Research Article

Physicochemical Characterization and Antioxidant Study of *Allium cepa* Leaf Extracts

JG. Pohare, GR. Shendarkar*, AK. Daswad, DP. Shelke, SN. Wangawar and AK. Dhadwe

PG Department, Nanded Pharmacy College, Shyam Nagar, Nanded – 431605, Maharashtra, India.

ABSTRACT

The present study reports physicochemical characterization and antioxidant activity of extracts from *Allium cepa* leaves collected from local region of Nanded, Maharashtra, India. Different physical parameters like ash values, extractive value, Loss on drying, solubility etc were evaluated for powdered drug. The extracts were obtained from Soxhlet method by using water and methanol as solvents for extraction and subjected for preliminary physicochemical evaluation and antioxidant studies. Total phenolic and flavonoids content were also analyzed. The presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phyto-chemical analysis. DPPH free radical scavenging assays showed strong antioxidant activities with increase in concentration of aqueous and methanol leaf extracts. Maximum percentage inhibition i.e. 80.97% was shown by aqueous extract at concentration of 150 μg/ml and was compared with Ascorbic acid as reference standard.

Keywords: *Allium cepa,* Aqueous and Methanolic extract, phytochemical screening, Antioxidant effect.

INTRODUCTION

Free radicals are recognized as the main products of lipid oxidation generating oxidative stress that plays a major role in the development of over 100 chronic diseases such as cancer, autoimmune disorders, aging, cardiovascular, and neurodegenerative diseases. These free radicals or reactive oxygen species (ROS) are highly reactive as they can interact with cellular molecules and metabolites leading to cellular damage. Free radicals include a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), superoxide ($O2^-$), etc. The action of hydroxyl radicals that initiates lipid peroxidation and causing DNA damage is facilitated by the rapid decomposition of H_2O_2 into oxygen and water.

The ability of the antioxidants to inhibit the free radical reactions thereby protecting the human body from diseases has led to the increasing interest in the discovery of new antioxidant phytochemicals. The use of synthetic antioxidants such as butylated hydroxyl anisole and butylated hydroxyl toluene are highly discouraged due to its carcinogenic properties. Hence, natural antioxidants like phenolics and flavonoids from fruits, vegetables, spices, and herbs can be exploited as a substitute to these synthetic antioxidants.

Economically important *Allium cepa* vegetable contains antioxidant components such as volatile organosulfur compounds and flavonoids. These compounds can act either directly as an antioxidant or indirectly by modulating the pro-apoptotic pathway or activating the endogenous antioxidant system. Therefore, it is necessary to identify specific food groups that ameliorate the effects of oxidative stress and the progression of cancer. Green onions belonging to the family *Alliaceae* are consumed for their immature bulbs as well as green foliage. Green onions produce a mild flavor during tissue disruption and can be eaten raw or cooked. Numerous cultivars have been developed economically for various parameters such as size, form, color, storability, and climatic adaptations. Cultivars are divided into the common onion group (*Allium cepa* var. *Cepa*), that includes the cultivars grown for green or salad onions and the Aggregatum group. Quercetin is the flavonoids that are abundant in green onions that benefits health by preventing the

formation and development of certain cancer. Therefore, it was thought worthwhile to unveil the antioxidant effect of leaves of *Allium cepa* by using suitable *in vitro* method.

MATERIAL AND METHOD

Collection of Plant material & Authentication

The leaves of *Allium cepa* (*AC*) were collected from local region of Nanded, Maharashtra. Herbarium sheet was prepared and sent for authentication by Taxonomist. Authentication number NPC/M. Pharm/Herbarium 2016-17/ H- 08 was obtained for the drug.

Fig. 1: *Allium cepa*

Plant profile:

Kingdom : Plantae
Division : Magnoliophyta
Class : Liliopsida
Order : Asparagales
Family : Alliaceae
Genus : Allium
Species : *Allium cepa*

Pharmacognostic study

The microscopic study of the fresh leaf section showed presence of upper and lower epidermis, palisade tissue and vascular tissues i.e. xylem and phloem. The powdered material showed presence of lignified fibres, lignified xylem fibers, and simple isolated starch grain.

