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# A Comprehensive Study on Antioxidant Activity and Antimicrobial Analysis of Broccoli (*Brassica oleracea)* Juice

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**ABSTRACT**

*In the present investigation the study on broccoli juice was carried out. firstly the sample held for preliminary inspection with respect to acidity (1.06%), pH (6.2),Total phenolic content (270mg/100g), Total caretenoid content (40mg/100g), Total ascorbic acid content (145mg/100g). The antioxidant activity of the compound Sulforaphane was determined by the use of DPPH. The antioxidant activity of Sulforaphane was found to be 83 mol /g FW. Finally the sample evaluated for Antimould and antibacterial analysis of different PPM solutions broccoli juice. Results obtained for the antimould analysis of Sulforaphane show that the 10 PPM concentration of Sulforaphane was found to have the best antimould activity as there was no any mould growth observed in the media containing 10 PPM sample till 10$^{th}$ day of observation. The 10 PPM solution prepared from the extracted compound was found to be the most suitable for the purpose of prevention of mould growth in the products prone to get spoiled by moulds.*

***Key words:*** *Broccoli, Brassica oleracea, Antioxidant Activity, Antimicrobial Analysis Antimould Analysis, Antibacterial Analysis*

## INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Antioxidants are now a day used as a source to cure the different types of cancer. The sulphoraphane is an antioxidant derived from the plant Broccoli. Broccoli is having many useful properties.

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Broccoli is a plant of the mustard/cabbage family Brassicaceae (formerly Cruciferae) given by Stephens, James. "Broccoli — *Brassica oleracea* L. (Italica group)" University of Florida p. 1 Retrieved 2009-05-14 It is classified in the Italica cultivar group of the species *Brassica oleracea*. Broccoli has large flower heads, usually green in color, arranged in a tree-like fashion on branches sprouting from a thick, edible stalk. The mass of flower heads is surrounded by leaves. Many varieties of broccoli are perennial. As per the Murray Michael; Lara Pizzorno *The Encyclopedia of Healing Foods* Simon & Schuster Adult Publishing Group p. 172 ISBN 9780743480529, Broccoli most closely resembles cauliflower, which is a different cultivar group of the same species. Broccoli evolved from a wild cabbage plant on the continent of Europe. Indications point to the vegetable's being known 2,000 years ago. Since the Roman Empire, broccoli has been considered a uniquely valuable food among Italians.

**COMPOSITION OF BROCCOLI**

George Mateljan Foundation 2009, Broccoli is high in vitamins C, K, and A, as well as dietary fiber; it also contains multiple nutrients with potent anti-cancer properties, such as diindolylmethane and small amounts of selenium. As per 2007 "Diindolylmethane Information Resource Center at the University of California, Berkeley A single serving provides more than 30 mg of Vitamin C and a half-cup provides 52 mg of Vitamin C.

**Fig. 1: Broccoli**

"Diindolylmethane Immune Activation Data Center", 2007, The 3,3'-Diindolylmethane found in broccoli is a potent modulator of the innate immune response system with anti-viral, anti-bacterial and anti-cancer activity. Dixon, G.R. Broccoli also contains the compound glucoraphanin, which can be processed into an anti-cancer compoundsulforaphane, though the benefits of broccoli are greatly reduced if the vegetable is boiled more than ten minutes. Clout, Laura 2009A high intake of broccoli has been found to reduce the risk of aggressive prostate cancer.

**Chemistry of sulforafane**

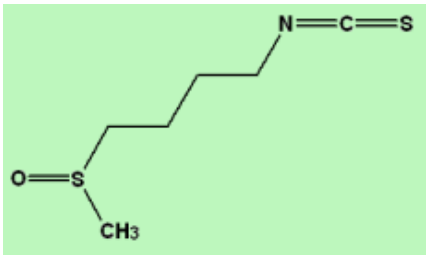


**Fig. 2: Molecular structure of Sulforafane**

Sulforaphane is a phytochemical belonging to the family of isothiocyanates, which means it contains the typical NCS group.

Sulforaphane is an antioxidant and stimulators of natural detoxifying enzymes, it can reduce the risk of breast cancer and prostate cancer.

