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Comparative Study Of Quercetin Level In Five Different Varieties Of Indian Onion Wastes

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ABSTRACT

The onion, also commonly known as bulb onion, is a vegetable and is the most widely cultivated species of the genus *Allium*. The name “wild onion” is applied to a number of *Allium* species, but among them *Allium cepa* is exclusively known for cultivation. The onion is mostly biennial or a perennial plant. But it is usually treated as an annual plant and harvested in its first growing season. *Allium cepa* is a common vegetable used in older days for treating number of diseases. There are so many varieties of onion are cultivated in the world among which the Indian variety onion has got the more demand in foreign market due to its taste, size and colour. India is the second largest producer of onion in the world next to China and ranks third in export of onion next to Netherland and Spain. Maharastra is the leading onion producing state in India followed by Karnataka and Gujarat. During the process of exportation the brown skin and the damaged onions are being dumped which act as a pollutants in the environment. An effective, economical and beneficial method should be developed to minimize the effect of these pollutants on the environment. Since onions are rich in flavonoids my reach is to check quantity of Quercetin in five different varieties (South Bellary Onion, Nasik Bellary Onion, Shallot / Podisu Onion, Rose Onion, Onion peels of all four onions) of Indian onion wastes.

Keywords: *Allium* Species, Quercetin, flavonoid, recycling of onion waste.

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INTRODUCTION

INTRODUCTION TO FLAVONOIDS

Fruits and vegetables are rich in antioxidant. Flavonoids occurs naturally in fruits, vegetables and beverages (Middleton EJ et al.,1998;). Among which Quercetin is the major flavonoid , belongs to the class flavonols. Generally flavonoids are natural substances with variable phenolic structure. Based on their beneficial effects on health flavonoids are considered as effective compound. Around 4000 varieties of flavonoids have been identified (De Groot H et al.,1998;). Flavonoids occurs as glycone, glycosides and methylated derivatives. It consists of a benzene ring (A) condensed membered ring (C) which is in the second position carries a phenyl ring (B) as a substituent (Narayana K Raj et al.,2001). Flavonoids are classified into seven groups and are as follows, Flavanone, Anthocyanin, Flavonol, Proanthocyanin, Flavanol, Isoflavone and Flavone (Murphy Karan J et al.,2003).

QUERCETIN – THE FLAVONOID

Quercetin is a member of the class flavonoids called Flavonoles. Generally Quercetin is an a glycone. But in plants it is present as glycone. It consist of three rings and five hydroxyl groups (Moskuag JO et al.,2004;). Common chemical names of Quercetins are 117-39-5, Sophoretin, Meletin, Xanthaurine, Quercetine, etc. The molecular formula of Quercetin is $C_{15}H_{10}O_7$ and the molecular weight is 302.238g/mol (Quercetin pubchem).

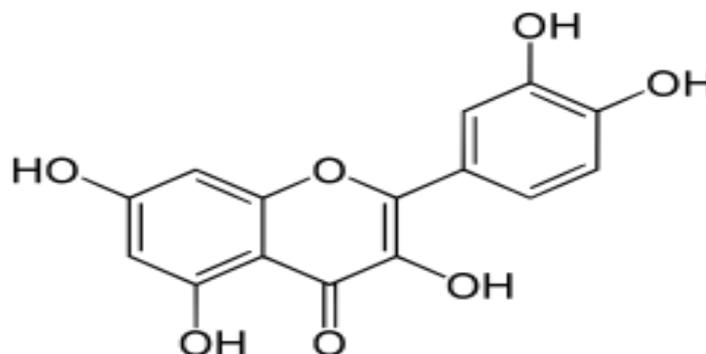


Figure 1: Structure of Quercetin

MECHANISM OF ACTION OF QUERCETIN

- Anti oxidation action.
- Inducible Nitric oxide synthesis inhibitory action.
- Xanthine oxidase inhibitory action.
- Direct radical scavenging action.
- Decreasing leukocyte immobilization.
- Modulation of gene expression.
- Interaction with other enzymes system (Parul Lakhanpal et al.,2007;).

