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# Identification and Characterization of the Antimicrobial and Active Components of Tea (*Camellia Sinensis*)

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***Abstract:*** *Four main types of processed tea samples (white, green, oolong and black tea) are collected from Badamtam tea garden of Darjeeling under Goodricke. White tea is only withered, Green tea is non-fermented, Oolong tea is partly fermented, and Black tea is fully fermented. Green and white tea contains catechins as main polyphenols which are responsible for the major antioxidant activity. After collection of these tea samples, various biochemical and microbiological assays were performed like the - Antioxidants potential assay, Total flavonoids assay, Antimicrobial assay (using different bacterial samples). Identification of active metabolites (mainly responsible for antioxidant and aroma like rutin and quercetin) in different solvents were done like Aqueous Extracts, DMSO extracts, Acetone extracts, Methanolic and Ethanolic extracts. Rutin and Quercetin are flavonoid group of compounds found in tea. Rutin is quercetin glycoside and by the action of beta D glucosidase as rutin by series of reactions; flavonol like quercetin is produced which are responsible for tea aroma and antioxidant capacity. So, to identify and characterize them for a phytochemical drug designing, it is necessary to find out in which solvent the active component is best extracted out to manifest antimicrobial and antioxidant potential.*

*Aim of this paper is to find out the comparison of antimicrobial and antioxidant capacities of four different types of tea according to manufacture difference (White, Green, Black and Oolong), and to differentiate between fresh leaves and manufactured of the same garden (Runlee Rungliot) . Our aim is also to screen the causative active metabolites which is responsible to show such activities and to check the presence or absence of the active components on different solvents as well.*

***Keywords:*** *- Tea, Antimicrobial, Antioxidant, Rutin, Quercetin, Flavonoid, Polyphenol*

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## I. Introduction:

Tea is the most preferred drink in the world after water which is under extensive research in recent years due to its fascinating chemical composition. There are four main types of tea which can be produced based on how the leaves of tea plants are processed: White tea is only withered, green tea is nonfermented, Oolong tea is partly fermented, and Black tea is fully fermented (Saha et al., 2018). Green and white tea contains catechins as main polyphenols and black tea contains tannins as polyphenols.

Rutin and quercetin they are flavonoid group of compounds found in tea. Rutin is quercetin glycoside. By the action of beta D Glucosidase enzyme from flavonoid glycoside as rutin by series of reactions flavanol like quercetin is produced which are responsible for tea aroma and antioxidant capacity (Bhattacharya and Sen-Mandi). Both of them possess antimicrobial activities as well (Shyam Choudhury et al., 2015). Different solvents are able to extract all those active metabolites in different aspects. So, to identify and characterize them for phytochemical drug designing it is necessary to find out in which solvent the active component is best extracted out to manifest antioxidant and antimicrobial potential.

Aim of our paper is to find out the comparison of antimicrobial and antioxidant capacities of four different types of tea according to manufacture difference (white, green, oolong and black), and to differentiate between fresh leaves and manufactured tea of same garden. Our aim is also to screen the causative active metabolite which is responsible to show antimicrobial and antioxidant. The main target is to find out the best solvent where active components can be isolated well.

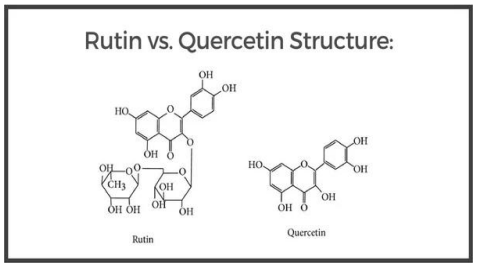



**Fig A:** The structure of Rutin and Quercetin

## II. Materials and Methods:

**Samples taken**:

White tea, black tea, green tea, oolong tea were taken from Badamtam garden of Goodricke, Pvt. Ltd. and fresh leaves and corresponding manufactured black tea was collected from Runglee Rungliot garden.


