https://doi.org/10.26651/allelo.j/2024-61-1-1472 0971-4693/94 Euro 20.00
Allelopathy Journal 61 (1): 97-104 (January, 2024) *International Allelopathy Foundation* 2024
Table: 5, Figs: 3

Camellia Sinensis flavonoids potential to Combat Ovarian Cancer

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(Received in revised form: September 15, 2023)

ABSTRACT

Nowadays, *Camellia sinensis* (L.) O. Kuntze is used as a health drink as well as a medication worldwide. Quercetin, kaempferol, and myricetin are different flavonoids present in it. Therefore, it was decided to extract and isolate the flavonoid content of *Camellia sinensis* with an evaluation of their anticancer activity. In the methodology, the extraction of the dried leaves was performed with the help of the Soxhlet apparatus. The extractives were obtained with different solvents. For the isolation of desired flavonoid column chromatography was applied using solvents of different polarity. Different polarity solvents utilization helped in determining a better medium for the extraction and isolation of flavonols. The flavonol quercetin obtained from *Camellia sinensis* was subjected to the estimation of anticancer activity. Anticancer ability was determined on the ovarian cancer cell line by *in vitro* method. The results depicted that the flavonoids especially quercetin, work as a good antineoplastic agent. The outcomes revealed that naturally available anticancer agents having good potential and lesser toxicity can be obtained from easily available natural sources like green tea leaves. Thus, the work in the future may be elaborated to find new targets for quercetin.

Keywords: Natural Sources Anticancer, *Camellia sinensis*, Flavonoids, Flavonols, Quercetin.

INTRODUCTION

Ovarian cancer is the second most frequent cancer after breast cancer in women over 40 years of age, especially in developed countries. It also has the poorest diagnosis and the greatest fatality rate of all cancers. Despite having a lesser frequency than breast cancer, ovarian cancer is three times more fatal, and its mortality rate is expected to increase remarkably by 2040 (9). Because ovarian cancer is asymptomatic at an early stage, more than 70 % of patients are detected only when it is progressed, where a survival rate of 5 years is found in less than 30 %. According to studies, up to 90 % of all ovarian cancers have an epithelial origin, with the remaining having a non-epithelial origin (germ cell and sex-cord-stromal) (11). Although there are several chemotherapeutic agents developed to fight against ovarian and different types of cancers nowadays, they are lacking in treatment because of different toxic effects and due to the growing ability of cancerous cells to develop drug resistance by various mechanisms (6). Hence finding a more efficient therapy for treating the cancer of ovaries is required.

There are numerous reported flavonoids showing anticancer activity. Chemotherapy, radiation therapy, hormonal therapy, and surgery are frequently effective against all varieties of cancer, but cancer patients must contend with resistance, unfavorable adverse or side effects, and the high cost of chemotherapy (4). Due to their enormous pharmacological relevance, natural products are currently regarded as a significant source in conjunction with chemotherapy to counteract all the aforementioned limitations. Due to their high selectivity,

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low cost, and minimal toxicity, natural products, and their derivatives could be superior chemotherapeutic agents. The majority (> 60 %) of anticancer pharmaceuticals currently in clinical use that have demonstrated significant efficacy against cancer are derived from natural products derived from plants, marine organisms, and microbes (10).

Multiple studies demonstrate the presence of significant phytochemicals in *C. sinensis*, such as quercetin, myricetin, and kaempferol, which exhibit various essential biological activities (1,15). There are numerous pharmacological applications for quercetin, including antioxidant, anti-diabetic, anti-inflammatory, and anti-proliferative properties. Multiple studies have demonstrated quercetin's anti-cancer activity against a variety of cancer types. *C. sinensis* is a commonly available plant (shown in Figure 1) that is grown all over the world in more than 32 countries covering an area of more than 2.5 million hectares (14). Thus, on the basis of the literature's descriptions of various flavonoid extraction and isolation methodologies, it was decided to extract and isolate flavonoids (primarily quercetin) from the leaves of *C. sinensis* and assess their anticancer potential.

Figure 1. Plant of *Camellia sinensis* in population and alone.

MATERIAL AND METHODS

2.1. Plant material and Chemicals

Dried leaves of *Camellia sinensis* were obtained from Shyali Products Private Limited, Delhi. The different chemicals used were Ethanol, Methanol, Ethyl acetate, Distilled water, Chromatographic quality Silica gel, Petroleum ether.