PHARMACOLOGICAL IMPORTANCE OF QUERCETIN

Quercetin is a versatile molecule with many pharmacological properties including antioxidant, neurological, anticancer, cardiovascular, antimicrobial, anti-inflammatory and anti-obesity agent (Aneela Maalik et al.,2014).

ANTICANCER ACTIVITY OF QUERCETIN

Quercetin because of its antioxidant property and metal ion chelating capacity, it has been reported to be effective compound in inflammation, arteriosclerosis, allergy and swelling .In addition, epidemiological data suggests that quercetin is an pharmacological compound associated with reduced risk of cancers and

hence can be used as drug for anti cancer activity. Because of its anti oxidant activity, Quercetin compound is considered to be one of the most potent flavonoid which is capable of interacting and modulating the activity of a variety of enzyme systems including cyclooxygenase, lipooxygenase, and tyrosine kinase. But the major problem with quercetin compound is the very low bioavailability when used as drug. Generally quercetin compound is present in the form of glycosides. Glycosides are too polar to penetrate the intestinal membranes and so it can't be easily absorbed. It can only be absorbed when the glycosides are released as aglycone due to action of microfloral enzymes. Though the aglycones are permeable through intestinal membrane, the bioavailability of that compound is not high due to low solubility proper and higher extent of conjugation. Thus, the bioavailability of quercetin is an important challenging one since factors like solubility, permeation across membrane and metabolism involves in affecting the bioavailability of Quercetin. Apart from these factors the number of hydroxyl groups, presence of a methoxy group in the B ring and lipophilicity also involves in affecting the bioavailability of flavonoid the Quercetin. Hence they have modified the quercetin structure in two different ways as Q-Cl and Q-OCH₃ by modifying phenolic-OH groups of ring A and ring B. These modifications are done in order to enhance the lipophilicity property of Quercetin compound by replacing one of the phenolic-OH group in ring B is replaced by a -Cl and a -OCH₃ group to get Q-Cl and Q-OCH₃, respectively. Using molecular dynamics (simulation, they have attempted to understand the effect of these groups on the binding of modified quercetin compound (Q-Cl and Q-OCH₃) to the lipid bilayer. And finally these two modified compounds were tested for their antioxidant, anti-inflammatory and anticancer activity. Based on these results and by correlating the bioactivity of Quercetin compound ,they have deduced structural activity relationship of the modified Quercetin compound. (Parul Lakhnpal et al.,2007;).

INTRODUCTION TO INDIAN ONION

The onion, also commonly known as bulb onion , is a vegetable and is the most widely cultivated species of the genus *Allium*. The name "wild onion" is applied to a number of *Allium* species, but among them *Allium cepa* is exclusively known for cultivation. The onion is mostly biennial or a perennial plant. But it is usually treated as an annual plant and harvested in its first growing season. *Allium cepa* is a common vegetable used in older days for treating number of diseases. There are so many varieties of onion are cultivated in the world among which the Indian variety onion has got the more demand in foreign market due to its taste, size and colour. India is the second largest producer of onion in the world next to China and ranks third in export of onion next to Netherland and Spain. Maharastra is the leading onion producing state in India followed by Karnataka and Gujarat.

TYPES OF INDIAN ONION

Onion is grown in many parts of South Indian states, which is one of the major cultivated crop and also a major crop for export.

- South Bellary Onion
- Nasik Bellary Onion
- Shallot / Podisu Onion
- Rose Onion

SOUTH BELLARY ONION

The South Bellary Onions are dark red and round shaped bulb. This variety is suitably cultivated during the early winter season in the state of Karnataka areas such as Chitradurga, Hubli, Dharwad, Challakeri. They mature within 120-130 days with average yield from 7.5-8.5 tons/hectare. This variety of onion is available only during the month of late August to mid of December, this is not to stable for storage.



Figure 2: South Bellary onion

NASIK BELLARY ONION

The Nasik Bellary Onions are dark pink or red and round shaped bulbs. This variety is cultivated in the state of Maharashtra during the late monsoon rainy season. They mature within 110-120 days with average yield of 15-17tons/hectare. This variety of onion is available throughout the year since they are very stable and can be stored or stocked for very long time.