Fig. 2: T S of *AC* leaf

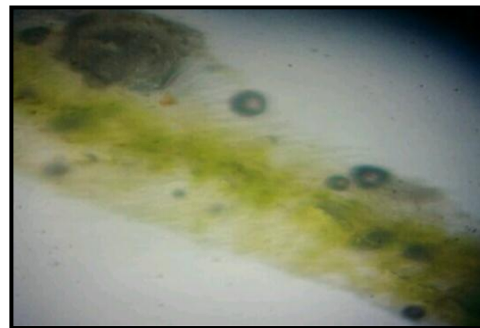


Fig. 3: Powder characteristic

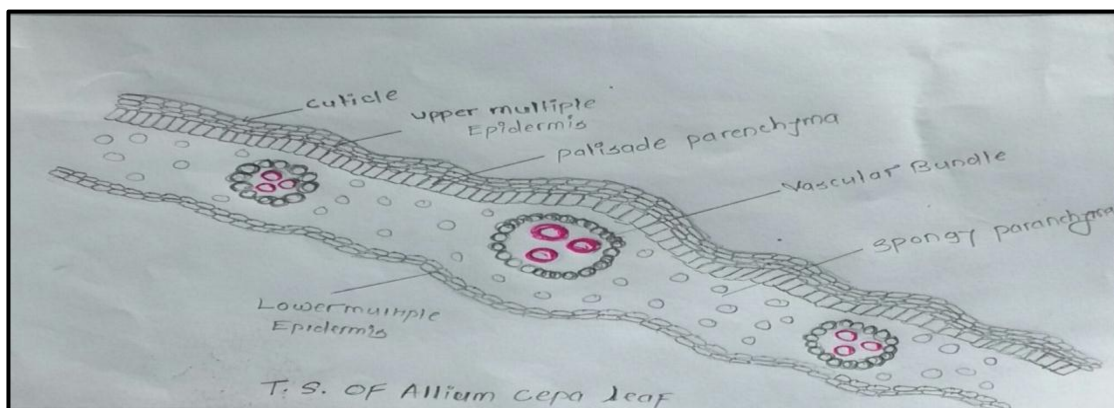


Fig. 4: Microscopic characters of *AC* leaf

Physical evaluation of powder

It has been done for following parameters.

1. Solubility
2. Melting point
3. Ash value
4. LOD
5. Extractive value

Method of extraction

Fig. 4: Aqueous extraction

Fig. 5: Methanolic extraction

About 150gm of the shade dried powdered plant material *Allium cepa* was extracted with Water (fig.04) and Methanol (fig.05) by using Soxhlet extractor. Then extracts were concentrated and evaporated to dryness. The extracts obtained were subjected to qualitative test for the identification of various phytoconstituents.

Phytochemical evaluation of extract

Test for identification

The extracts were tested for detect the presence of different phytoconstituents like by performing test for alkaloids, glycosides, tannin, flavonoids, amino acid, carbohydrates, steroids etc. It was further tested for evaluation of total Phenolic and flavonoids content by using following methods.

Total Phenolic content

The total phenolic content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1m) of extract or standard solution of Gallic acid [2,4,6,8,10µg/ml] was added to 10ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% $NaHCo_3$ solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer.

Total flavonoids content

Total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% NaNO2, after five minutes 0.3 ml 10 % AlCl3 was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm.

Thin layer chromatography

TLC fingerprinting for both the extracts was done by using suitable solvent systems and the obtained spots were estimated for their respective RF values. Solvent system used for aqueous extract was Chloroform: Methanol (Solvent system I) at the proportion of (8:2) and for methenolic extract was Toluene:Acetone:Ethyl acetate (Solvent system II) at the proportion of (9:0.5:0.5). The respective RF values are shown in Table no.05.

