



QUALITATIVE SCREENING OF SELECTED ONION VARIETIES.

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ABSTRACT

Phytochemicals are complex group of primary metabolites and secondary metabolites. Primary metabolites are essential for plant's growth and reproduction. Secondary metabolites (alkaloids, steroids, flavonoids, terpenoids, glycoside, saponin, tannins, phenolic compounds etc.) are active principles that are end products of primary metabolites such as carbohydrates, amino acid, and chlorophyll and lipid are synthesized by plants. The identification and isolation of such active compounds makes it more effective therapeutic application to various diseases. A large number of diseases such as asthma, arthritis, cancer etc. can be cured not only through pharmaceuticals chemicals but also by plant based drugs without any side effects leading to "MAN FRIENDLY MEDICINES". Separation of active compounds in *Allium* species using solvents (polar protic and polar aprotic) using standard protocol. The screening for extracts prepared in solvent distilled water and acetone showed the presence and/or absence of various phytoconstituents.

KEYWORDS : Onion Variety, Secondary Metabolites, Phytochemicals, Qualitative Screening.

INTRODUCTION:

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. Hippocrates (ca. 460–377 BC), one of the ancient authors who described medicinal natural products of plant and animal origins, listed approximately 400 different plant species for medicinal purposes. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and Egyptian (Sarker S and Nahar L, 2007). According to the World Health Organization (WHO), a medicinal plant is any plant which, in one or more of its organs (leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds), contains substances that can be used for therapeutic purposes which are precursors for chemopharmaceutical synthesis (Doughari JH, 2012).

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active and naturally occurring chemical compounds which are found in plants and provide health benefits for humans. They protect plant from disease and damage and contribute to the plant's color, aroma and flavor. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. It is said that primary metabolites are essential in plant growth and reproduction. In the absence of these that processes do not occur properly. While secondary metabolites are not as essential as primary. Primary metabolites include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary metabolites are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glycosides.

PLANT MATERIAL:

Scientific Name: - *Allium cepa* L. from Latin cepa "onion")

Family: - Liliaceae

Common Name: - Onion, Dungali, Kanda, Pyaj

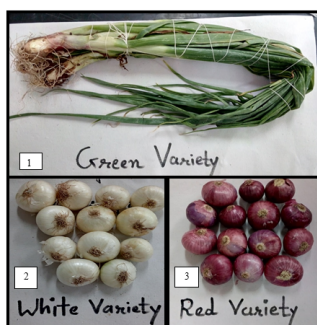


Fig.1: Different varieties of Onions.

Green variety: The plant is native to Central Asia. It is also called scallions and spring onions, are young onions harvested when their tops are green and the underdeveloped bulbs are 13mm 0.5inch) or less in diameter having a mild flavor. The entire onion, including top, stem and bulb, is used raw in salads and sauces, as a garnish and as a seasoning for prepared dishes (Fig. 1.1).

White variety: The plant is native to Central Asia. It has a thin, dry paper sheath with a crisp translucent pearl white flesh which is pungent, savory and warm. Its barely sweet finish can be attributed to its higher moisture content than yellow onions. It is used in Mexican foods or complementing the flavors of other ingredients. It can be sautéed to a dark brown color and served to provide a sweet and sour flavor to other foods (Fig. 1.2).

Red variety: It is a native plant of Southwestern Asia. It found to flourishly grow in three distinctly different regions, Turda in Romania, Tropa in Italy and Wethersfield, Connecticut within the United States. Red onions are shallow-rooted and need a friable soil that retains moisture well, especially after cultivation (Laura G et. al. 2002). They are often consumed raw, grilled or lightly cooked with other foods, or added as a decoration to salads. They tend to lose their color when cooked and are available throughout the year. The red color comes from anthocyanidins such as cyanidin and it contains high amount of flavonoids. They can be stored for 3 to 4 months at room temperature. They are used in various ways like culinarily, non-culinarily and medicinally (Fig. 1.3).

METHODS:-

Collection: Plant samples were collected from a local market near Navrangpura, Ahmedabad in the month of January 2017.

Sample preparation: The plant materials were oven dried at 50°C, and extracted using solvents distilled water and acetone.

Extraction: 10gm finely ground plant powder was taken and kept in 100ml solvent (Distilled water and Acetone) for 24 hours. The solution was then filtered using Whatmann filter paper No.42 (125mm) and kept at room temperature for the evaporation of the respective solvents (Fig.2). The dried extracts were then weighed for obtaining the extractive values of each plant material. The yield value of each extract was calculated by using the formula (Shilpakar A et. al. 2009):

$$\frac{\text{Extraction Obtained}}{\text{Total amount of crude drug}} \times 100$$



Fig.2 Extraction of Selected Onion Varieties.