Figure 3: Nasik Bellary onion

SHALLOT/ PODISU ONION

Shallot is bulb shaped with copper, reddish or gray skin. These varieties are mostly cultivated in the state of Tamil Nadu, area such as Tirupur, Coimbatore, Dindugal, etc. They are cultivated during the post monsoon period and harvested during the early winter season. There are two varieties and they are as follows,

- Podisu
- Janda

Podisu onion matures in smaller in size that is it varies from 20-30mm in diameter. Janda onion matures within 110-120 days. It is bigger in size that is 27-35mm in diameter. This variety of onion has the most health benefits and this variety is stable and can store for very long period of time.



Figure 4: Sambar/Podisu onion

ROSE ONION

Rose onion is round shaped with dark purple reddish skin. These varieties are mostly cultivated in the state of Andhra Pradesh and area round it such as chintamani, hosur and bagepalli. They are cultivated during the monsoon and post monsoon period and are harvested during the rainy and the early winter period. Rose onion matures in the 120 days and the size ranges from about 15-30mm in diameter. This variety of onion is also cultivated in the area krishnapuram, Andhra Pradesh. This variety of onion is not used in the local market, they are completely exported and only when there is much demand and high price of other varieties these are used that to especially the cargo sized below 20mm in diameter.

The completely dried cargo can be stored for 6 months in an bamboo basket under air conditioned.



Figure 5: Rose onion

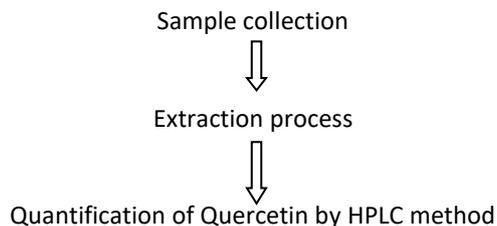
USES OF ONION

- Onions act as a very good source of Vitamin C, B₆, Biotin, Chromium and Calcium. In addition to this, they have got a good amount of Folic acid, Vitamin B₁ and Vitamin K.
- An intake of One hundred grams of onion as cooked or fried along with foods helps in providing 44 calories, mostly as complex carbohydrates, with 1.4gms of fiber.
- Onions contain an enzyme called alliinase. This enzyme is released when an onion is cut or crushed which causes our eyes to water.
- Onion contains flavonoids, which act as pigments in all vegetables and helps in providing colour. These compounds because of their antioxidants properties have a direct effect against tumor. And also helps in enhancing immune properties.
- Due to large amount of sulphur content in onion they are especially good for the liver. Since act as sulphurous food, they can easily mix best with proteins and involves in stimulating the action of the amino acids present in the brain and nervous system.
- Onion is considered as the richest dietary source of quercetin, a potent antioxidant which a class of flavonoids (also found in shallots, yellow and red onions), which is found in the bulb and near the skin. And this compound is particularly linked to the health benefits.