Chloroform: Methanol (8:2) Toluene: Acetone: Ethyl acetate (9:0.5:0.5)

Fig. 6: Aqueous extract

Fig. 7: Methanolic extract

ANTIOXIDANT ACTIVITY

DPPH assay (2, 2-Dipheny l-1-Picrylhydrazyl)

The free radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (50mg/ml) in methanol was used as reference standard.

Principle

2, 2 Diphenyl 1 -1- Picryl Hydrazyl (DPPH) is a stable free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity.

Method

Different concentrations (50, 100 & 150 µg/ml) of both the extracts were prepared with methanol and 1ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 30 min. After 30 min, the absorbances of the mixtures were measured at 517 nm. 1ml of DPPH and 1 ml of methanol was taken as control.

$$\% \, RSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where,
RSA is the free Radical Scavenging Activity;
Abs control is the absorbance of DPPH + Methanol;
Abs sample is the absorbance of DPPH + extract.

RESULTS

Powdered drug evaluated for different physical parameters showed the following results.

Table 1: Physical Parameter

Sr. no	Physical parameter	Drug name & Obtained value (%w/w)
1	Total Ash Value Water soluble ash Acid insoluble ash	10.50 % 3.5 % 4.0 %
2	**Extractive Value** Water Methanol	 23.2% 13.6%
3	LOD	8.66 %
4	Melting point	232^0 C
5	Solubility	Methanol, Aqueous solvent

The aqueous and methanolic extract screened for Phytochemical evaluation showed presence of following phyto constituents in it.

Table 2: Phytochemical screening of extract

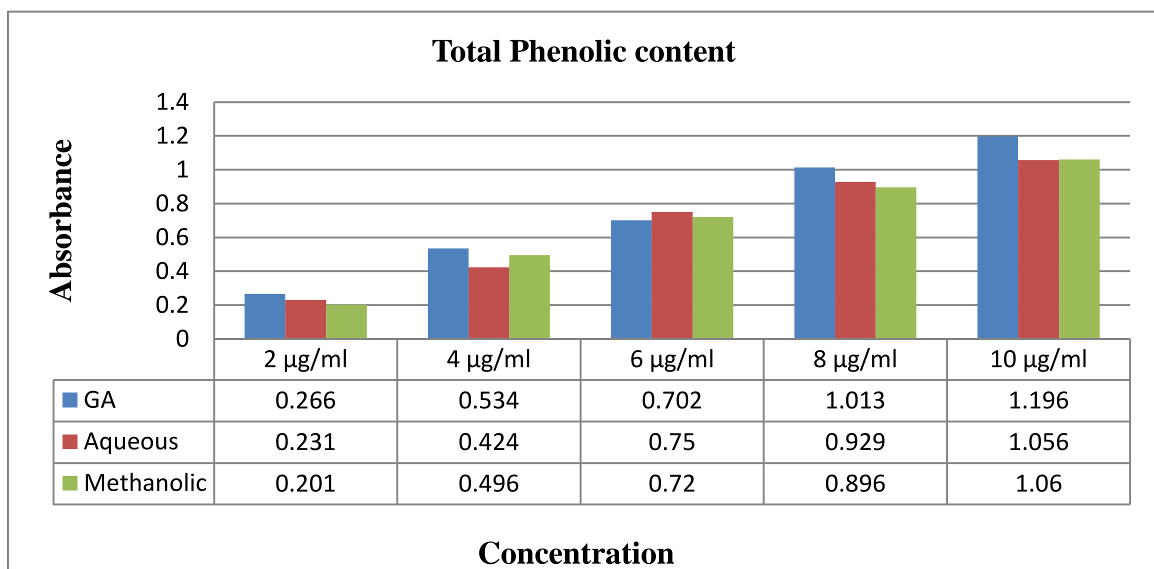
	Aqueous extract		Methanolic extract	
Sr. no	Test Name	Observation	Test Name	Observation
1	**Flavonoids** Shinoda Test Alkaline test NH_4OH test Zn test	 + + + -	**Flavonoids** Alkaline test NH_4OH test	 + +
2	**Glycosides** Keller Killani test Modified Borntragers test	 + -	**Glycosides** Keller Killani test Modified Borntragers test	 + +
3	**Steroids** Salwoski test Libberman test	 + -	**Steroids** Salwoski test Libberman test	 + -
4	**Protein** Biuret test Million test	 - +	**Protein** Biuret test Million test	 - +