2.2 Pharmacognostical value

The pharmacognostic evaluation of the *Camellia sinensis* leaves was performed as described in the parameters below:

2.2.1. Macroscopical Characters

Color, flavor, size, shape, touch, and breakage of *Camellia sinensis* leaves were all noted. These are the qualities that can be directly visualized and assessed.

2.2.2. Losses while drying

The loss on drying was determined by adding 2 g of crude powdered medication to a narrow china dish with a gauged level of 105 to 100 degrees C was used to dry the food in a hot air oven. The heating process was repeated until there was a 0. 5 mg difference between two subsequent weighings (13).

2.2.3. Determining the ash value

The relevance of ashes is influenced by the purity and properties of untreated substances, especially in power hierarchies. The overall inorganic content was indicated by this figure. The main objectives are to perform an ash value execution and get rid of any organic matter leftovers. Ash is typically produced when carbonates, phosphates, and silicates are used to burn crude medications. The total ash, acid-insoluble ash, and water-soluble ash values were determined.

2.2.4. Phytochemical screening

Phytochemical screening is useful to determine whether various phytoconstituents included in the herbal medication are present or not. Numerous tests for each of their respective categories were carried out, including testing for alkaloids, glycosides, flavonoids, tannins, saponins, phenolics, and carbohydrates.

2.2. Extraction from *Camellia sinensis*

The extraction was performed using the Soxhlet apparatus. For the extraction 250g of coarsely powdered dried leaves were taken and raped in a thimble then, put in the extraction chamber, the temperature was maintained between 60-70 ºC, and the extraction was performed continuously for 12 hours using 250 ml of the solvent system. Once extraction was done using methanol as solvent and second time using ethanol as solvent. The yield of extraction procedures was measured by utilizing a rotary evaporator to evaporate the solvent under a vacuum (12,8).

2.3. Isolation by Column Chromatography

At the top of the silica gel a thin cotton plug was placed. First a blank of hexane was run through the column. Then slurry of extract was prepared with suitable solvent and poured slowly from sides of column without disturbing the silica gel. Slowly, without disturbing the sample layer in the column, the solvent system was added, and the sample extract was allowed to run drop by drop. The dried extract was packed over a silica gel column and subsequently, a solvent with increasing polarity was run through the column, once for ethanolic extract and then for methanolic extract. First petroleum ether was flown through the column. Elution started by running hexane blank then the polarity was increased using solvent ethyl acetate, ethanol, and ethanol by adjusting the ratio. The fractions were collected, and TLC was performed for each fraction to verify the similarity with the standard. The R_f value of the fraction was compared with the standard quercetin. The fraction whose R_f value was matching with the standard was collected and dried. The standard quercetin and the isolated samples were compared by performing RP HPLC and FTIR analysis (2,3).

2.4. Spectral characterization of the isolated compounds

Using RP-HPLC, FTIR, 1HNMR, and 13CNMR spectral graphs, the chemical structure of the flavonoid isolated from ethanolic and methanolic extract was confirmed and compared to the standard quercetin sample. Figure 2 depicts all the derived spectral plots.

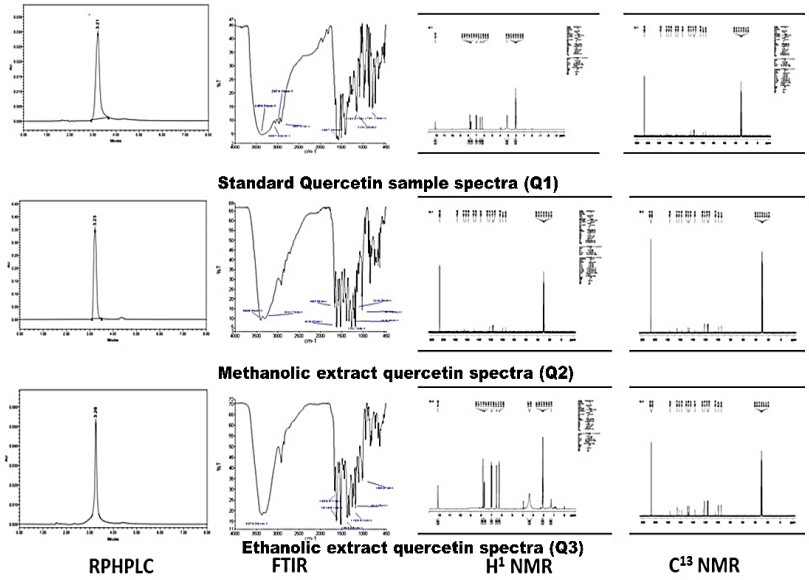

Figure 2. Spectra of standard (Q1), Methanolic extract (Q2) and Ethanolic extract sample (Q3)

