

Phytochemical Investigation and Evaluation of Free Radical Scavenging Potential of *Benincasa hispida* Peel Extracts

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ABSTRACT

The crude extracts of *Benincasa hispida* i.e. Methanolic extract (M.E.) and aqueous extract (A.E.) were studied for the presence and detection of phytochemical such as alkaloids, saponins, steroids, carbohydrates and flavonoids using standard procedures. On the basis of the results, the extracts were further used for in vitro evaluation of antioxidant activity. The present study was designed to study the phytochemical screening and to investigate the free radical scavenging potential of aqueous and methanolic extract of dried ripe peels of *Benincasa hispida*. The free radical scavenging potential was evaluated by DPPH(1,1-diphenyl-2-picrylhydrazyl). The extracts showed significant potential in a dose dependant manner when compared with the ascorbic acid. The highest scavenging activity of ME was found to be 87.87% at a concentration of $100\mu\text{g mL}^{-1}$ and that of aqueous 86.5% at concentration of $100\mu\text{g mL}^{-1}$. Thus both the ME and AE may be useful as a natural antioxidants in the near future.

Key words- *Benincasa hispida*, phytochemical investigation, DPPH, methanolic extract, aqueous extract.

INTRODUCTION

The plants kingdom serves as an inexhaustible source of new drugs and chemical entities. It represents an extraordinary reservoir of novel molecules. Of the estimated 250,000-500,000 plants species around the globe, only a small percentage has been investigated phytochemical and the fraction subjected to biological or pharmacological screening is even Lower (current science, 2000). The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The rapid disappearance of tropical forests and important vegetative areas it is Essential to have access to methods for identification and investigation of bioactive potential of natural products. Over the centuries there has been a tremendous use of botanicals as medicinal products in almost all the corners of the world. The associated risk of allopathic medicines has led to shifting of faith towards the herbal products. The safety and efficacy of natural products is an important so as to promote and rationalise their use. *Benincasa hispida* belongs to family cucurbitaceae, is traditionally used as a laxative, diuretic, tonic. It is used to control urinary discharges; removes foul taste from the mouth; heart tonic (Kirtikar and Basu, 1985). From 30 to 40 per



cent. of a thick, red fixed oil, an acrid resin, considered to be the tæniifuge principle, starch, sugar, fatty acids and the proteids, myosin and vitellin, the myosin precipitating from an infusion saturated with NaCl, and the addition of CO₂ separating out the vitellin, apparently identical with that of egg yolk (Material medica, edition 4th). Numerous evidences show that consumption of fresh fruits and vegetables reduce the risk of several pathological events such as cancer, cardio vascular and cerebrovascular diseases. Plants are reported to contain flavonoids, triterpenes, vitamin-c which are responsible for the antioxidant activity. Highly reactive free radicals and oxygen species those are present in human body that cause degenerative processes by oxidising nucleic acid in the cells. In addition, involvement of oxygen derive free radicals such as superoxide anions, hydrogen peroxide and hydroxyl radicals are well established in the injury of gastric mucosa and in the other models of gastric mucosal damage induced by nonsteroidal anti-inflammatory drugs and *H.pyroli* ethanol and feeding restriction stress (Beena V. Shetty *et al*, 2008). These free radicals scavenging may be involved in analgesic activity as these free radicals are involved in pain stimulation and anti oxidants show reduction in the pain (N.S. Gill, 2010). To the best of our knowledge, there have been no studies on the peels of the fruits.

MATERIAL AND METHODS

Collection and authentication of plant material- The fresh fruit was purchased in month of December 2010 from the local market and it was authenticated by Dr. Saroj Arora, Head of Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India (voucher no-0410/HRB).

Preparation of extracts- The peels were dried under the shade and then coarsely powdered with mechanical grinder. It was later stored in air tight containers for further uses. **Extraction procedure:** The dried powder of *Benincasa hispida* peels was put into successive solvent extraction. The powdered were defatted with n-hexane followed by chloroform, methanol and water. The solvents were recovered from distillation and further concentrated on water bath.