**Fig B:** Rungli Rungliot Tea garden

- **Preparation of tea extracts:**

All the tea extracts were made in 5 different solvents (Water, Methanol, Acetone, Ethanol, DMSO) taking 500 mg of leaves from each sample in 20 ml of the solvent.

- **Antioxidant capacity:**

Detected with 0.4% methanolic solution of DPPH with reduction at 517nm wavelength according to Ribeiro et al., 2002

- **Antimicrobial activity:**

The zone of inhibition was measured according to Radji et al.,2013 against *Klebsiella, Bacillus* sp., *Lactobacillus* sp. and *Staphylococcus* sp..

- **Flavonoid content:**

Flavonoid content was measured at 420 nm wavelength according to Zhisen et al, 1999 by using

- **Rutin and Quercetin content:**

Rutin and Quercetin content was measured at 430 nm and 405 nm respectively according to Mir et al., 2013. Stock solution was prepared in acid-ethanol as 0.2 % (w/v) rutin in 55% (v/v) ethanol and 5 % (v/v) acetic acid in water. A separate solution was prepared in methanol as 0.2 % (w/v), and diluted in methanol as and when required. Quercetin: Stock solution was prepared in acetic acid-ethanol as 0.2 % (w/v) quercetin in 55% (v/v) ethanol and 5 % (v/v) acetic acid in water.

## III. Result and discussion

**The distinction in antioxidant potential in different solvents:**

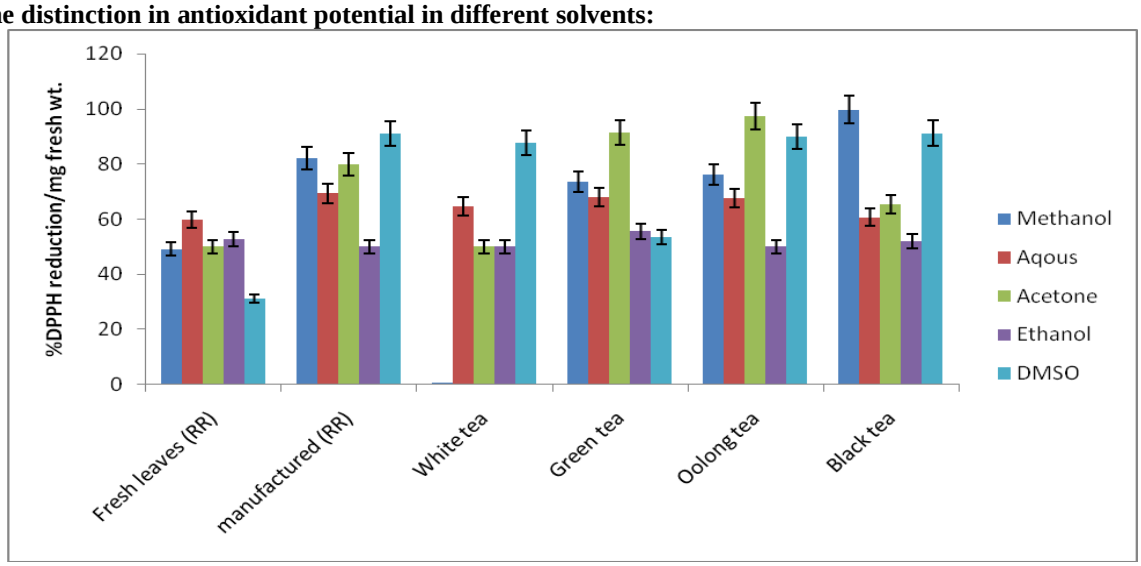



**Fig 1. Total antioxidant potential**

The results show that the fresh leaves has lower antioxidant capacities in all the solvents fresh leaves show lesser antioxidant action. Among all the solvents DMSO extracts are usually showing the best performance. The trend indicates the higher antioxidant activities in green and oolong tea in acetone extracts.