	Xanthoprotein test	-		
5	**Alkaloids** Wagners test Hagers test	 + -	**Alkaloids** Wagners test Hagers test	 + +
6	**Carbohydrates** Molish test Benedicts test Barfoed test	 + + -	**Carbohydrates** Molish test Benedicts test	 + +
7	**Amino acid** Ninhydrin test Tyrosine test Trytpohan test	 - + +	**Amino acid** Ninhydrin test Tyrosine test	 - -
8	**Tannins & Phenolic Compound** Lead acetate Dil. HNO_3 test $FeCl_3$	 + + -	**Tannins & Phenolic Compound** Lead acetate Pot. dichromate	 + +

Total Phenolic and total flavonoids content of the extracts were found to be as followed.

Table 3: Phenolic Content

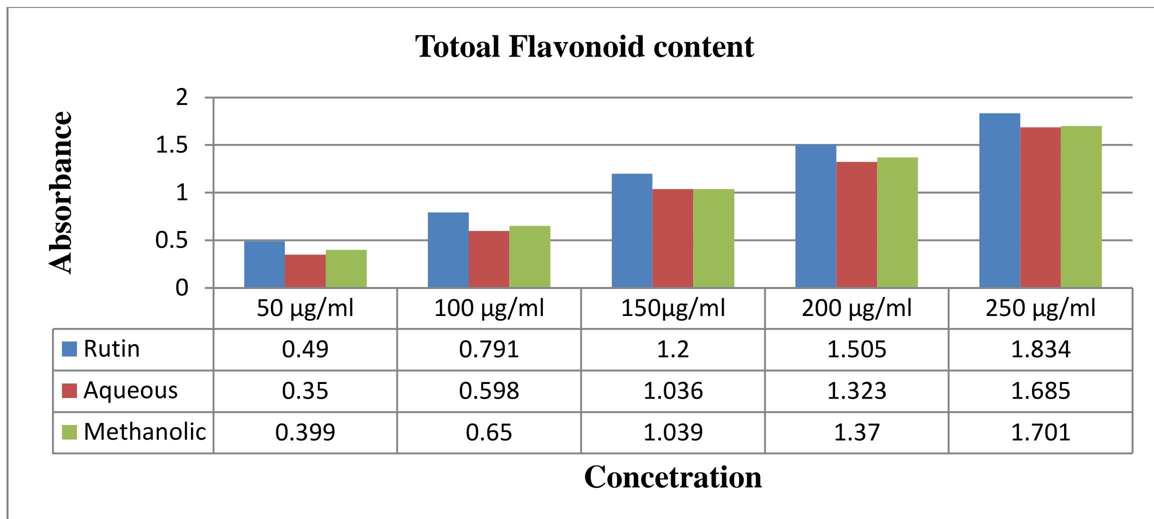
Conc^n of Extracts (µg/ml)	Absorbance
Aqueous extract(6µg/ml)	0.750
Methanolic extract(8µg/ml)	0.896



	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml
GA	0.266	0.534	0.702	1.013	1.196
Aqueous	0.231	0.424	0.75	0.929	1.056
Methanolic	0.201	0.496	0.72	0.896	1.06

Table 4: Flavonoid content

Conc^n of PHF (µg/ml)	Absorbance
Aqueous extract(150µg/ml)	1.038
Methanolic extract(100µg/ml)	0.650



In vitro antioxidant effect of the extracts determined by DPPH method show following results