- Quercetin present in onion helps in curing many diseases. It thins the blood, lowers cholesterol, raises good-type HDL cholesterol, and prevents blood clots, fights asthma, chronic bronchitis, hay fever, diabetes, atherosclerosis and infections. And more over it is specifically linked in inhibition of human stomach cancer.
- Quercetin compound helps in anti-inflammatory, antibiotic and antiviral. It also acts as a sedative. It is considered there is no better food source of quercetin than onion skins.
- Eat onion both raw and cooked to get rid of blood clots and to make the most health benefits of onions.
- Amongst cardiologists, prescribing onions to heart patients is a routine thing.
- List of the things that onions can do for our heart: (i) Boost beneficial HDL cholesterol (ii) Thin the blood (iii) Retard blood clotting (iv) Lower total blood cholesterol (v) Lower triglycerides (vi) Lower blood pressure.
- Studies have revealed that the antioxidants present in onions can protect us against cancer. This is done by reducing the DNA damage in cells caused by free radicals.
- All onions and onion relatives (garlic, leeks, chives and scallions or spring onions) are rich in organosulfur compounds. And all these vegetables are proven to have anticancer activity.
- An onion extract was found to destroy tumour cells and so it is considered to be a good antitumor agent. This is analysed by looking into the test tubes and arrest tumour growth when the cells were implanted in rats.
- The onion extract was shown to be unusually nontoxic. This is because a dose as high as 40 times that of the dose is required to kill the tumour cells and its had showed no adverse effect on the host.
- Apart from other onion the shallots have been proven to exhibit effective activity against leukaemia diseases in mice.
- Historically onions have been used to treat asthma. Their action against asthma is due to their property involved in inhibiting the production of compounds that cause the bronchial muscle to spasm and relax.
- Onions have potent antibacterial activity and involves in destroying many pathogens. In fact many disease-causing pathogens such as E. coli and salmonella are destroyed by means of onion extract.
- In onions the flavonoids are actually concentrated in the outer layers than that of the flesh part or bulb portion of onion. To get maximum health benefits its better to peel off as little of the fleshy. Hence than edible portion of onion the onion's outermost paper layer has got more flavonoid content. Even a very small amount of overpeeling of onion layer will result in unwanted loss of flavonoids present in onion. For an example it is said that on overpeeling the red onion can lose about 20 % of its quercetin level and almost 75 % of its anthocyanins.
- It is found that Onions have got a special property like rejuvenation properties. This is quite useful in tissue culture.
- Even onion extract can be well used as a spray in plant cultivation since it act as best antipesticide.
- Onions on regular consumption will leads to good memory power and helps in having strong nervous system.
- Onions act as a best remedy in curing menstrual disorders. Before a few days of the beginning of your menstrual cycle one should eat raw onions to get rid from menstrual disorders.
- One can use onion juice on hair /scalp to get rid of lice and hair fall. This is considered to be one of the most prominent benefits for hair.
- Onions generally contains water, protein, fats, starch, fibres, minerals, calcium, vitamin C, iron, B complex and mainly a rice source of flavonoids. (DR Arpita Sharma et al;).

MATERIAL AND METHOD

FLOW CHART



SAMPLE COLLECTION

A Four different varieties of onion is collected from avadi Market and the four different varieties onion are made into five samples as follows

- Bellary onion.
- South bellary onion.
- Shallot or sambar onion.
- Rose onion.
- Onion peels of four different varieties of Indian onion.

All the above samples are allowed for normal drying process for 15 to 17 days.

SOXHLET EXTRACTION

The dried sample is subjected to soxhlet extraction. The extraction is carried out with 10gms of sample and with 60% of 100ml ethanol. Since Quercetin is insoluble in water ethanol is chosen as solvent for effective extraction process. For each sample the extraction process is carried out for 48 hours. After completion of soxhlet extraction the sample in the thimble is subjected to acid hydrolysis.

ACID HYDROLYSIS

Acid hydrolysis is generally done after extraction process for getting effective extract of the sample. In this process it helps in conversion of rutin to Quercetin. Such that the complete Quercetin content in the sample (present in thimble) is extracted. For this 2.8M of HCL (10ml) and 40% ethanol (40ml) is added and left for five minutes. Then carried out with filtration and finally 0.4gms of ascorbic acid is added for preventing the oxidation of Quercetin.

Finally on rinsing the bottom vessel and acid hydrolyzed vessel with 50 ml, we get 250ml of extract which is later stored in volumetric flask.