QUALITATIVE SCREENING: Qualitative screening was performed as per the protocol followed by Prashant Tet. *al.* 2011 and Sahirabanu K and Cathrine L, 2015.

1. Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Extracts and dilute hydrochloric acid were taken in the ratio of 2:1 then the screening was done for detecting the presence of alkaloids.

Mayer's test: 1ml filtrate was taken and into it was added 1ml Mayer's reagent, yellow colored precipitates were observed that indicated the presence of alkaloids.

Preparation of Mayer's Reagent: Dissolve 1.358g of Mercuric chloride in 60 ml of water & pour into a solution of 5g of Potassium iodide in 10 ml of water. Add sufficient water to make 100 ml. White precipitate with most alkaloids in slightly acid solution.

Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.

Preparation of Wagner's reagent: Dissolve 2g of Iodine and 6g of Potassium Iodide in 100ml of water.

Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids.

Preparation of Dragendroff's reagent: Bismuth sub-nitrate 1.7g, glacial acetic acid 20ml, water 80ml and 50% solution of potassium iodide in 100ml of water. Mix together and store as stock solution. 10ml of stock, 20ml glacial acetic acid and make up to 100ml with water gives the working solution.

2. Carbohydrates: Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test: 2 ml of filtrate was treated with a drop of alcoholic alpha-naphthol (1:2) solution in a test tube. The mixture was shaken well and few drops of concentrated sulphuric acid (0.6 ml) were added slowly along the sides of test tube. Formation of the violet ring at the junction indicated the presence of carbohydrates.

Benedict's test: 2 ml of filtrate was treated with 2 ml of Benedict's reagent and heated gently for 2 minutes. Orange red precipitate indicated the presence of reducing sugars.

Fehling's test: 2 ml of filtrates were hydrolyzed with 1ml of dilute Hydrochloric acid (1N) neutralized with 1 ml of alkali (10% NaOH) and heated with 1 ml of Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

3. Glycosides: Extracts were hydrolyzed with dilute Hydrochloric acid and then subjected to test for glycosides.

Modified Borntrager's test: 2 ml of extract was treated with 2 ml of 5% Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of Benzene. The benzene layer was separated and treated with 1 ml of ammonia solution. Formation of rose pink color in the ammoniacal layer indicated the presence of anthranol glycosides.

Legal's test: 2 ml of extracts were treated with 1 ml of 5% Sodium Nitropruside in 1 ml of pyridine and 1 ml of 10% sodium hydroxide. Formation of pink to blood red color indicated the presence of cardiac glycosides.

4. Saponins:

Froth's test: 0.5gm of extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

Foam test: 0.5gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicated the presence of saponins.

5. Phytosterols:

Salkowski's test: 2 ml of extracts were treated with 1 ml of chloroform and filtered. The filtrates were treated with few drops of Concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicated the presence of triterpens.

Liebermann Burchard's test: 2 ml of extracts were treated with 1 ml of chloroform and filtered. 2 ml of filtrate was treated with few drops of acetic anhydride, boiled and cooled. 1 or 2 drops of concentrated Sulphuric acid were added. Formation of brown ring at the junction indicated the presence of phytosterols.

6. Phenols:

Ferric Chloride test: 1 ml of extract was treated with 3-4 drops of 5% ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

7. Tannins:

Gelatin test: To the extract, 2ml of 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

Ferric chloride test: 5 drops of 0.1% Ferric chloride was added to 2ml of extract, a brownish green or blue black color indicated positive result.

8. Flavonoids:

Alkaline Reagent test: 1 ml of extract was treated with 1 ml of 10% Sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of 2 ml of dilute acid (1 N HCl), indicated the presence of flavonoids.

Lead acetate test: 1 ml of extract was treated with 1 ml of 10% lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

9. Proteins and Amino acids:

Xanthoproteic test: The extracts were treated with few drops of Concentrated Nitric acid. Formation of yellow color indicated the presence of proteins.

Millon's test: To 1 ml of filtrate 3 ml of Millon's reagent was added. A white precipitate indicated the presence of proteins.

Ninhydrin test: 2 drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate. Appearance of purple color indicated the presence of amino acids.

Biuret's test: 1 ml of filtrate was treated with 1 ml of 2% Copper

Sulphate solution. To this 1ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink color ethanolic layer indicated the presence of protein.

10. Diterpenes:

Copper acetate test: 1 ml of extract was dissolved in water and treated with 1 ml of copper acetate solution. Formation of emerald green color indicated the presence of Diterpenes.

