# ANTIMICROBIAL, ANTIOXIDANT AND CHEMOPREVENTIVE POTENTIAL OF VITAMIN C RICH FRUITS

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**ABSTRACT:** The aqueous and methanolic extracts of Carambola, Guava. Kiwi, Papaya and Strawberry were evaluated for their antimicrobial, antioxidant and chemopreventive potential. The in vitro antimicrobial activity was performed by Agar well diffusion method Muller Hinton Agar medium against *E. coli*. and all the extracts showed significant antimicrobial activity. MIC values against E. coli. were also calculated by macrobroth dilution assay. The activity may be due to the high phenolic content in the fruits. Antioxidant capacity and chemopreventive ability of these extracts was also assayed in which Kiwi fruit was best among all the selected fruits. Natural compounds from these fruits can be further extracted and used as an alternative to synthetic drugs.

**Key words:** fruits, antimicrobial activity, antioxidant capacity, MTT Assay.

## INTRODUCTION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman T.A., 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, R.E. 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds.

Vitamin C is considered to be one of the most prevalent antioxidative components of fruit and vegetables, and it could exert chemopreventive effects without apparent toxicity. It has also been used as a dietary supplement intended to prevent oxidative stress–mediated chronic diseases such as cancer, cardiovascular disease (Khaw K. T., 2001), hypertension (Duffy S.J., 1999), stroke (Kurl S., 2002), and neurodegenerative disorder (Engelhart M.J., 2002). Although it has generally been acknowledged that vitamin C protects cells from oxidative DNA damage, thereby blocking the initiation of carcinogenesis, some studies have shown that dietary vitamin C supplementation is not beneficial but, rather, may cause DNA damage. These findings suggest that dietary components other than vitamin C may play an important role in cancer prevention. Moreover, the chemopreventive mechanism of vitamin C may be linked to the inhibition of other processes- in particular, tumor promotion- rather than to that of tumor initiation. Now-a-days natural products of plants are being treated as replacement of synthetic agents in nearly all filed of medicine. Antimicrobial and antifungal agents are also a part of this. The demand of natural food additives is increasing day and day. Natural products from plants can be a better option for the replacement of synthetic antimicrobial and chemopreventive agents.

In this article we are going to assay antimicrobial, antioxidant and chemopreventive potential of vitamin C rich fruit extracts.

## MATERIALS AND METHODS

**Standard and reagents:** Casein enzymic hydrolysate, yeast extract, sodium chloride, agar, gentamicine, distilled water, methanol, gallic acid, sodium carbonate, folin-ciocalteu reagent, sulphuric acid, sodium phosphate, ammonium molybdate, 3-(4,5-dimethyl thiazole-2yl)2,5 diphenyl tetrazolium bromide, dimethyl suphoxide, HeLa cell line.

**Extract preparation:**

Fruits were brought from local market. For compounds extraction, a fine powder (20 mesh) of samples (3g) was extracted using 50 ml of methanol and distilled water at 25ºC. The extracts were filtered through whatman no. 1 filter paper and evaporated in rotary vaccum evaporator to dryness. All the samples were redissolved in water at concentration of 20 mg/ml.

**Determination of antimicrobial activity:**

**Agar well diffusion method**

The antimicrobial activity was assayed by a modification of the agar diffusion method (Kirby-Bauer). In brief the bacterial culture was grown in LB medium at 37 °C. After 6 h of growth and at a concentration of $10^6$cells/ml, it was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, 100 µL of extracts were used in 8 mm wells for antimicrobial assay. The plates were incubated at 37 °C for 24 h. After this period, it was possible to observe inhibition zone. Overall, cultured bacteria with halos equal to or greater than 8 mm were considered susceptible to either the tested extract. Water was taken as negative control and Gentamicin was taken as positive control.

**Minimum inhibitory concentration**

For this analytical test LB broth is prepared. For preparing 500ml of media it requires 5gm bacto tryptone, 2.5gm bacto yeast extract, 5gm sodium chloride. The contents are mixed properly in distilled water and volume is raised to 500ml by adding more distilled water to it. The pH is maintained to 7.2-7.5. The media is sterilized and inoculated with *E. coli* culture. When the inoculated culture media is ready after 24 hrs 2 ml of culture media is transferred into test tube ($C_1$) and 1ml fruit extract is added to it. Now take 1ml media in $C_2$ test tube and add 1ml distilled water and 1ml from $C_1$ test tube. Likewise prepare 6 test tubes and label them as $C_1$, $C_2$, $C_3$.....$C_6$. Now incubate these test tubes in incubator for 24 hrs and note the absorbance at 540nm.