2.5. Anticancer activity assessment

The activity of the quercetin to fight against ovarian cancer was determined by MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] assay. In this assay, the living cells change the pale yellow MTT substrate to the dark blue product of formazan. As a result, the amount of MTT cleaved is directly correlated with the number of live cells, which is measured using colorimetric techniques. To achieve the concentrations of a range of test concentrations, the compounds were briefly dissolved in DMSO and serially diluted with a complete medium. All samples had DMSO concentrations of less than 0.1 %. OVCAR3 cells kept in the proper conditions were plated in 96 wells, treated with varying quantities of the test samples, and incubated for 96 hours at 37 °C with 5 % CO_2. Following the addition of MTT reagent to the wells and a 4-hour incubation period, the dark blue formazan product that the cells produced was dissolved in DMSO in a safety cabinet and analyzed at 550 nm. With the concentrations used to determine the IC50 values, percentage inhibitions were calculated and shown (5,7).

RESULTS AND DISCUSSION

The morphological characteristics of the dried leaves of *C. sinensis* as shown in Table 1 include its dark green color characteristic odor, bitter or acrid taste, and other details. The loss on drying was found to be 12.5 % as shown in Table 2. Several tests were performed for determining the phytoconstituents present in the extracts of *C. sinensis.* According to the data in Table 3, the methanol and ethanol extracts of *Camelia sinensis* leaves were shown to contain significant amounts of flavonoids, glycosides, alkaloids, tannins and phenolic compounds, saponin, sterols, and terpenoids.

Table 1: Morphological characters of *C. sinensis*

Characteristics	**Observations**
Colour	Dark green
Odour	Characteristic
Taste	Bitter or acrid
Appearance	Coarse powder
Shape	Obovate, lanceolate

Table 2: Loss on drying of *C. sinensis* Leaves course powder

Sample	Initial crucible weight	Final fixed weight of the crucible	LOD
Crucible 1	63.14 gm	62.89 gm	12.5%

Table 3: Determined ash values

S.NO	**Determination of Ash value**	**%**
1	Total Ash	9
2	Acid insoluble Ash	12.7
3	Water soluble ash	17.31

The structure of the flavonoid isolated was confirmed from the spectral analysis as shown in Figure 2. The results of the MTT assay revealed the ability of the samples in suppressing the growth of the cancerous cells. The samples are showing inhibition at a minimal concentration of 0.001µg/ml, and the inhibition of the cell growth goes increasing with the increase of concentration as the values of percent inhibition at different concentrations as shown in Table 4. From the graph, the IC_{50} value was calculated as 8 µg/ml, 5.5 µg/ml, and 10 µg/ml for samples Q1, Q2, and Q3 respectively. The results revealed that sample Q2 is showing prominent percent inhibition at a lower concentration as compared to samples Q1 and Q3 shown in Figure 3.

Table 4: Phytoconstituents testing of *Camellia sinensis* leaves

S. No.	Phytochemical test	Presence or absence of phytochemical test
1.	**Alkaloids**	
1.1	Mayer's test	Present
1.2	Dragendroff's test	Present
1.3	Hager's test	Present
2.	**Phenolic and Tannin Compounds**	
2.1	Gelatin test	Present
2.2	Lead Acetate test	Present
2.3	Ferric Chloride test	Present
3	**Flavonoids**	
3.1	Alkaline reagent test	Present
3.2	Lead Acetate test	Present
4	**Saponin**	
4.1	Foam test	Present
5	**Triterpenoids & Steroids Test**	
5.1	Salkowski's test	Present
5.2	Libbermann-Burchard's test	Present
6	**Glycoside**	
6.1	Killer- Killiani test	Present
6.2	Legal's test	Present
6.3	Borntrager test	Present