Epidemiological studies have shown that people who eat a lot of cruciferous vegetables have reduces incidences of cancer. Test with animals have demonstrated that feeding reduced the frequency, size, and number of tumors. During the fight against cancer cells our body produces special enzymes, called phase 2 enzymes. Sulforaphane is a phase 2 enzyme inducer, thereby neutralizing carcinogens before they can damage DNA. Sulforaphane inhibits benzo[a] pyrene-DNA and 1, 6-dinitropyrene-DNA adducts formation. A study by James D. Brooks et al entitled Potent Induction of Phase 2 Enzymes in Human Prostate Cells by Sulforaphane has shown that Sulforaphane induces phase 2 enzyme expression and activity in human prostate cells. This study may help to explain the lower prostate cancer risk with men who consume more cruciferous vegetables.

Fahey JW, Haristoy X, Dolan PM, *et al*. explained that Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo-a-pyrene-induced stomach tumors. Sulforaphane is an organosulfur compound that exhibits anticancer, antidiabetic, and antimicrobial properties. It is obtained from cruciferous vegetables suchas broccoli.

The enzyme myrosinase transforms glucoraph anin, aglucosinolate, into sulforaphane upon damage to the plant (such as from chewing).

**OBJECTIVE**

1. To detect the antioxidant activity of cancer preventing compound Sulforafane from broccoli.
2. To detect the antimicrobial activity of the compound sulforafane.
3. To optimize the extraction process used for the sulforafane extraction.
4. To facilitate the use of broccoli as a nutraceutical.
5. To purify the inducer of vitamins Sulforaphane.

## MATERIAL AND METHODS

**Raw Materials:-**

Broccoli:- The fresh raw broccoli used for the process of extraction were purchased from the local market, Aurangabad. The Broccoli used for the extraction was selected as a ripened one with more no. of sprouts so as to get the maximum yield.

**Chemicals Used:-**

A. Catchol:- To calculate the total phenolic content of the Broccoli as per the procedure given by Ranganna, the catchol pigment required was obtained from the sreya chemicals.
B. 2, 6-diphenol indophenol dye:- The analytical grade dye was made available from the local market.
C. DPPH (diphenylpicrylhydrazyl):-The diphenylpicrylhydrazyl in an appropriate quantity was used for the determination of antioxidant activity.
D. Dimethyl formamide:- It was used to dissolve powdered extract for the final purification by evaporation.
E. Acetonitrile:-Acetonitrile was used as a solvent for the preparation of extract for isolation of Sulforaphane.
F. Potato Dextrose Agar:- The PDA was require for the preparation of the media for the growth of yeast which was required for judging the antimicrobial activity of the Sulforaphane.
G. Nutrient Agar:-The nutrient agar was required for the bacterial growth (i.e. Antibacterial analysis) of the compound Sulforaphane.
H. Others:- The other chemicals like sodium hydroxide, hydrochloric acid etc. required for general tests like acidity of analytical grade were purchased from the local chemical supplier.

## METHODS

**1) Preparation of extract:-**The extract was prepared as per the procedure explained

- The Ripe broccoli was obtained from the local market and was stored at -20$^{o}$ C.
- The stored broccoli was then allowed to get sprouted. The broccoli sprouts were cut into pieces and the broccoli juice was obtained with the double volume of water.
- The juice so obtained was freeze dried at the temperature of around -45$^{o}$C.
- The lyophilization required almost 6 days for the powder formation.
- The lyophilized samples were then -20$^{o}$ C.
- The portions 1800 mg of these powders were taken and were mixed with the 30ml of Acetonitrile and then were extracted for around 6 hrs.
- The extracts so obtained were filtered through the sintered glass funnel. The filtered extracts were then evaporated in rotating evaporator at less than 40 $^{o}$ C.
- The residues obtained were then dissolved in water.
- The different ppm solutions of the compound were obtained.
- The 10,20,30,50,100,200,500 PPM solutions were used for the antimicrobial analysis.

**2) Determination of total phenolic content**

Total soluble phenol in ethanol extracts were determined with Folin-Ciocalteau reagent using catechol as a standard (13). Results were expressed as mg100g-1 wet weight catechol as equivalents.

**3) Determination of ascorbic acid**

- Ascorbic acid was quantitatively determined according to 2, 6-dichlorophenolindophenol dye method.
- The ascorbic acid of fresh samples 10g was extracted by grinding in a suitable medium with a small amount of sand and using 3% met phosphoric acid (v/v) as a protective agent.
- The extract was made up to a volume of 100ml mixed and centrifuged at 3000g for 15 min at room temperature.
- Ten milliliters was titrated against standard 2, 6-dichlorophenolindophenol dye, which was already standardized against standard ascorbic acid. Results were expressed as mg 100g-1 on fresh weight (fw) basis.