**Total flavonoid content:**

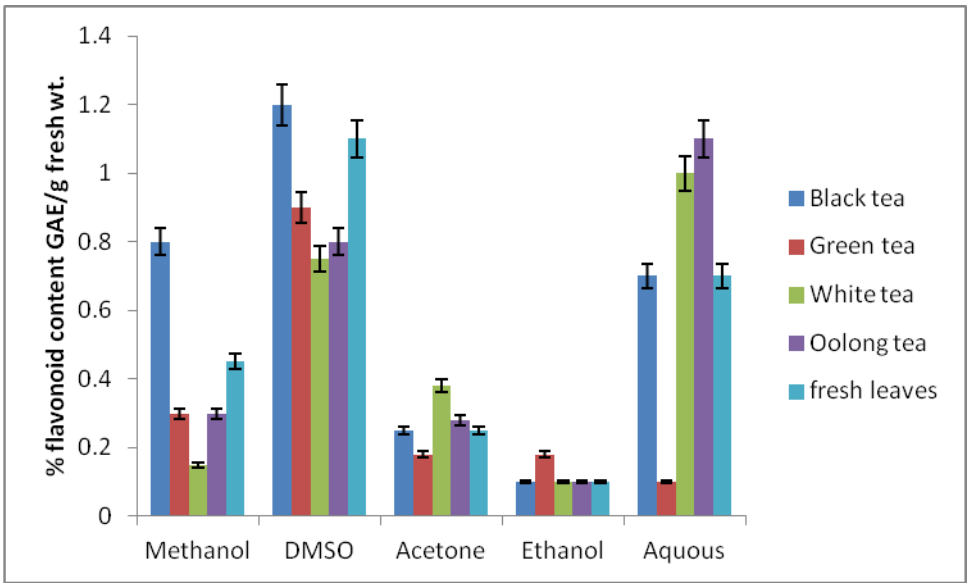



**Fig 2a. Total flavonoid content of all tea varieties**

It shows that the higher content of flavonoids are extracted in DMSO solvent. Among all black, fresh leaves is showing higher content. But in acetone least amount of flavonoids are extracted.

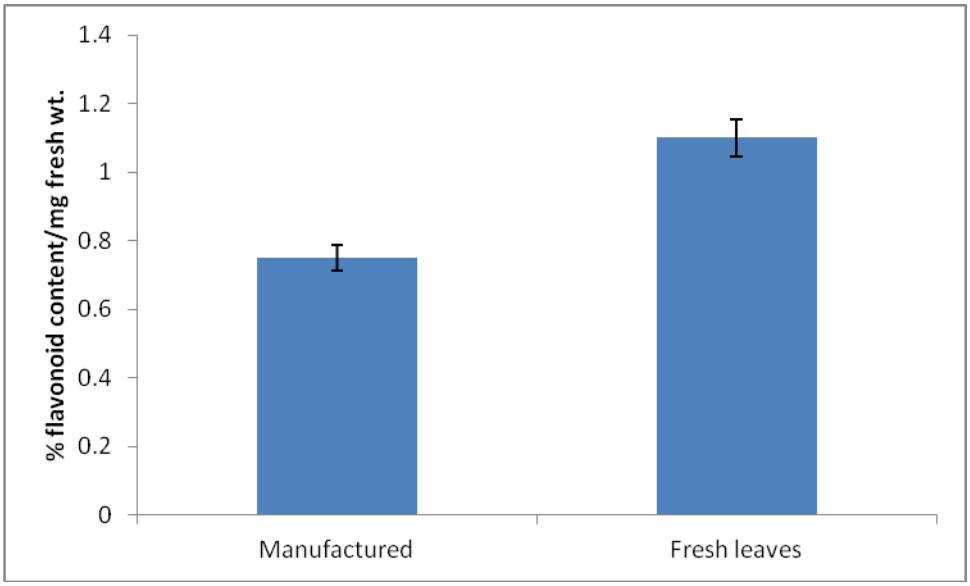



**Fig 2b. The flavonoid content of Runglee Rungliot fresh and manufactured leaves**

The manufactured leaves contains lesser amount of flavonoids than the fresh leaves of same garden (Runglee Rungliot)

