ejpmr, 2018,5(9), 338-341

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ROLE OF FRESH JUICE AND DRY POWDER PREPARATIONS OF *BENINCASA HISPIDA* AND *LAGENARIA SICERARIA* FRUITS IN INHIBITING CELLULAR STRESS BY MEASURING DPPH ASSAY

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Article Received on 23/06/2018 **Article Revised on 13/07/2018** **Article Accepted on 02/08/2018**

ABSTRACT

Introduction: During the past few decades, the drugs from natural products have gained importance in the field of medicine. Many plants and their products exhibit marked pharmacological activities. Benincasa Hispida and Lagenaria siceraria fruits are one of those important natural plants which are used in the treatment of various disorders. The present study was designed to analyze the antioxidant property of fresh juice and dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* fruits in inhibiting cellular stress by chemical testing and DPPH assay. **Materials and Methods:** Fresh fruits were obtained from the local market and washed thoroughly in the running water. Then the dry powder and fresh juices of *Benincasa hispida* and *Lagenaria siceraria* were made by using hot air oven at 60 degree Celsius made and used for DPPH assay and chemical testing of Phenolic and Flavonoid compounds. **Results:** Only dry powder of *Benincasa hispida* fruit showed positive for both phenolic and flavonoid compounds. In *Lagenaria siceraria*, both dry powder and fresh juice revealed positive only for flavonoid compounds. In DPPH assay, dry powder preparartions of *Benincasa hispida* and *Lagenaria siceraria* proved better free radical scavenging activity compared to their fresh juice forms. **Conclusion:** Enhanced antioxidant property of *Benincasa hispida* and *Lagenaria siceraria* dry powders possess good therapeutic potential and can be useful as an effective antioxidant in clinical use of various diseases.

KEYWORDS: Antioxidant property, Dry powder, Fresh juice, *Benincasa Hispida*, *Lagenaria Siceraria*.

INTRODUCTION

The fruit of *Benincasa hispida*, commonly called as ash guard, belonging to cucurbitaceae family. Scientific studies disclose its anti-inflammatory, diuretic, anti-Alzheimer, antidiarrheal, antiulcer, anorectic, antihistaminic activities and its antioxidant activity.[1,2] Several studies have shown the antioxidant property of Benicia *hispida* fruit in various forms of its extraction (ethanol, methanol).[3]

Lageneria siceraria commonly called as Bottle guard is a rich source of vitamin B & C and used by the cardiac patients to prevent atherosclerosis. It is an edible fruit and has got many medicinal values. Many studies in the past have shown the antioxidant property of *Lagenaria siceraria* in different forms of its extraction (ethanol, Acetone).[4,5] No studies have analyzed the antioxidant property of fresh juice and dry powder preparations of *Benincasa Hispida and Lagenaria Siceraria* fruits.

Since these fruit are edible, easily available, and have got lot of medicinal values, it becomes essential to see their antioxidant property in fresh juice and dry powder forms.

AIM

To analyze the property of fresh juice and dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* fruits in inhibiting cellular stress by chemical testing and DPPH assay.

MATERIALS AND METHOD

The fresh fruits were obtained from the local market and washed thoroughly in the running water. Then under sterile condition seeds were removed and the pulp of the fruits were cut into pieces and weighed. After weighing, both the fruit pieces were grinded separately and filtered using sterile cloth to extract juice from both the fruits. From this half quantity of the juices were used for dry powder preparations and the remaining were stored for chemical test analysis in the refrigerator. For dry powder preparation, both the juices were measured and filled in

the separate bowls and kept in hot air oven at 60 degree Celsius under sterile condition till all the water content of the juices get evaporated and the fine particles get settled. Now the settled powder particles were been dried, collected and weighed again. Finally, the dry powder and the fresh juice of both the fruits were used for the chemical testing and DPPH assay.

Chemical Testing[6]

Detection of phenolic compounds

a. Ferric chloride test: Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were added to 5% FeCl3 reagent. Formation of deep blue colour was not appeared in juice and dry powder preparations of both fruits.

b. Lead acetate test: Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were added to 10% lead acetate solution and mixed. Formation of white precipitate was present in dry powder preparation but not in the juice of *Benincasa hispida*. Whereas, in *Lagenaria siceraria* white precipitate was not present in both dry powder and juice forms. Thus the test was positive only for the dry powder preparation of *Benincasa hispida.*

Detection of flavonoids

a. Aqueous sodium hydroxide test: The Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were treated along with aqueous NaOH solution. Formation of yellow-orange colour was not found in juice but present in dry powder preparation of *Benincasa hispida* and the test was found to be positive in both juice and the dry powder preparation of *Lagenaria siceraria.*

b. H2SO4 test: The Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were added to concentrate H2SO4 and observed for the formation of orange colour. Only the dry powder preparation of *Benincasa hispida* was found to be positive for the presence of flavanoids and not in the fresh juice form. In *Lagenaria siceraria,* the test was positive in both juice and the dry powder preparation.

Table No. 1: Results of the chemical testing.