**4) Determination of total caretenoid content**

- Total carotenoids mg100g-1 were determined by a modified method of Ranganna (12) using acetone and petroleum ether as extracting solvents and measuring the absorbance at 450nm.
- Two grams of sample was thoroughly crushed and homogenized in mortar pestle with10 ml of 80% ethanol.
- The extract was centrifuged at 10000g for 15 min at 40C. The pellet following were centrifuged and the resulting supernatant was combined with initial extract. Triplicate supernatant extractions were made for each sample.

**5) Determination of antioxidant activity**

- All the apparatus were cleaned by using deionized water and dried in hot air oven.
- All the compounds were weighed and stock solutions were prepared.
- Dilution of standard, test and blank was prepared. methanol was used as a solvent.
- 100 µL DPPH solutions were added to each dilution by using micropipette.
- Absorbance was taken after 5 minute on UV-visible spectrophotometer at wavelength 517 nm.

**6) Antimicrobial analysis**

As per the references given by the handbook of food preservation, Dr. Shefiur Rehman, 1998 the antimicrobial analysis was carried out in the following manner.

**7) Antimould Analysis**

- For antimould and antiyeast activity the potato dextrose agar was prepared by taking 39 gms solids in 1000ml water as indicated by the bottle.
- The Media so formed was sterilized in an autoclave along with the petridishes and other containers at 135 $^{o}$ C for 45 min.
- The media was then poured into the petriplates within an aseptic condition obtained by keeping the 2 spirit lamps on both sides of the media container.

- The streak plate method was then used for the streaking of the different PPM solutions.
- The samples were then kept in an incubator for around 3-4 days and were then analyzed on each day.

**8) Antibacterial analysis**

- Nutrient agar was used for the antibacterial analysis of the extracted compound.
- Nutrient agar was also poured in petridishes by streaking plate method.
- The NA was then provided the different PPM concentrations of Sulforaphane.
- The samples were incubated at 32 $^{o}$ C for around 6 days and each day samples were observed under the digital colony counter for the microbial growth.

**Instruments Used**

**Table 1: Instruments used in Entitled research**

| Sr. No. | Apparatus | Model/ Make | manufacturers |
|---|---|---|---|
| 1 | Ultrasonic Cleaner | UCB40 | Spectra lab, |
| 2 | Vacuum Pump | Rocker 300 | Taiwan |
| 3 | Rotary Vacuum Evaporator | Laborota 400l efficient | Heidolph, |
| 4 | UV- Visible Spectrophotometer | Jasco-530 UV-Vis spectroscopy | Japan |

## RESULTS AND DISCUSSION

**Preliminary Inspection**

The acidity of broccoli juice was estimated with the ascorbic acid. The acidity was found to be 1.06%. The high acidity of broccoli was responsible for the longer shelf life of the product. The pH of the broccoli juice was found to be 6.2.The low pH of juice was responsible for increasing the shelf life of the produce. The total phenolics present in broccoli were estimated by standard procedure and it was found to be 270 mg/100g. The total caretenoid content of the broccoli juice was found to be 40mg/100g. The total ascorbic acid content in the broccoli juice was found to be 145mg/100g.

**Table 2: Preliminary Inspection in broccoli juice**

| Sr. No. | Parameter | Result |
|---|---|---|
| 1 | Acidity | 1.06% |
| 2 | pH | 6.2 |
| 3 | Total phenolic content | 270 mg/100g |
| 4 | Total caretenoid content | 40mg/100g |
| 5 | Total ascorbic acid content | 145mg/100g |

The analysis of above preliminary inspection shows that the low pH value and high acidity of the broccoli juice was responsible for the long shelf-life of the broccoli. Plant breeders and food producers are increasingly identifying specific genotypes and varieties of fruits and vegetables rich in functional ingredients comprising of nutritive and non-nutritive antioxidants. Many studies have demonstrated that cruciferous vegetables contain a wide array of phytochemicals.

The measurable content of vitamin C, Phenolic compounds and the carotenoids showed that the broccoli is the very good source of different nutrients and all these compounds are having the medicinal properties and hence broccoli is the very good nutraceutical.

**Antioxidant activity of Sulforaphane**

The antioxidant activity of the compound Sulforaphane was determined by the use of DPPH. The antioxidant activity of Sulforaphane was found to be 83 mol/g FW The antioxidant activity of Sulforaphane is responsible for its application as a anticancer compound. The antioxidant activity is the very much important property of the Sulforaphane to use it in the prevention of Brest, prostate, lung cancers.