**Rutin and Quercetin content:**

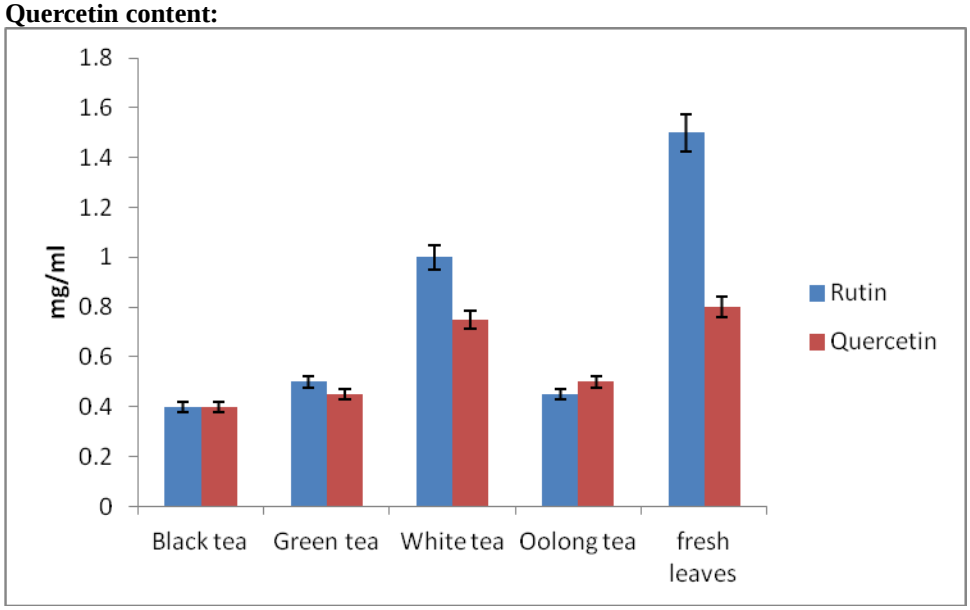
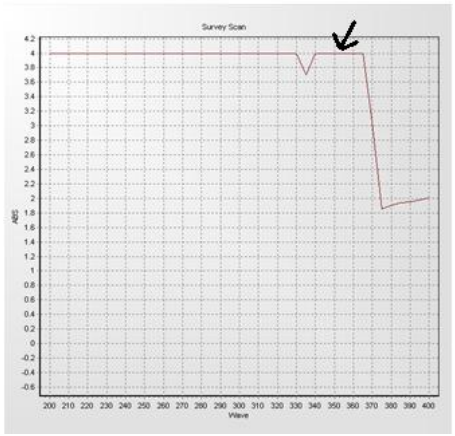
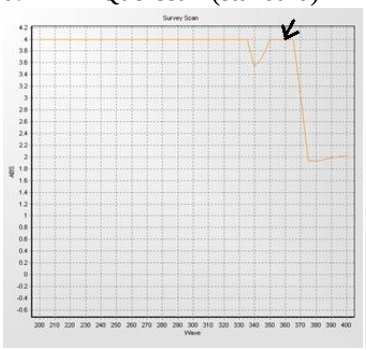



**Fig 3. The rutin and quercetin content of methanolic extracts**

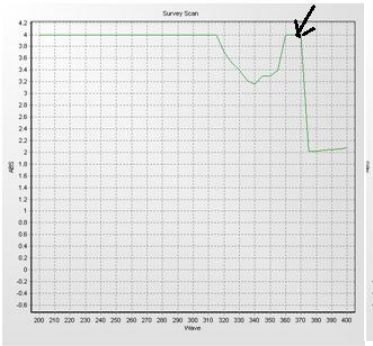
The levels of quercetin and rutin are highest in fresh tea leaves (Runglee Runliot)and the trend for quercetin content is like White tea > green tea> oolong tea> black tea. In case of rutin content the trend is similar.