Table 5: Antioxidant Activity

Sr. no	Concentration (µg/ml)	Ascorbic acid (%inhibition)	Aqueous extract (%inhibition)	Methanolic extract (%inhibition)
1	50	61.95 %	38.04 %	22.43%
2	100	95.12 %	64.87%	68.29 %
3	150	96.22 %	**80.97 %**	**74.14%**

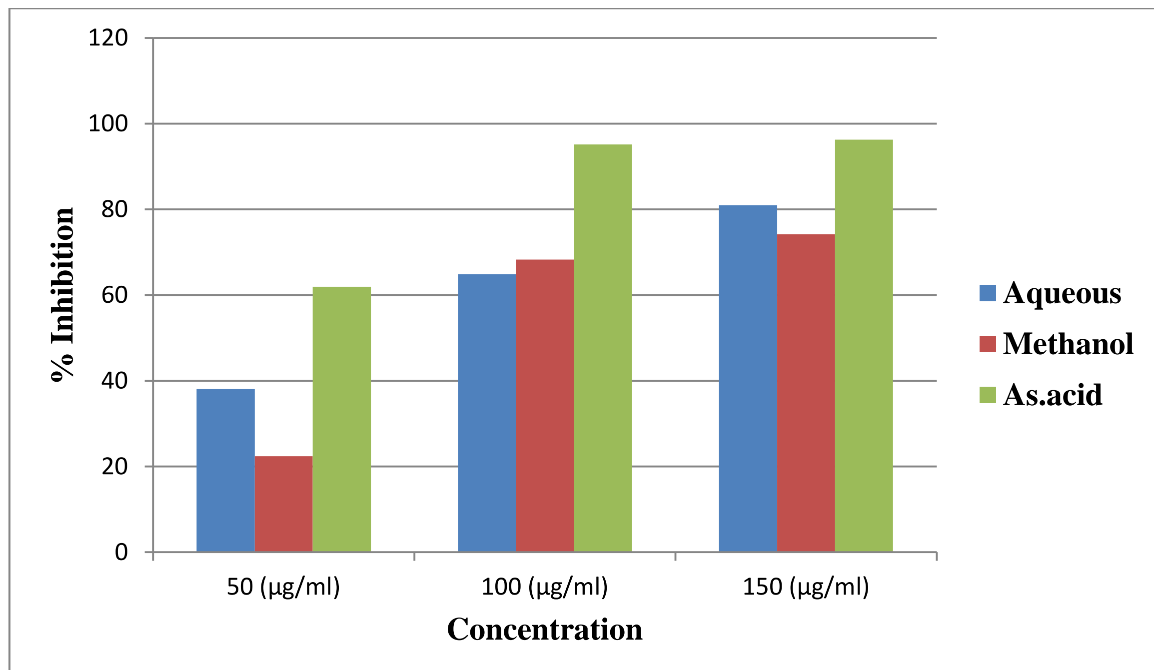


Table 6: R. F. Value table

Sr. no	Extract name	Solvent system		Extract name	Solvent system	
		No. of Spot	R_f Value		No. of Spot	R_f Value
1	Aqueous extract	1	0.81	Methanolic extract	3	0.52 0.72 0.88

CONCLUSION

The result obtained from above study indicates the presence of flavonoids, steroids, Glycosides & Tannins in the methanolic extract of leaf. TLC fingerprinting indicates presence of 3-Hydroxyflavone (0.81) in aqueous extract and presence of 6-Hydroxyflavone (0.52) Galangin (0.72) Flavone (0.88) at their respective Rf values. The antioxidant screening done by using DPPH method showed that the free radical scavenging effect of aqueous extract at concentration of 150 µg/ml (i.e. 80.97 %) and methanolic extract at concentration of 150µg/ml of (i.e.77.2%) showed maximum % inhibition of free radicals. From the study we can conclude that aqueous and methanolic extract of *Allium cepa* leaves possess promising antioxidant activity which can be considered as base for further pharmacological evaluation.

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