**Antimicrobial Analysis**

Antimicrobial analysis was carried out by using the different PPM solutions of the extracted compound Sulforaphane. The antimicrobial inspection was carried out in 2 stages *viz.* 1. Antimould analysis, 2 Antibacterial analysis The results obtained using the both ways are summarized below:-

**1) Antimould analysis**

The result shown that when the different PPM solutions were used for the analysis following observations were obtained.

**Table 3: Antimould analysis of different PPM solutions broccoli juice**

| CONC. | DAY 1 (C.F.U) | DAY 2 (C.F.U) | DAY 3 (C.F.U) | DAY5 (C.F.U) | DAY 8 (C.F.U) | DAY10 (C.F.U) | DAY15 (C.F.U) | DAY20 (C.F.U) |
|---|---|---|---|---|---|---|---|---|
| **Control** | Nil | Nil | 24 | 35 | 46 | 169 | 1112 | 45666 |
| **10 PPM** | Nil | Nil | Nil | Nil | Nil | Nil | 38 | 57 |
| **20 PPM** | Nil | Nil | Nil | 24 | 36 | 458 | 542 | 1168 |
| **30 PPM** | Nil | Nil | 10 | 35 | 88 | 679 | 5444 | 75755 |
| **50 PPM** | Nil | Nil | Nil | 46 | 64 | 646 | 5745 | 45455 |
| **100PPM** | Nil | Nil | 13 | 76 | 188 | 574 | 5757 | 333585 |
| **500PPM** | Nil | Nil | 35 | 144 | 454 | 1045 | 7678 | 174555 |

The above results obtained for the antimould analysis of Sulforaphane show that the 10 PPM concentration of Sulforaphane was found to have the best antimould activity as there was no any mould growth observed in the media containing 10 PPM sample till $10^{th}$ day of observation.

**2) Antibacterial analysis**

The result shown that when the different PPM solutions were used for the analysis following observations were obtained.

**Table 4: Antibacterial analysis of different PPM solutions broccoli juice**

| CONC. | DAY 1 (C.F.U.) | DAY 2 (C.F.U.) | DAY 3 (C.F.U.) | DAY5 (C.F.U.) | DAY 8 (C.F.U.) | DAY 10 (C.F.U.) | DAY 15 (C.F.U.) | DAY 20 (C.F.U.) |
|---|---|---|---|---|---|---|---|---|
| **Control** | Nil | Nil | 65 | 268 | 1795 | 56788 | 698788 | 97672742 |
| **10 PPM** | Nil | Nil | 29 | 169 | 1575 | 45757 | 576886 | 71747571 |
| **20 PPM** | Nil | Nil | Nil | Nil | Nil | Nil | 156 | 277 |
| **30 PPM** | Nil | Nil | 35 | 156 | 1435 | 35575 | 478787 | 57575762 |
| **50 PPM** | Nil | Nil | Nil | 88 | 1112 | 42885 | 617575 | 67291721 |
| **100PPM** | Nil | Nil | 47 | 139 | 1755 | 35885 | 535757 | 75752167 |
| **500PPM** | Nil | Nil | Nil | 96 | 568 | 56285 | 57575 | 72671276 |

The different PPM samples were analyzed for antimicrobial activity of the anticancer compound Sulforaphane. The antimould and antibacterial activities of the compound were determined. The 10 PPM solution prepared from the extracted compound was found to be the most suitable for the purpose of prevention of mould growth in the products prone to get spoiled by moulds. The mould growth prevention property of the compound Sulforaphane makes it act as a preservative in the food products which get affected by mould. Ex. Jam Jellies etc

Similarly the study was carried out to analyze the samples for the antibacterial activity. The antibacterial activity was also determined in more or less same manner. The result obtained had shown that the 20 PPM solution of Sulforaphane when added to the media it showed no signs of bacteria for the longer period of time. The antibacterial activity of this compound helps to retain the vegetable for long period of time. The property also was facilitating the use of Sulforaphane as a preservative in the food products prone to the bacterial spoilage.

## CONCLUSION

Broccoli is high in vitamins C, K, and A, as well as dietary fiber; it also contains multiple nutrients with potent anti-cancer properties, such as diindolylmethane and small amounts of selenium. This benefits we can prolonged by maintaining the 10 PPM of broccoli juice. The commercialization of production of Broccoli juice may have great market potential in future.

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