Phytochemical screening: The chemical tests were carried out using standard procedures mentioned in references. The tests were carried out for the presence of phytochemicals such as alkaloids, tannins, saponins, flavonoids, steroids, triterpenoids, carbohydrates, amino acids using standard procedures (Harborne, 1973). The alkaloids were tested using various reagents mentioned in the texts such as Dragendroff's reagent (Potassium bismuth iodide solution), Hagers solution (Picric acid solution), Mayer's reagent (Potassium iodide solution). The triterpenes were detected by Liberman's Burchard's test (blue green colour with acetic anhydride plus sulphuric acid). The carbohydrates were detected by Fehling's solution. For the detection saponins foam test was performed and for the steroidal compounds- Salkowlis reagent was used. The flavonoids were detected by concentrated nitric acid (crimson or magenta colour with concentrated nitric acid).

Free radical scavenging activity: DPPH radical scavenging activity: The radical scavenging activities of the plant extract was determined by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method (G.A. Ayoola, 2008). Briefly, the ascorbic acid was used as a reference drug. The dilution were made of different concentrations

Table 1. Phytochemical screening of different extracts

Sno.	Tests	Extracts		
		Chloroform	Methanol	Water
1	Alkaloids	-	+	-
2	Glycosides	+	-	+
3	Flavonoids	+	+	+
4	Carbohydrates	-	+	+
5	Saponins	-	-	+
6	Phenolic compounds	+	+	+
7	Steroidal compounds	-	+	+
8	Resin	+	-	-

+ : presence of chemical constituents , - : Absence of chemical constituents

Table 2. Percentage scavenging of DPPH radical

Conc. of extracts ($\mu\text{g mL}^{-1}$)	Percentage scavenging of DPPH radicals		
	Methanolic	Aqueous	Ascorbic acid
10	84.54	81.81	57.72
20	85.3	83.18	59.24
40	86.36	84.39	66.66
60	87.87	85.00	75.75
80	87.92	86.36	79.92
100	88.02	85.50	90.74

using methanol as solvent starting from $10\mu\text{g mL}^{-1}$, 20 g mL^{-1} , 40g mL^{-1} , 60 g mL^{-1} , 80g mL^{-1} and 100g mL^{-1} .

10 mg of DPPH was dissolved in 250 ml of methanol and 10ml was transferred into each dilution made above.

The mixture was shaken vigorously and allowed to stand at room temperature for 50 minutes. The absorbance was measured at 517 nm using spectrophotometer (Shimadzu UV). A blank solution of DPPH dissolved in methanol was taken as a negative control. The DPPH radical scavenging activity was calculated using the Equation (1):

$$\% \text{ scavenging of DPPH radical} = 100 \times \frac{A_1 - A_2}{A_1} \quad (1)$$

Where, A_1 is absorbance of the negative control and A_2 is the absorbance of the sample.

RESULTS AND DISCUSSION

Phytochemical screening: The results of the phytochemical screening of the methanolic and aqueous extracts are shown in Table-1. The results revealed the presence of several desired photochemical constituents.

DPPH radical scavenging method: A decrease in absorption is shown due to reaction of DPPH with antioxidants to form 1,1-diphenyl-2-picrylhydrazine. The decrease occurs due to acceptance of hydrogen atom (Arun and Prakash). The concentration-dependent DPPH radical scavenging was visible in both ME and AE. The highest radical scavenging activity of ME was found out to be 88.02% at a concentration of $100\mu\text{g mL}^{-1}$

and that of AE was found out to be 86.50% at a concentration of 100 µg mL¹. The results are shown in the Table-2. In the present study, the methanolic and aqueous extracts of *Benincasa hispida* peels were evaluated for its free radical scavenging potential. The results of both phytochemical screening and anti oxidant activity revealed that both the extracts possess *in vitro* free radical scavenging activity. Hence the extract could be further evaluated for *in vivo* analgesic or antiinflammatory activity. The ME and AE showed significant free radical scavenging activity, so this can also be responsible for reduction of inflammation and pain (Kim *et al*). The triterpenoids might also be responsible for the free radical scavenging activity and analgesic activity.

CONCLUSION

The phytochemical investigation depicted several compounds which possess one or more therapeutic applications. Among those screened are flavonoids, triterpenes which are responsible for marked free radical scavenging potential. The methanolic and aqueous extracts showed remarkable antioxidant activity. This could prove beneficial for future if the extracts were to be evaluated for analgesic activity. The scope for isolation of desired compound could be a advantageous step in field of drug discovery from natural sources.

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