RESULT AND DISCUSSION:

Yield Extractive Value:-The yield extractive value was higher in aqueous extract than acetonetic extract. Result showed that the yielding extractive value was higher in white variety and lower in green variety for aqueous extract, while for the acetonetic extract the yielding extractive value was higher in the green variety and lower in the red variety (Fig. 3).

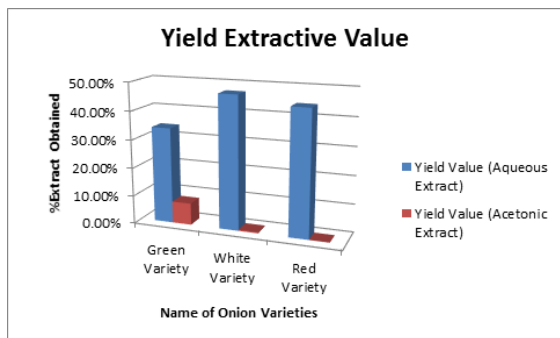


Fig.3: Yield Extractive Value for Selected Onion Varieties.

Qualitative Screening:

The phytochemical screening of the *Allium* species was performed according to the standard protocol of Prashant T et. al.2011 with required modification. The screening of the extracts prepared in solvents distilled water and acetone showed the presence of Alkaloids, Saponins, Glycosides, Carbohydrates, Phytosterols, Flavonoids, Proteins and Amino Acids, Diterpenes, Phenols and Tannins and the absence of Cardiac Glycosides (Table. 1 and 2) (Fig. 4 and 5).

Table 1: Qualitative Screening of Selected Onion Varieties (Aqueous Extract)(where +=present and -=absent).

Phytochemicals	Tests	Varieties of Onions		
		Green	White	Red
Alkaloids	Wagner's test	+	+	++
	Dragendroff's test	+	+	++
Saponins	Froth's test	+++	+	++
	Foam test	+++	+	++
Glycosides	Modified Borntranger's test	+	++	+++
Carbohydrates	Molish's test	+	+	+
	Fehling's test	+	+	+
Phytosterols	Salkowski's test	+++	+	++
	Liebermann Burchard's test	+	+++	++
Flavonoids	Alkaline Reagent test	++	+	+++
	Lead Acetate test	+	+	++
Proteins And Amino Acids	Millon's test	+	+	+
	Biuret's test	+	+	+
Diterpenes	Copper Acetate test	+	+	+
Phenols	Ferric Chloride Test	++	+	++
Tannins	Ferric Chloride Test	+	+	+

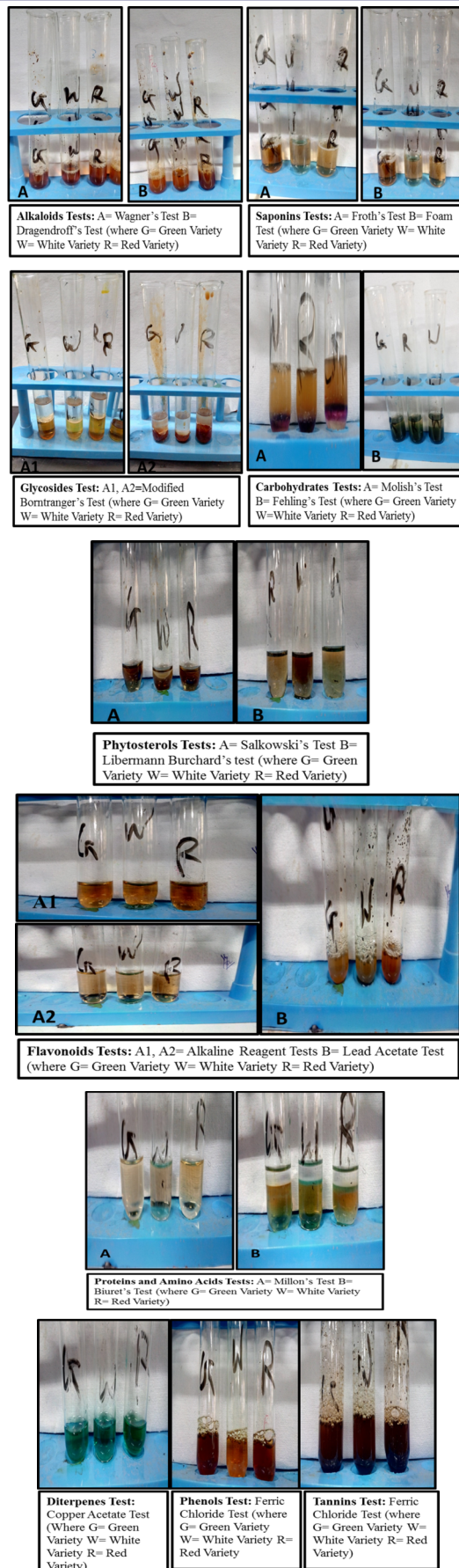


Fig.4: Qualitative Screening of Selected Onion Varieties (Aqueous Extract).

