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Research Article

IN VITRO ANTIOXIDANT AND PHYTOCHEMICAL ANALYSIS OF METHANOLIC AND AQUEOUS EXTRACT OF *ALLIUM CEPA*

Vandana Singh[1] and Ramesh Kumar[2]*

Department of Biochemistry, Bundelkhand University, Jhansi, India

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ABSTRACT

Introduction: *Allium cepa* commonly known as "onion" belongs to family Amaryllidaceae is herbecous monocot plant having potential to decrease cancer, tumor initiation, promate healing of stomach ulcers, reduce the cholesterol, blood pressure and symptoms associated with diabetes mellitus, inhibit platelet aggrergation and prevent inflammatory process associated with asthma. Keeping in view of the above beneficial effects of onion bulb, we sought to analyse the photochemicals present in methanolic as well as in aqueous extract. **Materials and Methods:** We collected the onion bulb from the local market and washed with tap and distilled water. After drying the onion bulb at room temperature it was crushed and used for aqueous and methanolic extractions. **Results:** Phytochemical analysis of the onion bulb had most of the important phyto constituents like alkaloids, flavonoids, glycosides but we use more than one test reagents for detection of these photochemical constituents and they shows different response for every tests. Alkaloids, carbohydrate, reducing sugar, flavonoids, glycosides test all was show almost positive result for aqueous extract whereas tannin and phenolic compound tests shows positive for methanolic extract. Both extract conform the presence of saponin, amino acid, protein, terpenoids, steroids by showing positive result of these tests and absence of cardiac glycosides. Carbohydrate (9.06mg/ml) and protein (1.45mg/ml) is present in methanolic extract of onion bulb. It also shown 9 spots in methanolic extract7 & 6 spots in aqueous extract 5g and 10g respectively.
Conculsion: From above results, we conclude that the result is not only depend upon solvent concentration but also influenced by method which we employed for test. We observed that most of the phytochemical compounds found in aqueous extract than methanolic extract. Antioxidant activity observed is may be due to the presence of flavonoids and phenolic compounds.

INTRODUCTION

Allium cepa commonly known as onion belongs to family Amaryllidacea is a monocoat plants. Its close relatives include the garlic, shallot, leek, chive [1] and Chinese onion [2]. This genus also contains several other species variously referred to as onions and cultivated for food, such as the Japanese bunching onion (*Allium fistulosum*), the tree onion (*A. proliferum*), and the Canada onion (*Allium canadense*). The name "wild onion" is applied to a number of *Allium* species, but *A. cepa* is exclusively known from cultivation. Its ancestral wild original form is not known, although escapes from cultivation have become established in some regions [3]. The onion is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season.

Since ancient times onion have been an important dietary resource and have also been of interest for medical purposes [4]. *Allium* is the largest and important representative genus of the *Liliaceae* family comprises 450 species. Onion is a bulbous plant widely cultivated in almost every country of the world [5]. Onions are easily propagated, transported and stored. Onions are effective against common cold, heart disease, diabetes, osteoporosis, coughs and sore throat [6]. It is rich in proteins, carbohydrates, sodium, potassium and phosphorus [7]. Onion was consumed throughout Europe during the Middle ages and was later thought to guard against evil spirits and the plague, probably because of their strong odor [8]. Folk healers traditionally used onion to prevent infections is among the oldest cultivated plants used both as a food and for medicinal applications [9]. The plant is used as traditional remedy in the treatment of various disorders so it has particular medicinal importance [10]. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties [11]. Onion bulbs contain a good

Corresponding author:* **Ramesh Kumar
Department of Biochemistry, Bundelkhand University, Jhansi, India

number of phytochemicals, most of which are hydrocarbons and their derivatives [12].

Onions possess strong, characteristic aromas and flavours, which have made them important ingredients of food. Onions and onion flavours (essential oil) are important seasonings widely used in food processing. Recent research has demonstrated that onions possess several biological properties, such as antibacterial [13], antimutagenic [14] and antioxidant activities [15]. The medicinally most significant components of onion oil are the organosulfur-containing compounds [16,17]. These compounds are reactive, volatile, odour producing and lachrymatory [18].

Adding antioxidants is an effective means for storing food to slow down oxidation of food or even deter corruption [19]. The use of synthetic antioxidants is restricted because of their carcinogenicity [20,21]. Thus, there has been increasing interest in finding natural, effective, and safe antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases [22,23]. Antioxidants that retard the oxidation process may additionally exhibit antimicrobial activity [24,25]. The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections [26,27]. One such possibility is the use of essential oils as antioxidant and antimicrobial additives. So far, a variety of studies had been carried out to evaluate the antimicrobial and antioxidant activities of essential oils, and the results indicated that essential oil did have noticeable antimicrobial and antioxidant activities [28-30]. Thus, essential oils isolated from plants are a potential source of food additives in the food industry. Keeping in view of the above beneficial effects of onion, we sought to analyze the photochemicals present in methanolic as well as in aqueous extract. Anti-oxidant activity was also evaluated. Further, TLC was conducted to monitor the number of bioactive components (spots) present in the extracts.