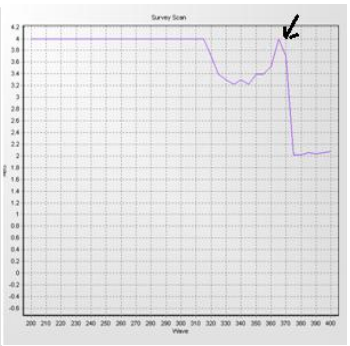
a. Quercetin (standard)



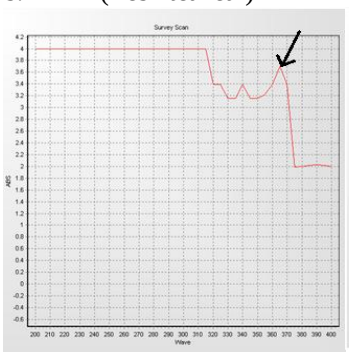
b. (fresh tea leaf)



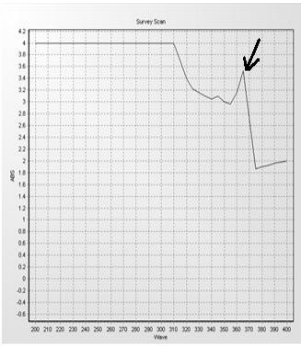
c. White tea



d. Green tea



e. Oolong tea



f. Black tea

**Fig 4:** UV spectral scan of different tea samples (b-f)between 200-400nm to show characteristics peak of Quercetin with resoect to standard Quercetin (a)

UV Spectral scan shows that quercetin (in methanolic solution) having absorption within (330-380nm) with respect to standard, is higher in content in fresh leaves then white tea, then green, then oolong and at last black tea.

**Antimicrobial activities in different solvents against different microorganisms**

**Table 1: Antimicrobial activities of Methanolic Extracts (70% methanol used)-Control: 70% methanol**

| | **Black Tea** | **White Tea** | **Green Tea** | **Oolong Tea** | **Fresh Tea (RR)** | **Manufactured Tea (RR)** | **Rutin (0.2%)** | **Quercetin (0.2%)** | **Control** |
|---|---|---|---|---|---|---|---|---|---|
| ***Bacillus sp.*** | 1.1cm | 1.45 cm | 1.3 cm | 1.2 cm | 1 cm | 1.3 cm | 0.8 cm | 0.7 cm | - |
| ***Klebsiella sp.*** | 1.1 cm | 1.35 cm | 1.2 cm | 1.25 cm | 1.15 cm | 1.2 cm | - | 1.0 cm | - |
| ***Staphylococcus sp.*** | 1.9 cm | 1.65 cm | 1.9 cm | 1.3 cm | 1.4 cm | 1.6 cm | 0.7 cm | 1.4 cm | - |
| ***Lactobacillus sp.*** | - | 1.2 cm | 1.4 cm | 1.25 cm | 1.15 cm | 1.4 cm | | | - |

The 70% methanolic extracts of White tea showed highest zone of inhibition against *Staphylococcus sp* (1.65 cm). Standard Rutin and Quercetin shows lower zone of inhibition than tea samples.

**Table 2: Antimicrobial activities of Ethanolic Tea Extract (95% ethanol used) -Control: 95% ethanol**

| | Black Tea | White Tea | Green Tea | Oolong Tea | Fresh Tea (RR) | Manufactured Tea (RR) | Control |
|---|---|---|---|---|---|---|---|
| ***Bacillus cereus*** | 2.0 cm | 1.85 cm | 1.8 cm | 2.2 cm | 2.1 cm | 1.2 cm | 1.0 cm |
| ***Klebsiella sp.*** | 1.65 cm | 2.05 cm | 1.72 cm | 1.675 cm | 1.55 cm | 1.25 cm | 1.1 cm |
| ***Staphylococcus sp*** | 2.0 cm | 2.0 cm | 1.6 cm | 1.9 cm | 1.9 cm | 2.0 cm | 1.7 cm |
| ***Lactobacillus*** | 1.7 cm | 1.7 cm | 1.85 cm | 1.85 cm | 1.6 cm | 1.9 cm | 1.4 cm |

95% ethanolic extract of white tea against *Klebsiella* sp. Shows highest zone of inhibition 2.05cm.