Table 2: Qualitative Screening of Selected Onion Varieties (Acetone Extract) (where, +=present and -=absent).

Phytochemicals	Tests	Varieties of Onions		
		Green	White	Red
Alkaloids	Wagner's test	++	+++	+
	Dragendroff's test	++	+	+
Saponins	Froth's test	-	+	+
	Foam test	-	+	+
Glycosides	Modified Boritranger's test	++	+	++
Carbohydrates	Molish's test	++	++	++
	Benedict's test	+	++	+++
Phytosterols	Salkowski's test	+	+	++
	Liebermann Burchard's test	+	+	+
Flavonoids	Alkaline Reagent test	+++	+	++
	Lead Acetate test	+	+	++
Proteins And Amino Acids	Millon's test	+	+	+
Diterpenes	Copper Acetate test	+++	+	++
Phenols	Ferric Chloride Test	+	+	+
Tannins	Gelatin Test	+++	++	+

DISCUSSION:-

For the extracts prepared in distil water the presence of Alkaloids, Saponins, Glycosides, Carbohydrates, Phytosterols, Flavonoids, Proteins and Amino Acids, Diterpens, Phenols and Tannins and the absence of Cardiac Glycosides were seen which was found to be similar with the experiment performed by Ponnulakshmi R and Balasubramanians E, 2013. While for the extracts prepared in acetone the presence of Alkaloids, Saponins, Glycosides, Carbohydrates, Phytosterols, Flavonoids, Proteins and Amino Acids, Diterpens, Phenols and Tannins and the absence of Cardiac Glycosides were seen, there was not a single review available for that. So, results for acetonic extract were new as far as the research papers were concerned (Table 1 and 2)

CONCLUSION:

Extraction of *Allium* species was done by using polar protic (Aqueous) and polar aprotic (Acetone) solvents. Distil water showed the extraction of maximum metabolites as compared to the acetone extracts. The yield extractive value is high in white variety and low in green variety for aqueous extract while for acetonic extract is high in green variety and low in red variety. According to the experimental results, during the phytochemical screening the metabolites present in the three selected *Allium* species are alkaloids, tannins, phenols, flavonoids, saponins, terpenes, carbohydrates and proteins except cardiac glycosides in both aqueous and acetonic extract. The presence of these secondary metabolites gives large scope for the separation and isolation of various phytoconstituents for studying their bio-efficacy.

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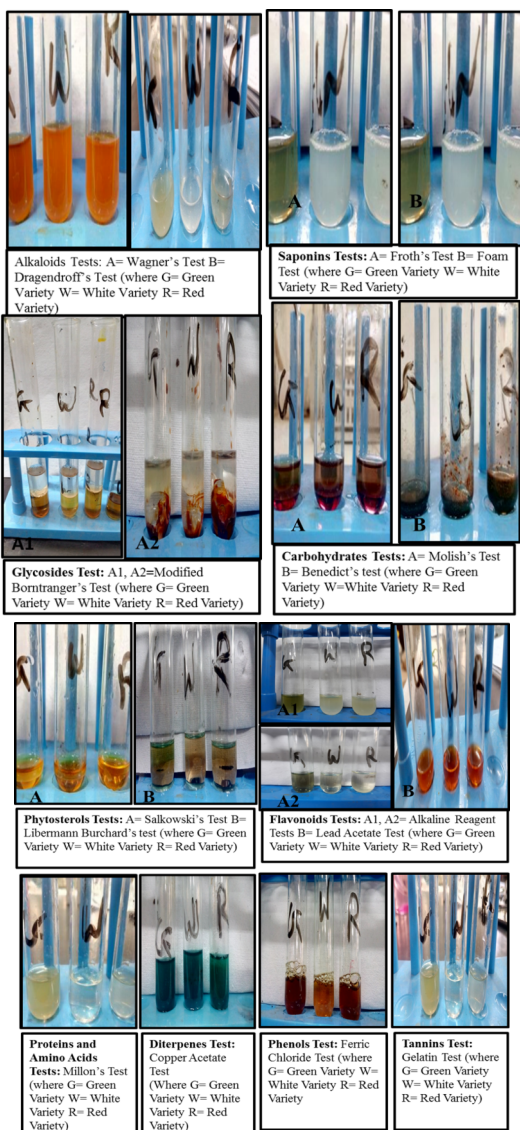


Fig.5: Qualitative Screening of Selected Onion Varieties (Acetonic Extract).