		Phenolic compound		Flavonoids	
Sr.No	**Test substances**	**Ferric chloride test**	**Lead acetate test**	**Aqueous sodium hydroxide test**	**Sulphuric acid test**
1	*Benincasa hispida* Fresh juice	-	-	-	-
2	*Benincasa hispida* Dry powder	-	+	+	+
3	*Lagenaria siceraria* Fresh juice	-	-	+	+
4	*Lagenaria siceraria* Dry powder	-	-	+	+

DPPH Assay

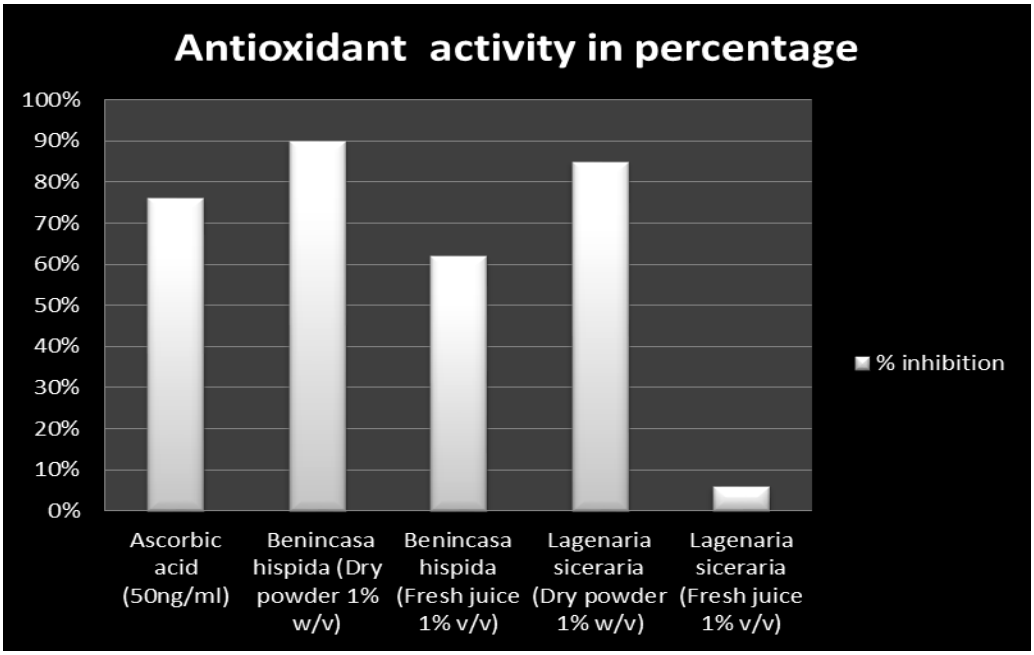
The percentage of antioxidant activity (AA %) of fresh juice and dry powder preparation of *Benincasa hispida* and *Lagenaria siceraria* were assessed by DPPH free radical assay. The dry powder and the fresh juice of *Benincasa hispida* and *Lagenaria siceraria* were prepared in 1% w/v and 1% v/v solutions. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of samples (Dry powder and Fresh juice), 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV VIS spectrophotometer. The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Reference standard compound being used was ascorbic acid 50 ng/ml. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.[7,9] Percentage inhibition was calculated by,

DPPH scavenging effect (%) = A0 - A 1 / A0 × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample. (Control =0.856).

Table No. 2: Absorbance and % inhibition of DPPH assay.

Samples	Absorbance	% inhibition
Ascorbic acid (50ng/ml)	0.206	76%
Benincasa hispida (Dry powder 1% w/v)	0.088	90%
Benincasa hispida (Fresh juice 1% v/v)	0.322	62%
Lagenaria siceraria (Dry powder 1% w/v)	0.131	85%
Lagenaria siceraria (Fresh juice 1% v/v)	0.808	6%



"Fig. 1: Antioxidant activity of fresh juice and dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* in percentage."

DISCUSSION

In this study, the dry powder of *Benincasa hispida* showed positive for both flavonoids and phenolic compounds by the chemical testing. These results were similar to the studies which showed the presence of flavonoids and phenolic compounds in the methanolic fruit extract of *Benincasa hispida*.[10] In DPPH assay, dry powder of *Benincasa hispida* revealed 90% inhibition of free radical scavenging activity compared to its fresh juice preparation which was about 62%. In the previous DPPH assay studies of *Benincasa hispida* proved that the maximum antioxidant activity was found to be in the methanolic extract compared to the alcoholic extract.[1,3]

In this study, both the dry powder and the fresh juice of *Lageneria siceraria* showed positive for flavonoids and negative for phenolic compounds by the chemical testing. These results were similar to the studies which showed the presence of flavonoids in the ethanolic fruit extract of *Legenaria siceraria*.[11] In DPPH assay, dry powder of *Lageneria siceraria* showed 85% inhibition of free radical scavenging activity compared to its fresh juice preparation which was about 6%. In the previous DPPH assay studies of *Lageneria Siceraria* showed that the maximum antioxidant activity was found to be in the acetone extract compared to the alcoholic extract.[4,5]

CONCLUSION

In this study, both the chemical testing and DPPH assay showed maximum antioxidant activity in the dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* compared to their fresh juice preparations. The result of enhanced antioxidant property of *Benincasa hispida* and *Lagenaria siceraria* dry powder possess good therapeutic potential and can be clinically useful as an effective antioxidant in various disease conditions after appropriate preclinical studies and clinical trials.

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