**Determination of antioxidant activity**

**Determination of total phenolic content**

Total phenolic content of methanolic and aqueous extract was determined by the method of Singleton and Rossi (1965) and then expressed as microgram/gram Gallic acid equivalents. In brief, a 100 µL-aliquot of the samples was added to 2mL of 0.2% (w/v) Na2CO3 solution. After 2 minutes of incubation, 100 µL of 500 mL/L Folin–Ciocalteu reagent was added and the mixture was then allowed to stand for 30 minutes at 25◦C. The absorbance was measured at 620 nm using a spectrophotometer (UV-2802, Unico Co. Ltd, Shanghai, China). The blank consisted of all reagents and solvents but without the sample.

**Determination of total antioxidant activity**

Total antioxidant capacities were determined by the method of Pan et al., (2008). An aliquot (0.1mL) of the fractions was combined with 1mL of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate).

The tubes were capped and incubated at 95◦C for 90 minutes. After the samples were cooled to 25◦C, the absorbance was measured at 620nm against blank. The blank contained 1mL of reagent solution without the sample. The total antioxidant activity was expressed as the absorbance value at 620 nm. The higher absorbance value indicates the greater antioxidant activity.

### Determination of chemopreventive activity

### MTT Assay

Seed the cell (100µl Hela cell line)in 96-well microtiter plate row A, B, C to all 12 wells in each row with 100 µl of extract. Now incubate the plate at 37°C under 5% CO2 for 3 days. After then remove media and add 100µl of 0.1mg/ml MTT. Now incubate it for 4 hrs at 37°C. Then remove MTT and add 100µl of DMSO and shake it gently now take the reading at 492nm with ELISA reader. Control wells received only the media without the tested samples.

## RESULTS AND DISCUSSION

**Determination of antimicrobial activity by agar well diffusion:** The antimicrobial activity of selected fruits extracts shows positive result against *E. coli* strain. The methanolic extract shows better inhibition as compared to aqueous extract. The maximum inhibition is shown by kiwi extract in both methanolic (36mm) as well as in aqueous extract (29mm), also low but significant value is obtained by guava, it shows an inhibition zone of 21mm & 20mm in methanolic and aqueous extracts respectively. All the extract has exhibited better results as compared to positive control (1.4 cm). The result of antimicrobial activity is shown in Table-1

**Table-1: Antimicrobial activity of Methanolic Extracts**

| S.NO | Sample | Inhibition zone in cm(mean± standard deviation) | |
|---|---|---|---|
| | | Aqueous extract | Methanolic extract |
| 1. | Carambola | 2.2±0.1 | 2.4±0.1 |
| 2. | Guava | 2.0±0.1 | 2.1±0.1 |
| 3. | Kiwi | 2.9±0.1 | 3.6±0.1 |
| 4. | Papaya | 2.7±0.15 | 2.7±0.1 |
| 5. | Strawberry | 2.5±0.1 | 2.6±0.05 |

**Determination of minimum inhibitory concentration:** Macro broth Dilution Assay was done for evaluation of Minimum Inhibitory Concentration of the extracts and the results of macrobroth dilution assay are shown in figure-1 & figure-2 MIC values of all extracts against *E. coli* are tabulated in table 2. Methanolic extracts were found to be more effective than aqueous ones. Kiwi fruit (methanolic extract: 0.3125mg/ml; aqueous extract: 0.625 mg/ml) was the most effective among the selected fruits whereas guava (methanolic extract: 5.0mg/ml; aqueous extract: 10.0 mg/ml) showed least yet significant value of MIC.

**Table -2: MIC value of Aqueous and Methanolic Extracts**

| S.NO | Sample | MIC (mg/ml) of Aqueous Extract | MIC (mg/ml) of Methanolic Extract |
|---|---|---|---|
| 1. | Carambola | 5mg/ml | 2.5mg/ml |
| 2. | Guava | 10mg/ml | 5mg/ml |
| 3. | Kiwi | 0.625mg/ml | 0.312mg/ml |
| 4. | Papaya | 2.5mg/ml | 5mg/ml |
| 5. | Strawberry | 2.5mg/ml | 5mg/ml |

