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RESEARCH ARTICLE

In Vitro Antioxidant and Anticancer Activity of Plant Extracts of *Tanacetum dolicophyllum* (Kitam.) Kitam

Gunjan Sharma[1], Zoya Zaidi[2], Mohammad Irfan Ali[3] and Sarmad Moin[1]*

[1]School of Applied Sciences, Suresh Gyan Vihar University, Jaipur – 302017, Rajasthan, India; moinsarmad@gmail.com

[2]Synthetic Organic Chemistry Research Lab, Department of Chemistry, Thanthai Hans Roever College, Perambalur – 621212, Tamil Nadu, India

[3]Faculty of Life Sciences, Mandsaur University, Mandsaur – 458001, Madhya Pradesh, India

Abstract

The objective of this study was the assessment of antioxidant and cytotoxic activity from *Tanacetum dolicophyllum.* Soxhlet extraction has been used for preparing plant extracts for which five solvents have been used, i.e., Petroleum ether, benzene, ethyl acetate, ethanol, and water. Proteins, tannins, phlobatannins, amino acids, carbohydrates, alkaloids, steroids, flavonoids, glycosides, cardiac glycosides, and phenols were detected in the sample extracts. In the phytochemical screening of plant extracts, ethyl acetate and ethanolic extract show the best results which further leads to the investigation of antioxidant activity. Ferric-Reducing Antioxidant Power (FRAP), 2,2-diphenyl-2-picrylhydrazyl (DPPH), Reducing Power assay, and Phosphomolybdenum assays were performed to estimate antioxidant activity. In the RPA and TAC, the absorbance of sample extracts increases as there is an increase in concentration. In DPPH and FRAP assay, ethyl acetate showed good results. DPPH assay for ethyl acetate extract with 172.73(µg/ml) and ethanolic extract with 171.07(µg/ml) of IC_{50} values. The ethyl acetate extract had the highest antioxidant activity. The MTT assay was used to investigate the anticancer activity. The MTT assay discovered that the ethyl acetate extract had the highest anticancer activity against HeLa cells with an IC_{50} 75 µg/mL as compared to a normal cell line J774A. Several compounds present in the extract of ethyl acetate acted as anticancer and contributed to cytotoxic activity.

Keywords: Alkaloids, Anticancer, Antioxidant, Cytotoxicity, *Tanacetum dolicophyllum*

1. Introduction

The assessment of all the drugs is based on pharmacological methods which lead to the finding of drugs stated to as natural products[1]. Plant parts like root, stem, flower, fruits, and seeds may contain bioactive components[2]. Plants are gifted with numerous phytochemicals such as vitamins, alkaloids, tannins, flavonoids, and other metabolites[3,4]. Studies have revealed that many anti-oxidant compounds possess anti-inflammatory, anti-bacterial, anti-microbial, anti-tumor, anti-carcinogenic, anti-mutagenic, and anti-viral activities[5,6]. The breakdown of antioxidants is related to reduced risks of various diseases linked with aging[7,8], and in current years, there's been worldwide movement for the natural phytochemicals present in leaves, fruits, flowers, and vegetables[9-11]. Environmental factors like altitude, rainfall, climate, and other may affect plant progress which in return affects the quality of constituents present in specific species[12].

Numerous medicinal plants have been known for their antioxidant properties. The natural antioxidants either are

*Author for correspondence

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operative to avert the critical methods caused by oxidative stress[13].

In biological cells, antioxidants either deactivate or stabilize free radicals. The role of free radicals is well-known and is elaborated in several acute and chronic disorders in humans, such as aging, immunosuppression, diabetes, and neuro degeneration[14]. Antioxidants have a special interest because of their free radical scavenging abilities[15].

The genus Tanacetum has about 150 species that shows medicinal values. Many treatments use Tanacetum species to cure diseases like ulcers, headache, fever, and menstrual cramps. Extracts of Tanacetum have been stated to exhibit anti-tumor[16], anti-oxidant[17], anti-inflammatory[18], and anti-microbial activity[19]. *Tanacetum dolicophyllum* (Asteraceae), shows many interesting and observed pharmacological aspects. *Tanacetum dolicophyllum* (Kitam.) Kitam. is an erect hairy herb with yellow bright heads and radical leaves (Figure 1). This herb raises in Alpine fields from August to October. Traditionally, *T. dolichophyllum* was used as fragrant and incense material in the Uttarakhand and Ladakh region[20].

