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Antioxidant Activity and Phytochemistry of Leaf Extract of Melodinus acutiflorus

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Abstract: Medicinal plants are the natural herbicidal source of different drugs being manufactured by local pharmaceutical companies in all over the world. These are the essential originator of modern pharmaceutical market. It is therefore to be needed to evaluate the medicinal plants for the pinpoint the innovative compounds that can be used in the drugs. The present study was designed to quantify the phytochemical profiling and antioxidant activity of *Melodinus acutiflorus*. The study revealed the presence of phytochemicals such as alkaloids, carbohydrates, phenols, amino acids, flavonoids, proteins cardiac glycosides, terpenoids, saponin, phlobatannins and steroids. DPPH free radical scavenging assay was used for antioxidant activity. The flavonoid contents were measured using UV-spectrophotometer. Antioxidant activity correlated positively with total content of flavonoid compounds.

Key words: Antioxidant Activity • Phytochemical Profiling • Melidonus Acutiflorus

INTRODUCTION

The medicinal plants are very vastly used for diagnostic purpose for the cure of distinct human diseases due to the existence of phytochemical constituents [1]. Phytochemicals are naturally occurring in the leaves, bark and roots of medicinal plants that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds [2]. Terpenoids are important in many biological activities i.e., anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities [3]. Terpenoids are very effective in attracting useful mites and consume the herbivorous insects [4]. Alkaloids are anaesthetic agents and are important constituents of medicinal plants [5]. It has long been noticed that naturally occurring constituents in higher plants possesses antioxidant activity. Recently, there has been increased interest in oxygen containing free-radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders.

Accordingly, attention is being focused on the protective biochemical functions of naturally occurring antioxidants compounds in the cells of the organisms containing them [6]. The main objective of our research work was to analyze the existence of different phytochemical constituents and antioxidant constituents in *Melodinus acutifloras* used for healing and curing of several diseases.

MATERIALS AND METHODS

Collection of Plant: *Melodinus acutifloras* can grow as either a small shrub or tree ranging in height from 0.9-6.1 meters with widely spaced thick succulent branches that are often covered with "knobby" protuberances. Fresh leaves of *Melodinus acutifloras* were collected from the local village (Mamashkhel) of Bannu in the month of March 2014. The plant was identified by Taxonomist Mr. Safiullah Khan Department of Botany Government Post Graduate College Bannu. The leaves were washed by deionized water and were shade dried at room temperature for 10 days; the dried leaves were then chopped and ground mechanically to mesh size of 1 mm. This was used for further studies.

Sample Preparation: A total of 800g fine powder was socked in 2 Liter of methanol for seven days at room temperature and filtered by using Whatman filter paper no 1. The filtrate was then collected and evaporated under reduced pressure in rotary evaporator. The crude extract was store at 4C° in the refrigerator for further investigation.

Analysis of Phytochemicals and Antioxidants: Chemical tests [7, 8] were carried out on the crude Methanolic extract of the leaves of Melodinus acutifloras using standard procedures to investigate the phytochemicals. Preliminary phytochemical screening of Methanolic extracts detects the presence of alkaloids, carbohydrates, phenols, proteins, amino flavonoids, steroids, cardiac glycosides, terpenoids, saponin and phlobatannins. The antioxidant activity of extract was done by the DPPH free radical scavenging method. The DPPH (2,2-diphenyl-1-picryl-hydrazyl) was reduced by the antioxidants present in the given sample. The reaction was indicated as, $DPPH^0+AH \rightarrow DPPH-H + A^0$

The reduction of DPPH is measured spectrophotometrically at 517nm by the action of antioxidants in a Methanolic solution until absorbance remains constant.

Different concentrations of Ascorbic acid were taken as the standard.

Procedure:

- 0.1ml of the prepared sample was taken in the test tubes
- 0.3g of DPPH was dissolved in 100ml methanol.
- 6ml DPPH solution was added to the test tubes.
- Test tubes were kept in the dark room (DPPH is light sensitive) for 25min.
- The O.D of the solution containing DPPH was read spectrophotometrically at 517nm.
- The O.D of the DPPH solution without sample was also read spectrophotometrically at 517nm.
- The difference in the O.D of DPPH solution and DPPH + sample solution was calculated.
- The decrease in OD with sample addition is used for calculation of the antioxidant activity.