**Table 3: Antimicrobial activities of Acetonic Tea extract (15% acetone used)-Control: 15% acetone**

| | Black Tea | White Tea | Green Tea | Oolong Tea | Fresh Tea (RR) | Manufactured Tea (RR) | Control |
|---|---|---|---|---|---|---|---|
| ***Bacillus cereus*** | 0.7 cm | 1.3 cm | 1.35 cm | - | 1.0 cm | 0.7 cm | - |
| ***Klebsiella sp.*** | 0.8 cm | 1.0 cm | 1.1 cm | - | 0.9 cm | 0.7 cm | - |
| ***Staphylococcus sp.*** | 0.9 cm | 1.3 cm | 1.2 cm | - | 0.5 cm | - | - |
| ***Lactobacillus sp.*** | 1.2 cm | 0.9 cm | 1.0 cm | 1.2 cm | 1.2 cm | 1.2 cm | - |

The highest zone of inhibition is found against *Bacillus cereus* with Green tea 15% acetone extract .

**Table 4: Antimicrobial activities of DMSO Tea Extract (15% DMSO used)-Control: 15% DMSO**

| | Black Tea | White Tea | Green Tea | Oolong Tea | Fresh Tea (RR) | Manufactured Tea (RR) | Control |
|---|---|---|---|---|---|---|---|
| ***Bacillus cereus*** | 1.05 cm | 1.1 cm | 1.1 cm | 1.15 cm | 1.0 cm | 1.1 cm | - |
| ***Klebsiella sp.*** | 1.1 cm | 1.05 cm | 0.7 cm | 1.2 cm | 0.7 cm | 1.0 cm | - |
| ***Staphylococcus sp.*** | 2.6 cm | 2.7 cm | 2.65 cm | 2.5 cm | 2.3 cm | 2.8 cm | - |
| ***Lactobacillus sp.*** | 1.3 cm | 1.1 cm | 1.35 cm | 1.3 cm | 1.2 cm | 1.5 cm | - |

The highest zone of inhibition is found in case of Manufactured Black Ranglee Rungliot variety against *Staphylococcus sp*. That is 2.8cm


**Fig 5.** Antimicrobial activities in acetone extract

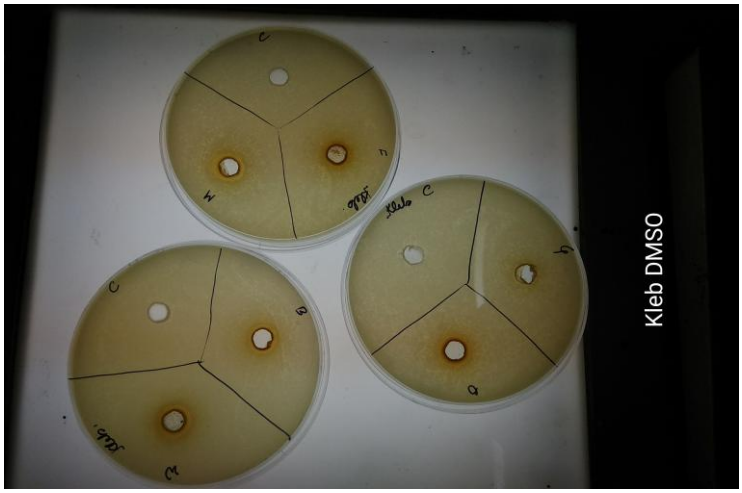



**Fig 6.** Antimicrobial activities in DMSO extract

The general trend shows Ethanolic>methanolic>DMSO>acetone extracts show antimicrobial activities.