The present study was to investigate the antioxidant activities and anticancer activities of the ethanolic and ethyl acetate extract of the whole plant of *T. dolicophyllum* to make drugs for treatment of cancer. To determine the presence of alkaloids, carbohydrates, proteins, anthracyanines, amino acids, steroids, cardiac glycosides, coumarins, flavonoids, saponins, phenols, phlobatannins, tannins, and terpenoids extracts were subjected to phytochemical analysis.

2. Materials and Methods

2.1 Plant Collection and Verification

The plant of *T. dolicophyllum* was collected from Choskore, Panikhar, Kargil region of Ladakh.

The plant was verified at the Botanical Survey of India, Jodhpur. The specimen was numbered BSI/AZRC/I.12012/Tech./20-21/418 and annotated with the locality that is Choskore, Ladakh.

Figure 1. *Tanacetum dolicophyllum* (Kitam.) Kitam.

2.2 Extraction of the Plant Materials

The plant materials were washed, shade dried, and stored in bottles for added investigation in lab[21]. The fine powder (90g) remained subject to the following extraction in different solvents (petroleum ether, benzene, ethyl acetate, ethanol, and water) by using Soxhlet. Dimethyl sulfoxide (DMSO) acting as a liquified solvent for this extract[22].

2.3 Phytochemical Screening

The qualitative preliminary phytochemical investigation was done on separate extracts by using standard procedures[23-39]. Quantitative analysis of phenols[40], flavonoids[41], carbohydrates[42], tannins[40], alkaloids[43], proteins[44], and steroids[45] was performed by using standard protocols.

2.4 Antioxidant Assays

2.4.1 Reducing Power Assay (RPA)

The RPA was based on the transformation of Fe (III) to Fe (II) in the presence of solvent fractions by using standard methods[46]. The higher absorbance of the reaction mixture indicates a higher RP value.

2.4.2 Phosphomolybdate Assay (Total Antioxidant Capacity)

The TAC of the sample extract was done by the phosphomolybdate method using ascorbic acid[47].

2.4.3 Ferric Reducing Ability (FRAP) Test

The improved FRAP method, as adopted by Benzie and Strain (1996) was used[48].

2.4.4 DPPH Radical Scavenging Assay

The scavenging activity of the plant sample was performed using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method[49].

2.5 Cell Culture

HeLa cell line was brought from NCCS, Pune. The experiments were performed in triplicates.

2.5.1 Cytotoxicity Assay

MTT assay was accomplished to evaluate the cytotoxicity of the extracts with some modifications[50].

2.6 Statistical Analysis

All antioxidant assays were carried out in duplicates except RPA and MTT assays (triplicate). Results are expressed as mean ± SD.

3. Results

3.1 Qualitative Analysis

In a qualitative study of carbohydrates, steroids, tannins, phlobatannins, coumarins, glycosides, and cardiac

Table 1. Qualitative phytochemical analysis of five plant extracts

S. No.	Qualitative tests	Petroleum ether	Benzene	Ethyl acetate	Ethanol	Water
1.	Carbohydrates	+	+	+	+	+
2.	Proteins	-	-	+	+	-
3.	Amino acids	-	-	+	+	-
4.	Steroid	+	+	+	+	+
5.	Glycosides	+	+	+	+	+
6.	Cardiac glycosides	+	+	+	+	+
7.	Anthocyanine	-	-	-	-	-
8.	Saponin	-	-	-	-	-
9.	Tannin	+	+	+	+	+
10.	Phlobatannin	+	+	+	+	+
11.	Coumarin	+	+	+	+	+
12.	Polyphenol	-	-	+	+	-

glycosides all extracts showed positive results. In the case of proteins, amino acids, and polyphenols; petroleum ether, benzene, and water showed negative results. The case of anthocyanine and saponin all showed negative results (Table 1). All the extracts further proceed for the quantification of phytochemical compounds.

3.2 Quantitative Analysis

All the extracts showed different amounts of phytochemicals. A total of 5 extracts were studied for the phytochemical screening. The concentration of extracts is shown in Table 2. Out of all the five extracts, ethyl acetate and ethanol showed the best results.