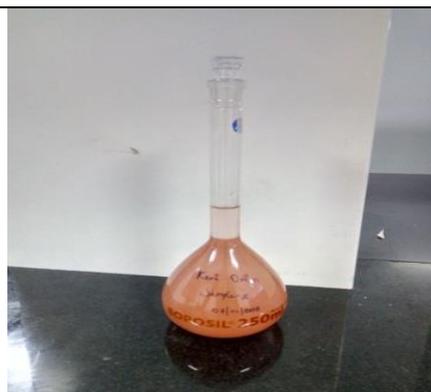
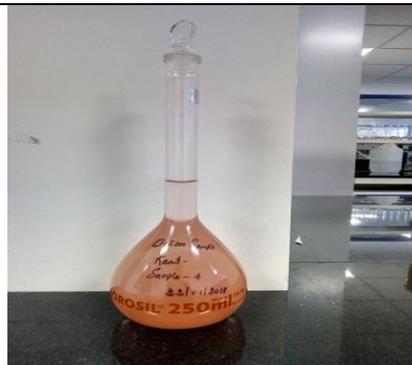
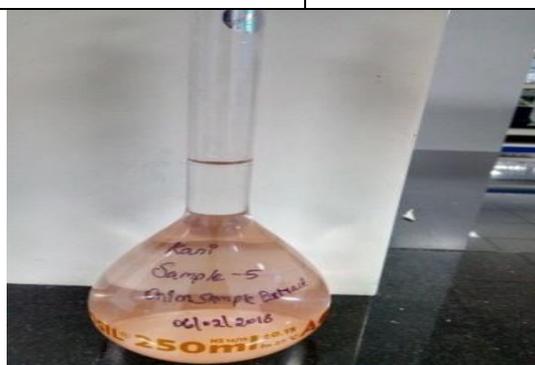
HPLC ANALYSIS

The stored extract is subjected to HPLC analysis for quantifying the amount of Quercetin in five different samples.

- Sample injected: 20 μ l
- Mobile phase: 0.1% of formic acid in methanol
- Stationary phase: C₁₈ Nova pak
- Column flow: 1 μ l/min
- Column temperature: 25^oc

RESULT AND DISCUSSION**EXTRACTION RESULT**

Extraction is a common process in every compound isolation and quantification Soxhlet extraction have been and are at present used as reference when conventional methods based on other principles have to be evaluated. In this context the methods most frequently compared with Soxhlet have been those based on the use of ultrasounds, shaking or stirring and on a combination of both of them. Most of these conventional methods have in common with Soxhlet, the fact that they are time-consuming and require large amount of solvent. On the other hand, they are relatively simple both in performance and fundamentals, so their development does not require specialized personnel. Finally, these methods are cheap, which has favored their widespread use particularly both in industries and routine laboratories.

**Figure 6: Nasik Bellary Onion Extract****Figure 7: South Bellary Onion Extract****Figure 8: Shallot onion Extract****Figure 9: Rose Onion Extract****Figure 10: Onion Peel Extract**

ONION WITH HIGH QUERCETIN LEVEL

It is said that Onions (*Allium cepa* L) had ranked highest in quercetin content in a survey of 28 vegetables and 9 fruits. And it is also found that Quercetin levels tend to be highest in red and yellow onions and the lowest in white onions. Amount of quercetin present in onions varies based on bulb color type and variety of onion (Parul Lakhanpal et al.,2007;).

FOOD	Quercetin mg/100g	Myricetin mg/100g	Kaempferol mg/100g
Broccoli, Raw	2.8	0.0	6.3
Carrots, Raw	0.4	0.0	0.0
Celery, Raw	3.5	----	----
Cocoa powder, Unsweetened	20.1	----	----
Cranberries, Raw	14.0	4.3	0.1
Kale, Raw	5.1	0.0	14.6
Lettuce, Looseleaf, Raw	2.0	0.0	0.0
Lingonberries, Raw	11.3	0.0	0.0
Onions, Raw	22.6	0.0	0.3
Tomatoes, Red ripe, Raw	0.5	0.0	0.1

From the table it is clearly known among all vegetables onion has got the maximum amount of Quercetin in them. There are papers which solidly prove that the amount of Quercetin is high in the outer layer of onion or the onion peel. Similarly on comparing the Quercetin level in five different varieties of Indian onion wastes it is found that the onion peel sample has got the highest amount of Quercetin in them. And the amount of Quercetin present in the onion peel was found to be 402.7 mg/kg.