MATERIALS AND METHODS

Collection of Plant Materials

Allium cepa was collected in the month of January from local market of Jhansi (U.P). Firstly the collected plant material was washed with tap water for 3-4 times and then with de-ionized water for two times. After washing, plants were kept in the dark for drying at room temperature and under the constant observation to avoid any contamination. After drying, it was crushed with the help of electric grinder. Powdered sample was stored for further use.

Extraction Procedure

Extraction was done by two methods i.e. Aqueous and Methanolic extraction.

Aqueous Extract

Different concentration of dry powder i.e. 5gm and 10 gm was taken in conical flasks having equal amount (100ml) of de-ionized water. Both the flasks were heated at 90°C in water bath for 1 hour. After 1 hour flasks were taken out from water bath and kept at room temperature for cooling purpose. Then the extract was filtered with the help of filter paper and stored at 4°C.

Methanolic Extract

The powdered material was extracted with absolute 80% methanol using Soxhlet apparatus. Different concentration of plant material and solvent were taken. After filling the soxhlet apparatus with plant material and solvent it was run at 60-80°C until it gets colorless and continuously flow of water to cool down the condenser. Finally the extract was collected in air tight bottles and stored at 4°C.

Phytochemical Analysis

Detailed phytochemical analysis was carried out for all the extracts as described elsewhere [31] with some of modifications.

Thin layer chromatography

Each of the extracts was to begin with, checked by thin layer chromatography (TLC) on analytical plates over silica gel-G of 0.2 mm thickness. These plates were developed in Butanol: Acidic acid: Water having a ratio of 2:1:1. The developed TLC plates were air dried followed by hot air oven for 20 minutes. Freshly prepared 0.2 % ninhydrin solution was used to detect the bands on the TLC plates.

The movement of the spots were expressed by its retention factor (Rf).

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Antioxidant activity

The total antioxidant capacity of the methanol extract of different parts of *Allium cepa* were evaluated by the phosphomolybdenum reduction assay method according to the procedure described by Prieto *et al*. [32]. The assay is based on the reduction of Mo (VI) to Mo (V) by the methanol extract of different part of garlic and subsequent formation of green phosphate/Mo (V) complex at acid pH. One mL of various concentrations (3- µg/mL) of the extract was combined with 1 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. BHT was used as a standard. A typical blank solution contained 3 ml of the reaction mixture and the appropriate volume of the same solvent used for the samples/standard. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer.

RESULTS

The preliminary Phytochemical analysis aqueous & methanolic extract of onion reveals the presence various of secondary metabolites (Table-1). Different phytochemical tests were performed for the qualitative detection of secondary metabolites and the presence or absence of the phytochemical constituents depends on the test applied. Hager's & Wagner's test shows presence of alkaloids in all the extract while Mayer's test shows negative results. Molisch's test and Barfoed's test were used for detection of carbohydrate. Barfoed's test showed negative result for both extracts (aqueous extract & methanolic) and Molisch test show positive

for aqueous extract (5 & 10 gm). Fehling's test show positive for both extract and Benedict's shows only for aqueous extract. Both tests were used for the detection of reducing sugar. Test for flavonoids included alkaline reagent, lead acetae and ammonia test. Ammonia and alkaline reagent tests shows absence of this metabolite in methanolic extracts while flavaonoids was detected by all the methods in aquous extraction. Borntragers' test shows presence of glycosides while Legal's test shows its absence in aquous extraction. Ferric chloride and Lead acetate shows absence of tannins and phenolic compounds in aquous extract while all other methods shows it presence in all the extracts with different methods. Cardiac glycosides was absent all the extracts. In contrast to cardiac glycosides, saponin, terpenoid, steroid, amino acids and protein was present in all the extracts.

TLC results reveals 9 spots with methanolic extraction. Aquous extraction with different conc. of onion shows different spot numbers. The Rf values are shown in Tables-2. Further, we observe the ant-oxidant activities in all the extracts.

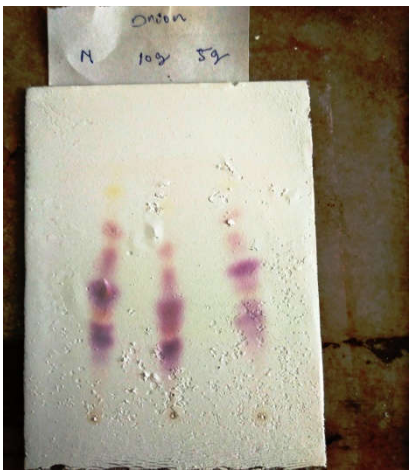

Fig 1 TLC Plate showing spots of different solvent extracts i.e. aqueous (5g & 10 g) and methanolic extraction of *Allium cepa*

Table 1 Phytochemicals screening of aqueous and methanolic extracts of Allium cepa