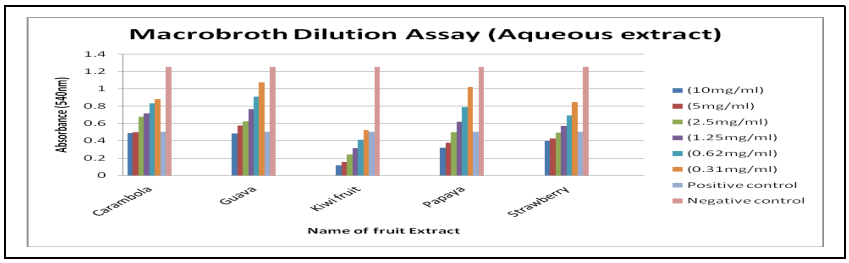


**Figure-1: Macrobroth dilution assay for MIC (Aqueous Extract).**

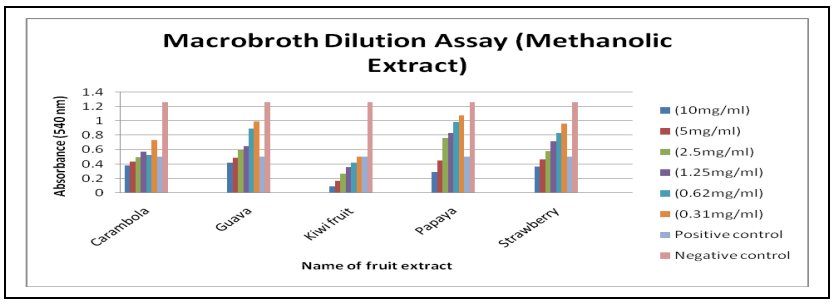


**Figure-2: Macrobroth dilution assay for MIC (Methanolic Extract).**

**Total phenolic content:** Results for phenolic content has been tabulated in table 3. Kiwi exhibits the maximum phenolic content in both type of extract (methanolic & aqueous) among tested species of fruits. The absorbance of aqueous extract of carambola, guava, kiwi, papaya, and strawberry at 620nm is 0.375, 0.454, 0.785, 0.126, and 0.144 respectively and methanolic extract of carambola, guava, kiwi, papaya, and strawberry at 620nm is 0.523, 0.198, 0.805, 0.083 and 0.292 against blank (0.043) at same absorbance value. The value compared to the blank shows that all extracts shows the positive results and has sufficient phenolic content in them. The maximum absorbance is shown by kiwi in methanolic & aqueous and least absorbance is shown by papaya in methanolic & aqueous.

**Total antioxidant activity:** the total antioxidant capacity of prepared extracts both methanolic & aqueous was measured by spectrophotometer at 620nm. A high absorbance value of the sample indicates its strong antioxidant activity. The maximum absorbance is reported by kiwi in methanolic and aqueous is 3.984 & 4.190 respectively whereas the least value is attain by papaya in methanolic (0.437) & aqueous (0.269). The blank absorbance is 0.073 at 620nm, this signifies that all the prepared extracts have a good antioxidant potential.

**Table -3: Phenolic Content of Aqueous and Methanolic Extracts.**

| S.NO | Sample | Absorbance at 620 nm (*mean± standard deviation)* | |
|---|---|---|---|
| | | Aqueous Extract | Methanolic Extract |
| 1. | Carambola | 0.375±0.023 | 0.523±0.006 |
| 2. | Guava | 0.454±0.005 | 0.198±0.003 |
| 3. | Kiwi | 0.785±0.006 | 0.805±0.011 |
| 4. | Papaya | 0.126±0.004 | 0.083±0.008 |
| 5. | Strawberry | 0.144±0.004 | 0.292±0.004 |