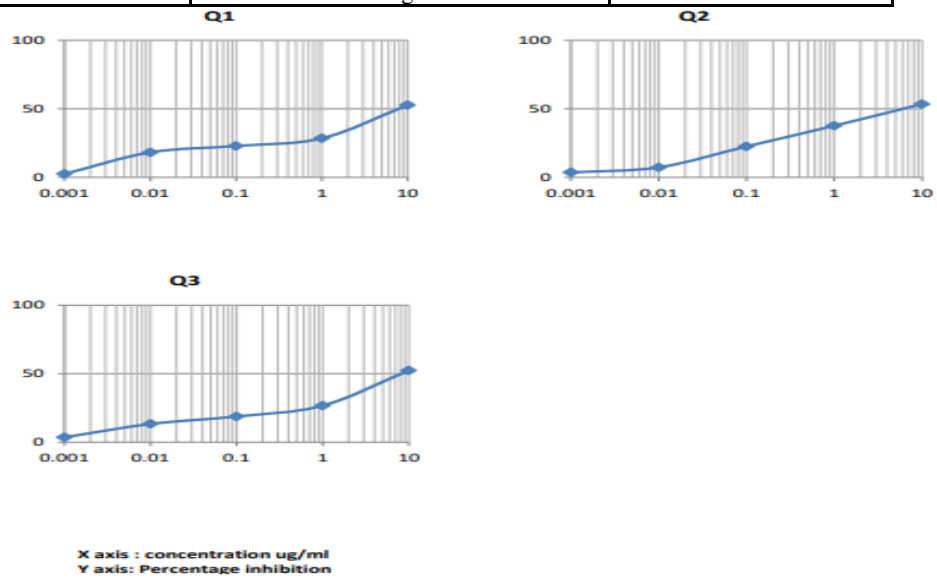

Figure 3. Suppression of the growth of the cancer cell line OVCAR 3 by the samples Q1, Q2, and Q3. OVCAR 3: Ovarian cancer cell line, Q1: Standard quercetin, Q2: Isolated quercetin from methanolic extract, Q3: Isolated quercetin from ethanolic extract

In the assay, the samples start inhibition of the cell growth at a very low concentration of 0.001 µg/ml. In the starting sample Q3 was leading in percent inhibition but on increasing the concentration, the highest percent inhibition shown was by Q2. The IC_{50} value was also the least of Q2, revealing it has the highest anticancer activity among the three as shown in Table 5.

Table 5: Percent inhibition of OVCAR 3 cell line by samples Q1, Q2, and Q3 at different concentrations

OVCAR3			
Concentration(µg/ml)	**Q1**	**Q2**	**Q3**
10	52.7	53.44	52.28
1	28.46	37.6	26.67
0.1	22.85	22.5	18.75
0.01	18.19	7.24	13.29
0.001	2.47	3.54	3.57
IC_{50} Value	**8**	**5.5**	**10**

Fewer research has been done on ovarian cancer treatment with quercetin obtained from natural sources. Despite the enormous benefits of quercetin, its pharmaceutical use is restricted by poor oral bioavailability caused by poor aqueous solubility, poor permeability, instability in physiological medium (stomach and intestine), short biological half-life, and extensive first past metabolism in the liver before reaching the systemic circulation. In addition, if the action of quercetin can be made more site-specific, it can result wonders in its pharmacological activity. Hence, it is required to develop new formulations of quercetin, that have good bioavailability and site-specific action. These results can be used in developing a good agent in the treatment of ovarian cancer a very common disease responsible for high rate of mortality in women around the world.

4. STUDY PERIOD

The work of extraction and isolation was done in a period of 7 months and a period of 5 months was consumed for the phytochemical and activity evaluation.

5. ACKNOWLEDGEMENT

The authors are thankful to the management of the Noida Institute of Engineering and Technology, Greater Noida, for providing the necessary facilities to carry out this research work.

AUTHORS' CONTRIBUTION

In the present research work, AS showed her work of isolation of flavonoid content from *Camellia Sinensis* with further testing of the isolated compounds to find its activity in combating Ovarian Cancer. She explained the methodology of the work and showed the results of the anti-cancer activity. RM, AM, and PT performed the systematic evaluation and elaborated on the conclusion. All authors read and approved the final manuscript.

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We have not excluded any author that substantially contributed to this manuscript. We have followed the ethical norms established by our respective institutions.

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