3.3 Antioxidant Tests

3.3.1 Reducing Power Assay

The standard curve of ascorbic acid with the RP of ethyl acetate and ethanolic extracts of *T. dolicophyllum* is given in Figure 2. The RP value of all the extracts increased with an increase in concentration. The RP of plant extracts of

Table 2. Quantitative phytochemical analysis of 5 plant extracts (concentration in mg/g)

Plant extracts	Phenols	Flavonoids	Carbohydrates	Tannins	Alkaloids	Proteins	Steroids
Petroleum ether	0.055	0.059	0.314	0.156	0.296	0.081	0.101
Benzene	0.075	0.172	0.095	0.043	0.134	0.045	0.086
Ethyl acetate	1.08	0.984	0.741	0.379	0.973	0.969	0.973
Ethanol	1.01	0.614	0.763	0.198	0.655	0.878	0.500
Water	0.11	0.329	0.378	0.029	0.158	0.033	0.210

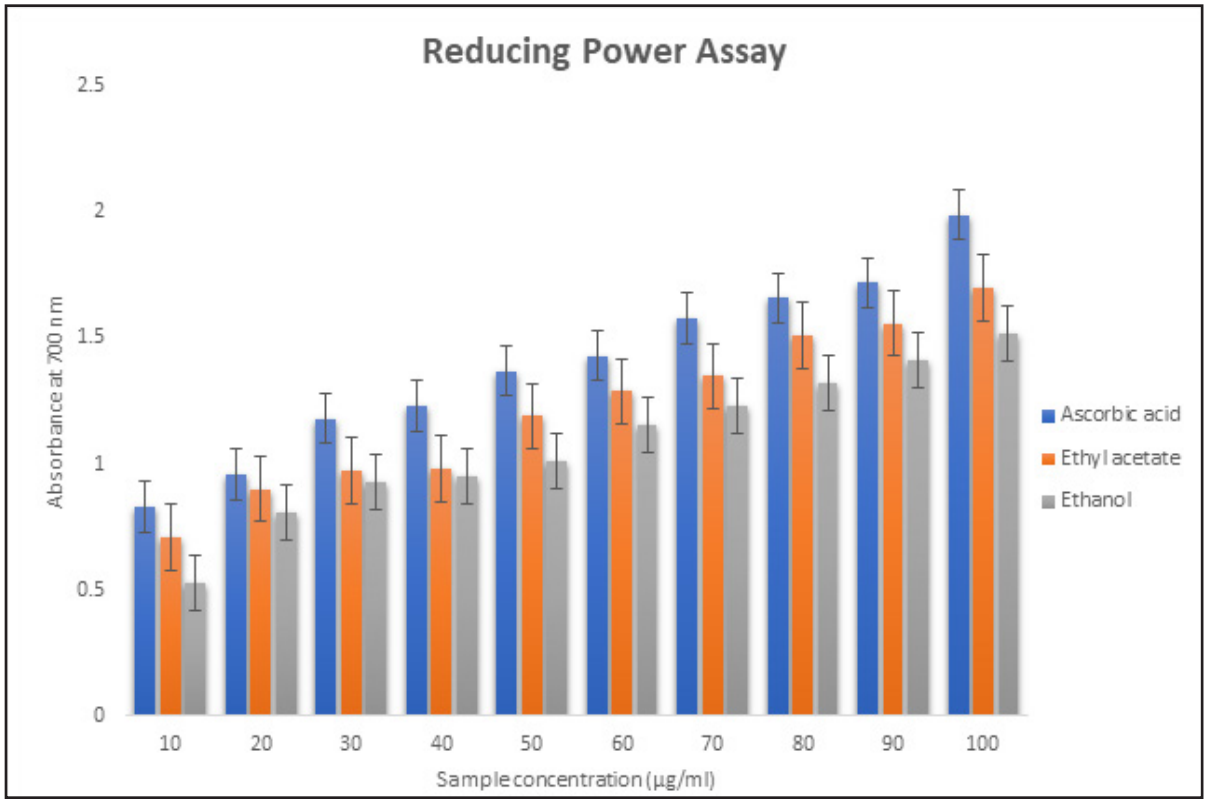

Figure 2. Reducing ability of various plant extracts and Ascorbic acid with an increase in concentration.

T. dolicophyllum was tested at varied concentrations. The absorbance increases with an increase in concentration. All tests were done in triplicate with a standard deviation of 0.1 for ascorbic acid, 0.13 for ethyl acetate, and 0.11 for ethanol.

3.3.2 Phosphomolybdenum Assay

The standard curve of ascorbic acid in phosphomolybdenum assay with ethyl acetate and ethanolic extracts of *T. dolicophyllum* is shown in Figure 3. The assay of plant extracts of *T. dolicophyllum* was tested at