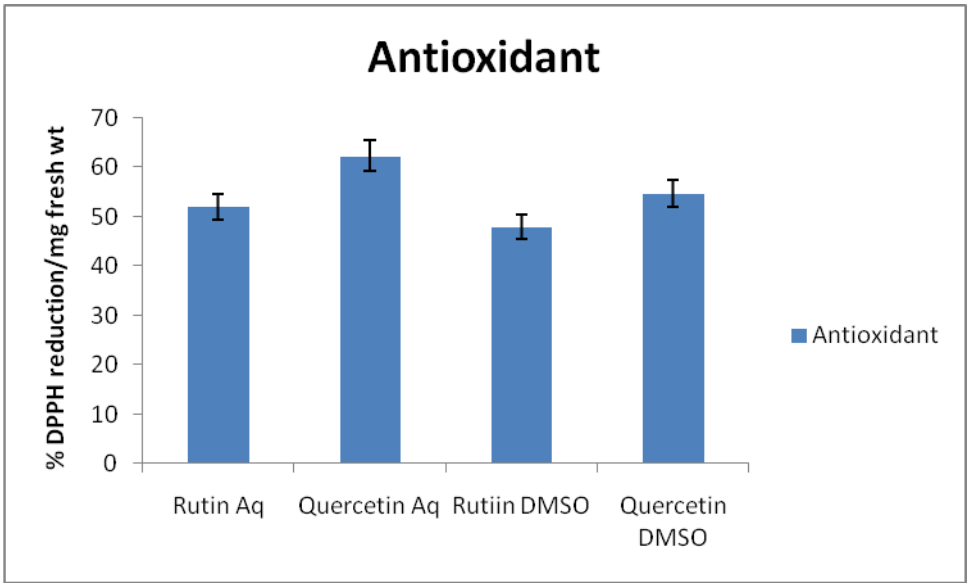



**Fig 7. Antioxidant capacity of Rutin and Quercetin in different solvents**

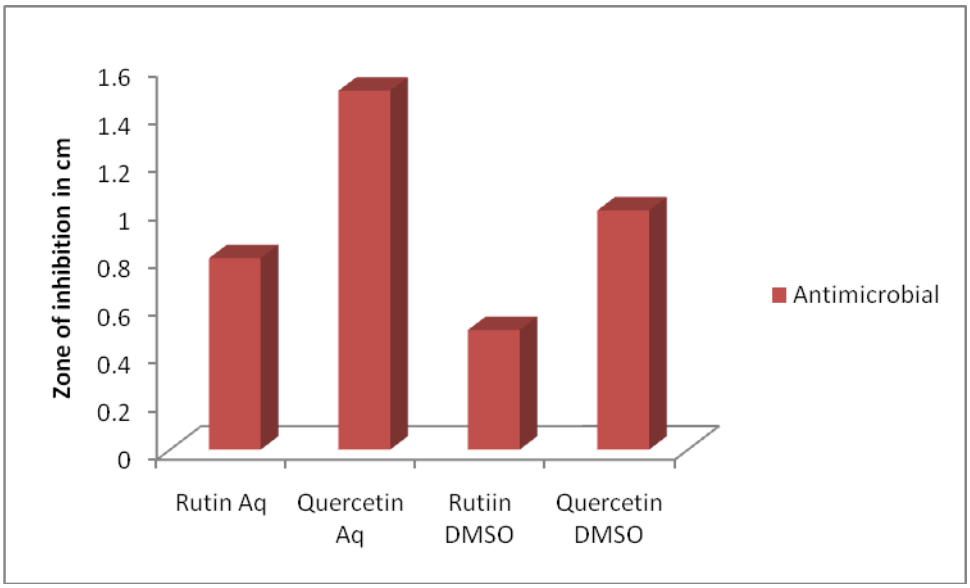



**Fig 8: The antimicrobial capacity of Rutin and Quercetin in different solvents**

The antioxidant and antimicrobial action of quercetin is higher in aqueous solution.
Abouzeed et al., 2018 had also shown the rutin and quercetin antioxidant and antimicrobial activities.

## IV. Conclusion:

So, the quercetin is the active component present in different amounts in different tea which is the responsible candidate to show antioxidant and antimicrobial activity. Thus in different tea varieties like white, green , oolong and black tea quercetin and rutin are present in different amount –both of them are responsible to show antioxidant and antimicrobial activities-so during manufacturing process the content of such active metabolites are getting changed. And it is pertinent to mention here that the content varies from fresh tea (Runglee Rungliot) to the same manufactured one.

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