to treat diseases of nervous system, digestive system, rheumatism and fever [25]. Reports have also shown that the plant posses a good anti-viral, antidiarrhoeal and immunostimulant properties [26]. The alkaloids mesaconitine and 3 acetylaconitine have shown to possess antiinflammatory activity [27]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and *in vitro* antioxidant activity of root of *A. heterophyllum* in Bhopal region of Madhya Pradesh.

2. MATERIAL AND METHODS

2.1. Plant material

The roots of plant *A. heterophyllum* were collected from rural area of Bhopal (M.P) in the month of February, 2020.

2.2. Chemical reagents

Ascorbic acid (AA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid and quercetin (QT) were acquired from Sigma-Aldrich, USA and All the other chemicals and reagents were of analytical grade and were purchased from S.D. fine Chemicals Pvt. Ltd., Mumbai, India and SRL Pvt. Ltd. (Mumbai, India).

2.3. Extraction by maceration method

The shade dried material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Fifty gm of dried plant material were exhaustively extracted with hydroalcoholic solvent (ethanol: water: 80: 20) using maceration method. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [28].

2.4. Qualitative phytochemical screening

Crude extracts were screened to identify the occurrence of primary and secondary metabolites, viz. carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, saponins, terpenoids, proteins and fixed oils, using standard screening test and phytochemical procedures [29-31].

2.5. Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso et al [32]. One ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g) [33].

2.6. DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method [32]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher is the antioxidant activity.

3. RESULTS AND DISCUSSIONS

The crude extract so obtained after the maceration extraction process, was further concentrated on water

bath. The solvent was evaporated completely to obtain the actual yield of extraction. To obtain the percentage yield, extraction is very important phenomenon in phytochemical extraction. To evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of hydroalcoholic root extract of *A. heterophyllum* was found to be 6.6% w/w. The phytochemical analysis of *A. heterophyllum* roots exhibited the existence of many important bioactive secondary metabolites in hydro-alcoholic extracts, such phenols, flavonoids and saponins (table 1). The content of flavonoid was estimated from the QT standard curve ($Y=0.048X + 0.021$, $R^2=0.997$, where X is the quercetin equivalent (QE) and Y is the absorbance) and the results were expressed as quercetin equivalent (mg/100mg) (table 2 & fig. 1).

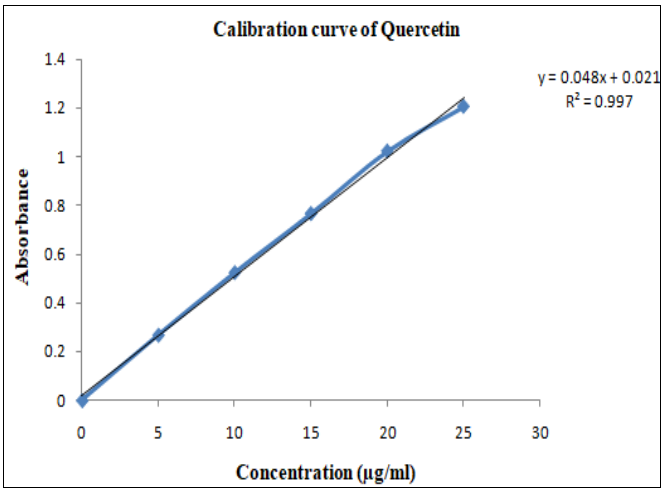

Fig. 1: Graph of estimation of total flavonoids content

The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the *in vitro* general antioxidant activity of pure compounds as well as plant extracts [34, 35]. The decrease in absorbance by the DPPH radical with increase in concentration of the extract which manifested in the rapid discolouration of the purple DPPH, suggest that the hydro alcoholic extracts of *A. heterophyllum* has antioxidant activity due to its proton donating ability. It was found that the extracts exhibited a dose-dependent activity which indicates that DPPH scavenging activity was increased proportionately to the increase in the extracts' concentration. Additionally, the IC_{50} values of scavenging DPPH radicals for the AA and extract were shown in Table 3. Comparing with AA, the IC_{50} value for DPPH radical activity of extract was found to be 297.05.

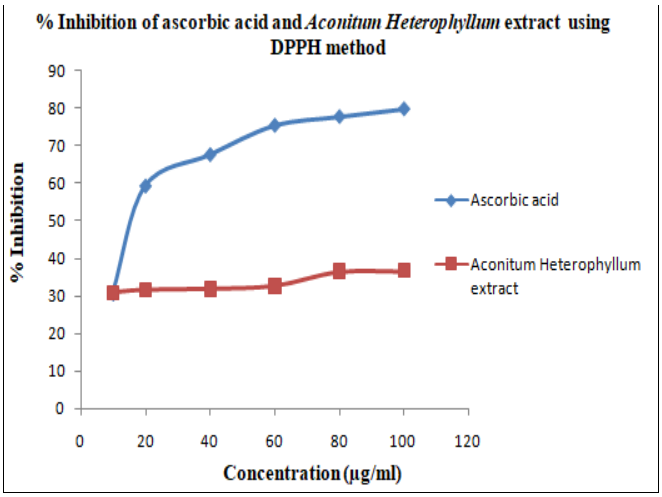

Fig. 2: Percentage inhibition of ascorbic acid and hydroalcoholic extract of *A. heterophyllum* (roots) using DPPH method

Table 1: Result of phytochemical screening of hydroalcoholic extract of *A. heterophyllum*

S. No.	Constituents	Roots extract
1.	**Alkaloids**	
	Wagner's Test:	-ve
2.	**Glycosides**	
	Legal's Test:	-ve
3.	**Flavonoids**	
	Alkaline Reagent Test:	- ve
	Lead acetate Test:	+ ve
4.	**Diterpenes**	
	Copper acetate Test:	-ve
5.	**Phenol**	
	Ferric Chloride Test:	+ ve
6.	**Proteins**	
	Xanthoproteic Test:	-ve
7.	**Carbohydrate**	
	Fehling's Test:	-ve
8.	**Saponins**	
	Froth Test:	+ve

Table 2: Estimation of total flavonoids content of hydroalcoholic extract of *A. heterophyllum*

S. No.	Hydroalcoholic extract	Total flavonoids content (mg/100mg of dried extract)
1.	Roots	1.731

Table 3: Percentage inhibition of ascorbic acid and hydroalcoholicextract of *A. heterophyllum* (roots) using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	*A.heterophyllum* extract
1	10	30.42	30.89
2	20	59.11	31.56
3	40	67.48	31.85
4	60	75.25	32.62
5	80	77.58	36.47
6	100	79.63	36.57
	IC_{50}	**18.69**	**297.05**

4. CONCLUSION

Despite ongoing scientific research on this species, this study constitutes the first attempt to determine the phytochemical compositions as well as the antioxidant, activities of *A. heterophyllum* root hydroalcoholic extracts that could be found despite the throughout literature survey so far as we know. The knowledge of phytochemical constituents of the plant is the basic approach to identify novel secondary metabolites as unmodified form, semi-synthetic or drug templates. This study delineates that hydroalcohol extracts could be potentials in free-radical scavenging activity. So, it can be assumed that different active secondary metabolites were present in these extracts. Furthermore, the activity of this plant constituent can help to elucidate the justification for the ethno medicinal use of this plant species scientifically. Based on our findings, further studies are necessary to elucidate the mechanism lying with these effects of the plant extracts and could be open a new window in the search for new bioactive drug lead components of this plant extracts.

Conflict of Interest

None declared

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