S.No	Phtochemical Tests	Aqueous Extract		Methnolic Extract
		5 Gm	10 Gm	
	Tests For Alkaloids			
1.	(A) Mayer's test	-ve	-ve	-ve
	(B) Wagner's test	+ve	+ve	+ve
	(C) Hager's test	+ve	+ve	-ve
	Test For Carbohydrate			
2.	(A) Molisch's test	+ve	+ve	-ve
	(B) Barfoead's test	-ve	-ve	-ve
	Test For Reducing Sugar			
3.	(A) Fehling's test	+ve	+ve	+ve
	(B)Bendict's test	+ve	+ve	-ve
	Test For Flavonoids			
4.	(A) Alkaline reagent	+ve	+ve	-ve
	(B) Lead acetate	+ve	+ve	+ve
	(C)Ammomia test	+ve	+ve	-ve
	Test For Glycosides			
5.	(A) Borntragers' test	+ve	+ve	-ve
	(B) Legal's test	-ve	-ve	+ve
	(C) 10% NaoH test	+ve	+ve	-ve
6.	Test For Cardiac Glycosides			
	(A)Keller killani test	-ve	-ve	-ve
	Test For Tannin And Phenolic Compound			
	(A) Ferric chloride 5%	-ve	-ve	+ve
7.	(B) Lead acetate	-ve	-ve	+ve
	(C) Dillute iodine	+ve	+ve	+ve
	(D) Ferric chloride10%	+ve	+ve	+ve
	(E)Hydrolysable tannin	+ve	+ve	+ve
8.	Test For Saponin			
	(A) Froth test	+ve	+ve	+ve
	Test For Amino Acid & Protein			
9.	(A) Ninhydrin test	+ve	+ve	+ve
	(B) Biuret	+ve	+ve	+ve
10.	Test For Terpenoid	+ve	+ve	+ve
11.	Test For Steroid	+ve	+ve	+ve

"+" = Positive; "-" = Negative; Aq. Ex.= Aqueous Extract; Met. Ex. = Methanolic Extract

Table 2 Rf value of aqueous and methanolic extation of *Allium cepa*

S. No.	Onion Bulb Extract	No. of Spots	Rf Value
1.	Meth. ext.	9	0.31,0.36,0.44,0.50,0.52,0.57,0.63,0.73,0.84
2.	Aq.ex. (5g)	6	0.29, 0.36, 0.44, 0.47,0.57,0.68
3.	Aq.ex. (10g)	7	0.36,0.42,0.47 ,0.52,0.57,0.68 ,0.78

Shenoy *et al*. [33] revealed the presence of tannins in chloroform extract and none of the phytochemicals were identified in petroleum ether extract. Phenolics, flavonoids, saponin, alkaloid, tannins were also determined in *Allium* sp. by Udu-Ibiam *et al* [34], Rekha and Shruti [35] and Huzaifa *et al* [36] in their study. But in the present study, we observed all the secondary metabolites present in both aqueous and methanolic extracts.

Flavonoids are water soluble antioxidants that prevent oxidative damage and have strong anti-ulcer activity [37]. A

reduced risk of cardiovascular disease has been reported in persons with high flavonoids intake [38]. Plants glycosides are used as medication for the elimination of poisons from the body [39]. Having tested that glycoside is present in the white onion extract, it can be used in the elimination of poisonous substances from the body [40]. Alkaloids are most efficient therapeutically significant plant substance. Both natural and synthetic alkaloids are used as basic medicinal agent because of their analgesic, antispasmodic and bacterial properties [41]. The saponin constituent is responsible for the possession of haemolytic property. This can give the plant the traditional medicinal use as cholesterol binding agent. The biological functions of flavonoids include protection against allergies, inflammation, platelets aggregation microbes, ulcer, viruses and tumors [42].

Numerous investigations of the antioxidant activity of plant extracts have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity [43,44]. Phenolic compounds are the major group contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials. The antioxidant activity of the compounds is partly due to one electron reduction potential that is the ability to act as hydrogen or electron donors [45]. Atoui *et al.* [46] mention that the antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

Antioxidant activity from a high intake of fruits and vegetables has been reported to prevent alteration of DNA by reactive oxygen species (ROS) and subsequent cancer development [47]. Flavonoids, ubiquitous in the plant kingdom, have been widely studied for their antioxidative effects [48,49]. Onions are known to contain anthocyanins and the flavonoids quercetin and kaempferol [50,51]. However, anthocyanin pigments, concentrated in the outer shell of red onions, are only minor constituents of the edible portion [51]. Kaempferol, while detectable in certain onion varieties, is present in much smaller quantities than quercetin [48,50]. Therefore, quercetin is the major flavonoid of interest in onions. Mechanisms of action include free radical scavenging, chelation of transition metal ions, and inhibition of oxidases such as lipoxygenase [52-54]. The antioxidative effects of consumption of onions have been associated with a reduced risk of neurodegenerative disorders [55], many forms of cancer [49,56], cataract formation [57], ulcer development [53], and prevention of vascular and heart disease by inhibition of lipid peroxidation and lowering of low density lipoprotein (LDL) cholesterol levels [58-60]. Another antioxidant effect of onions and their extracts includes the reduction of rancidity in cooked meats [61]. Phenolic compounds in general and flavonoids in particular have the ability to provide protection against oxidative stress. Thus in this study, the presence of flavonoids and phenolic compounds in the extract could be considered responsible for conferring antioxidant ability.

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