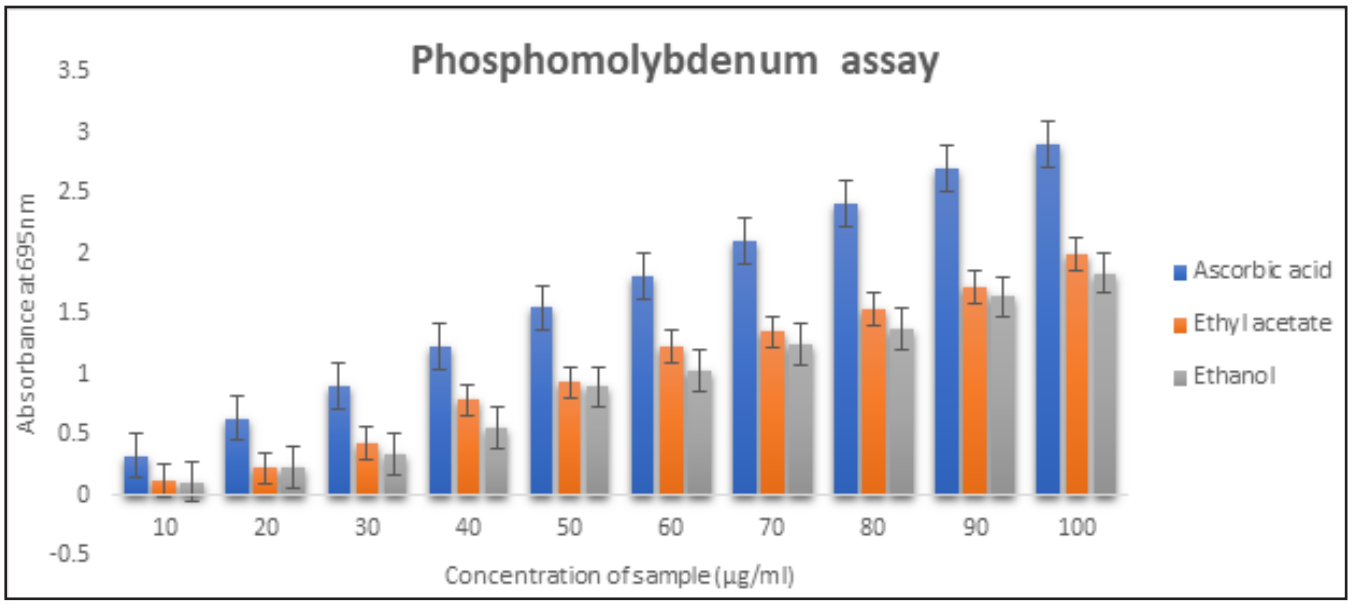

Figure 3. Phosphomolybdenum assay of various plant extracts and Ascorbic acid with an increase in concentration.

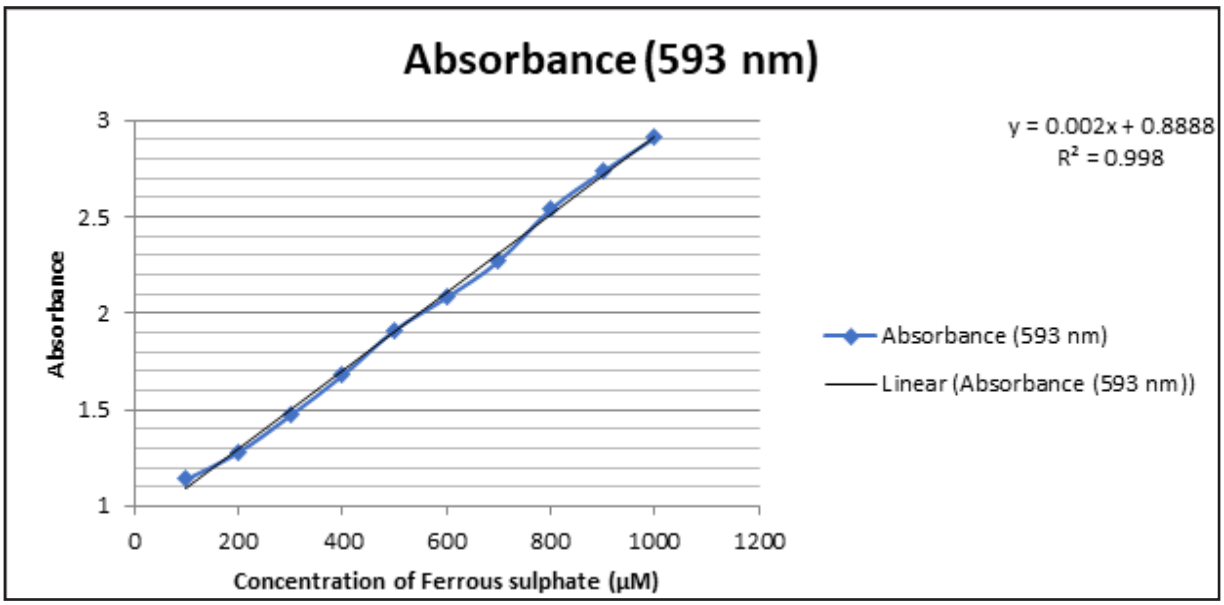

Figure 4. Standard curve of FRAP assay using ferrous sulphate at concentrations between 100 and 1000 M. The value is represented as the mean ± S.E.M.