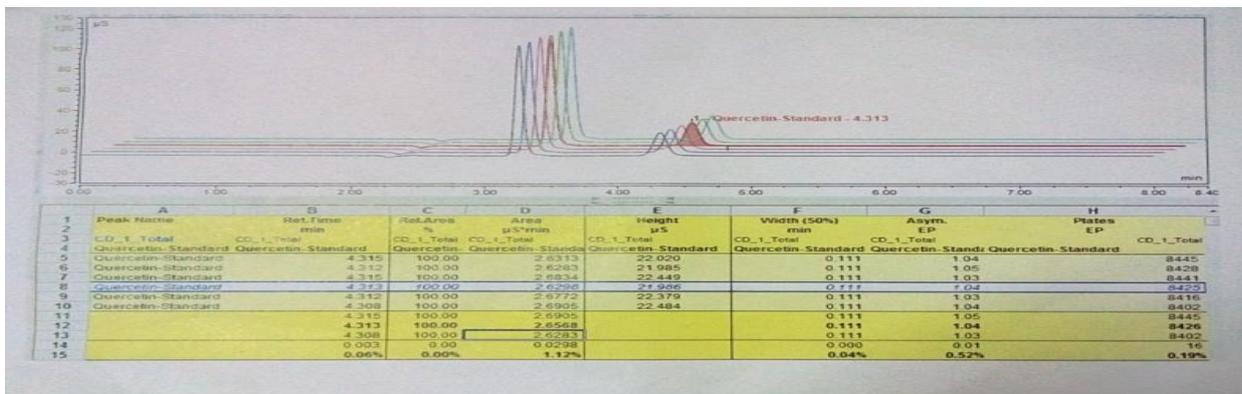


Figure 11: Chromatogram against standard Quercetin

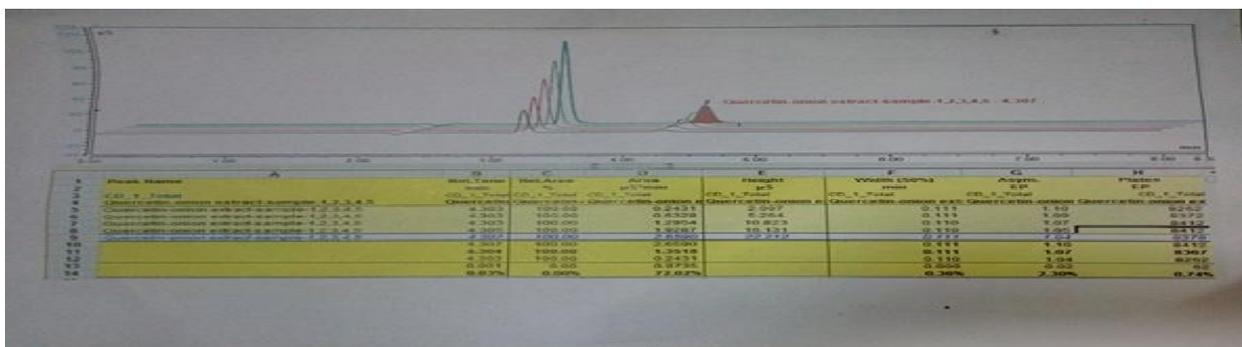


Figure 12: Chromatogram of Quercetin standard with five samples

TABLE SHOWING QUERCETIN AMOUNT IN THE SAMPLES

SAMPLES	AMOUNT OF QUERCETIN(mg/kg)
Sample 1	170.3
Sample 2	227.9
Sample 3	304.15
Sample 4	379.6
Sample 5	402.7

Thus from the result it is clear that Quercetin is present at higher amount in onion peel than onion bulbs. Hence the order of samples with high Quercetin level is as follows

1. Onion peel (sample 5)
2. Rose onion (sample 4)
3. Shallot or sambar onion (sample 3)
4. South bellary onion (sample 2)
5. Nasik bellary onion (sample 1)

CONCLUSION

Quercetin is best antioxidant property and metal ion chelating capacity, it has been reported to be effective compound in inflammation, arteriosclerosis, allergy and swelling. So the present study explores the quantity of active ingredient present in Quercetin contraction in different Onions of the country. It also gives us comparative study of quercetin level in five different varieties of Indian onion wastes and following by sample 1 contain 170.3 mg/kg, sample 2 contains 227.9 mg/kg, sample 3 contains 304.15 mg/kg, sample 4 contains 379.6 mg/kg and sample 5 contains 402.7 mg/kg. This study showed that the best result sample 5 (Onion peel) maximum amount of Quercetin.

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