Ascorbic acid was considered equivalent to the antioxidant capacity of plant extract, therefore ascorbic acid is taken as standard and various concentrations of ascorbic acid was prepared and added to the DPPH

solution. The decrease in OD is plotted against the concentration of ascorbic acid. The concentration of sample was calculated using the above standard curve.

RESULTS AND DISCUSSION

Herbal medicines are very popular in developing and underdeveloped countries. Medicinal plants play a vital role in the production of these herbal medicines. The latex of *Melodinus acutifloras* has been utilized in tropical regions for medicine for the treatment of different ailments like itches, swellings and fevers. In the Guiana's medicines are produced from *Melodinus acutifloras* for the treatment of skin eruptions and abscesses, dysentery, herpes, syphilis, coughs and as a purgative. Here in this study we investigated different phytochemical constituents of the leaf extract of *Melodinus acutifloras*.

In Table 1 the results revealed the presence of alkaloids carbohydrates, phenols, proteins, aminoacids, flavonoids, steroids, cardiac glycosides, terpenoids, saponin, phlobatannins.

In Table 2 the results revealed the total phenolic content of the crude Methanolic extract of leaf of *Melodinus acutifloras* (21.10%). The antioxidant activity of the phenolic compound is due to the redox potential which can play an important role in neutralizing free radicals [9].

In Table 3 the results revealed the total flavonoid content of Methanolic leaf extract of *Melodinus acutifloras*. The results showed the high concentration of flavonoid content i.e 97.45%.

Table 1: Phytochemical screening of Melodinus acutiflorus

S.no	Tested For	Sample
1	Carbohydrates	
	 Molish test 	+++
2	Phenols	
	 Lead Acetate test 	+++
3	Alkaloids	
	•Wanger's test	+
4	Protein	
	 Million's test 	+++
5	Amino acids	
	 Ninhydrin test 	++
6	Tannin's	
	 Ferric chloride test 	++
7	Flavonoids	+++
8	Steroids	
9	Phlobatannins	
10	Saponin	++
11	Terpenoids	+++
12	Cardiac glycosides	+++

Table 2: Total Phenolic content of Melodinus acutiflorus

	Sample concentration	Absorbance	Total Phenolic
S.no	(µg/ml)	(660nm)	content (leaf)
1.	5	0.096	
2.	10	0.187	
3.	15	0.298	
4.	20	0.408	
5.	25	0.501	
	Sample Melodinus acutiflorus		
1.	0.5ml	1.381	
2.	1 ml	1.001	21.10

Table 3: Total Flavonoid content of Melodinus acutiflorus

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	Sample concentration	Absorbance	Total Flavonoid	
S.no	$(\mu g/ml)$	(510nm)	content (leaf)	
1.	20	0.013		
2.	40	0.032		
3.	60	0.049		
4.	80	0.068		
5.	100	0.092		
	Sample Melodinus acutiflorus			
1.	0.5ml	0.955		
2.	1 ml	0.956	97.45%	

Table 4: Antioxidant activity of Melodinus acutiflorus

	Sample concentration	Absorbance	Total Antioxidant	
S.no	(µg/ml)	(517nm)	activity (leaf)	
1.	20	0.254		
2.	40	0.905		
3.	60	1.557		
4.	80	1.996		
5.	100	2.667		
Sample Melodinus acutiflorus				
1.	1.0ml	0.955		
2.	1.0ml	0.956	18.13	

In Table 4 indicate that plant have high antioxidant potential and the leaf extract showed high DPPH free radical scavenging activity. The antioxidant activity showed by the plant is due to the presence of high flavonoid and phenolic content in the plant [10].

CONCLUSION

From the above study it is concluded that the *Melodinus acutifloras* is the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, phlobatannins and reducing sugars. It plays a vital role in preventing various diseases. The study also concluded that natural antioxidants strengthen the antioxidant defense against reactive oxygen species (ROS) and this plant is used for discovering and screening of the phytochemical constituents which are very helpful for the production of new drugs.

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