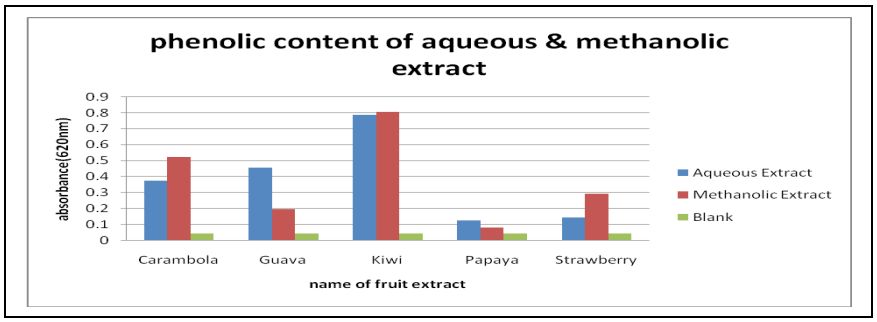


**Figure-3: Phenolic content of Aqueous & Methanolic extract**

**.Table-4: Antioxidant Activity of Aqueous and Methanolic Extracts.**

| S.NO | Sample | Absorbance at 620 nm(*mean± standard deviation)* | |
|---|---|---|---|
| | | **Aqueous Extract** | **Methanolic Extract** |
| **1.** | Carambola | 1.925±0.005 | 2.579±0.006 |
| **2.** | Guava | 4.075±0.022 | 0.952±0.012 |
| **3.** | Kiwi | 4.190±0.037 | 3.984±0.014 |
| **4.** | Papaya | 0.269±0.003 | 0.437±0.003 |
| **5.** | Strawberry | 1.973±0.011 | 0.968±0.004 |

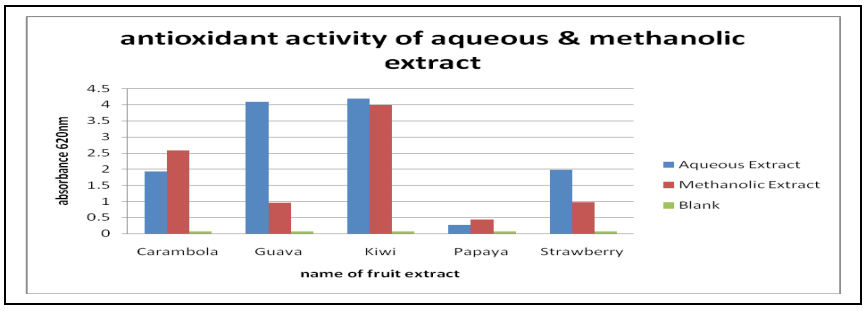


**Figure-4: Antioxidant activity of Aqueous & Methanolic Extracts.**

**Anticancerous activity:** The anticancerous activity of methanolic and aqueous extract of fruits were investigated using an 3-(4,5-dimethylthiazole-2yl)-2,5-diphenyl tetrazolium bromide assay on Hela cell line. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan and the amount of formazan is directly proportional to the number of viable cell. The anticancer activity of both type of extract is presented in table-5. The absorbance of aqueous extract of carambola, guava, kiwi, papaya, and strawberry at 492nm is 0.547, 0.779, 0.332, 0.302, and 0.308 respectively and methanolic extract of carambola, guava, kiwi, papaya, and strawberry at 492nm is 0.547, 0.779, 0.332, 0.302 and 0.308. The value of cell control is 0.573 at 492nm. The positive value among aqueous extract is shown by all extract except guava and among methanolic extract too. The Polyphenolic compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells.

**Table-5: MTT Assay of Aqueous and Methanolic Extracts.**

| S.NO | aqueous extract | Absorbance at 492 nm(*mean± standard deviation)* | |
|---|---|---|---|
| | | Aqueous Extract | Methanolic Extract |
| **1.** | Carambola | 0.547±0.050 | 1.281±0.025 |
| **2.** | Guava | 0.779±0.008 | 1.016±0.060 |
| **3.** | Kiwi | 0.332±0.026 | 0.295±0.015 |
| **4.** | Papaya | 0.302±0.079 | 0.663±0.122 |
| **5.** | Strawberry | 0.308±0.004 | 0.793±0.063 |

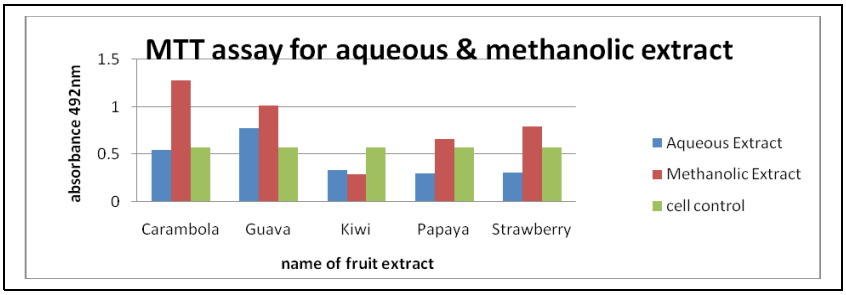


**Figure-5: MTT Assay of Aqueous & Methanolic Extracts.**

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