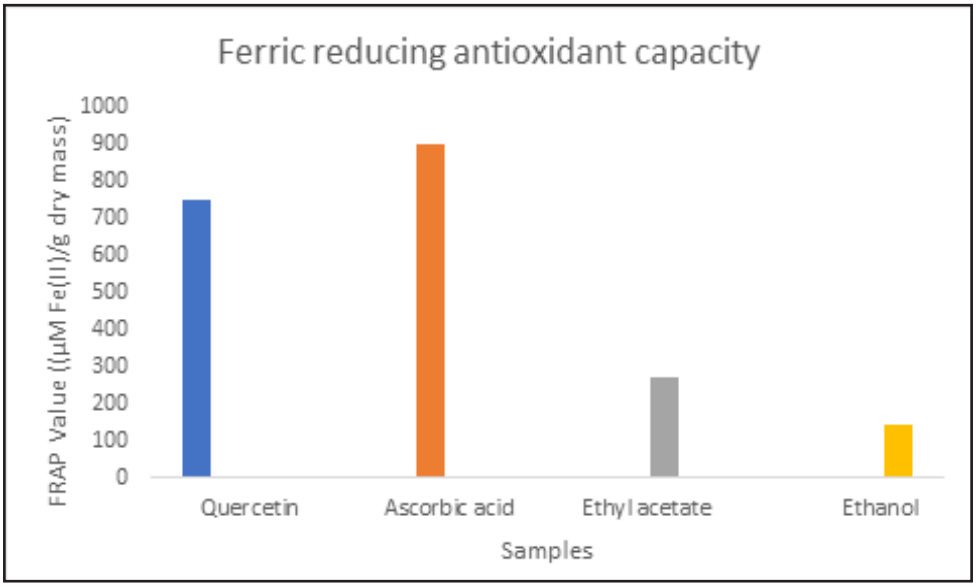

Figure 5. Total antioxidant activity of *T. dolicophyllum* extracts and standard controls (quercetin and ascorbic acid) as determined by FRAP assay. The concentration used for samples was 1 mg/ml dissolved in DMSO. The data expressed as the mean ± S.E.M.

Table 3. FRAP and DPPH of plant extracts

Plant extracts	FRAP (mg QE*/g extract)	DPPH [IC_{50} (µg/ml)]
Ethyl acetate	273.45	172.73
Ethanol	144.15	171.07

varied concentrations. The absorbance increases with an increase in concentration. All tests were done in duplicate with a standard deviation of 0.19 for ascorbic acid, 0.13 for ethyl acetate, and 0.17 for ethanol.

3.3.3 Ferric Reducing Antioxidant Power (FRAP)

FRAP is a redox potential-based that uses iron as the oxidant. FRAP of the plant extracts was obtained according to y = 0.002x+0.08888, R2 = 0.998 as quercetin equivalent (mg/g extract). The FRAP value was resolute by graph plotting of the $FeSO_4$ standard curve at the given concentration shown in Figure 4. The ethyl acetate extracts have a higher antioxidant capacity in comparison to the ethanolic extracts. FRAP value of the ethyl acetate extract, ethanolic extract, and the standards are shown in Figure 5 and Table 3.

3.3.4 DPPH Radical Scavenging Capacity

DPPH radical scavenging capacities of plant extracts of *T. dolicophyllum* were tested at various concentrations shown in Figure 6. A lower IC_{50} value shows a higher antioxidant activity of the extract. Values for the DPPH assay are given in Table 3. Ethyl acetate extract with 172.73(µg/ml) and ethanolic extract with 171.07(µg/ml) of IC_{50} values.

Ethyl acetate extract shows better results than ethanolic extract. So, ethyl acetate extract leads to the further investigation of anticancer properties.

3.4 Anticancer Activity

A former study showed that *T. dolicophyllum* is a source of anticancer chemo-preventive agents[51]. In the study, the anticancer effect of ethyl acetate extract of *T. dolicophyllum* against the HeLa and normal cell line J774A was investigated. The cytotoxic activity was examined using the MTT assay, a method for evaluating cell viability by mitochondrial impairment which relates to the number of active cells. After 24 hours of incubation with HeLa cells, the extract strongly reduced the viability of cancer cells. On increasing the concentration % viability decreases in the HeLa cells. The IC_{50} value of the MTT assay of HeLa is

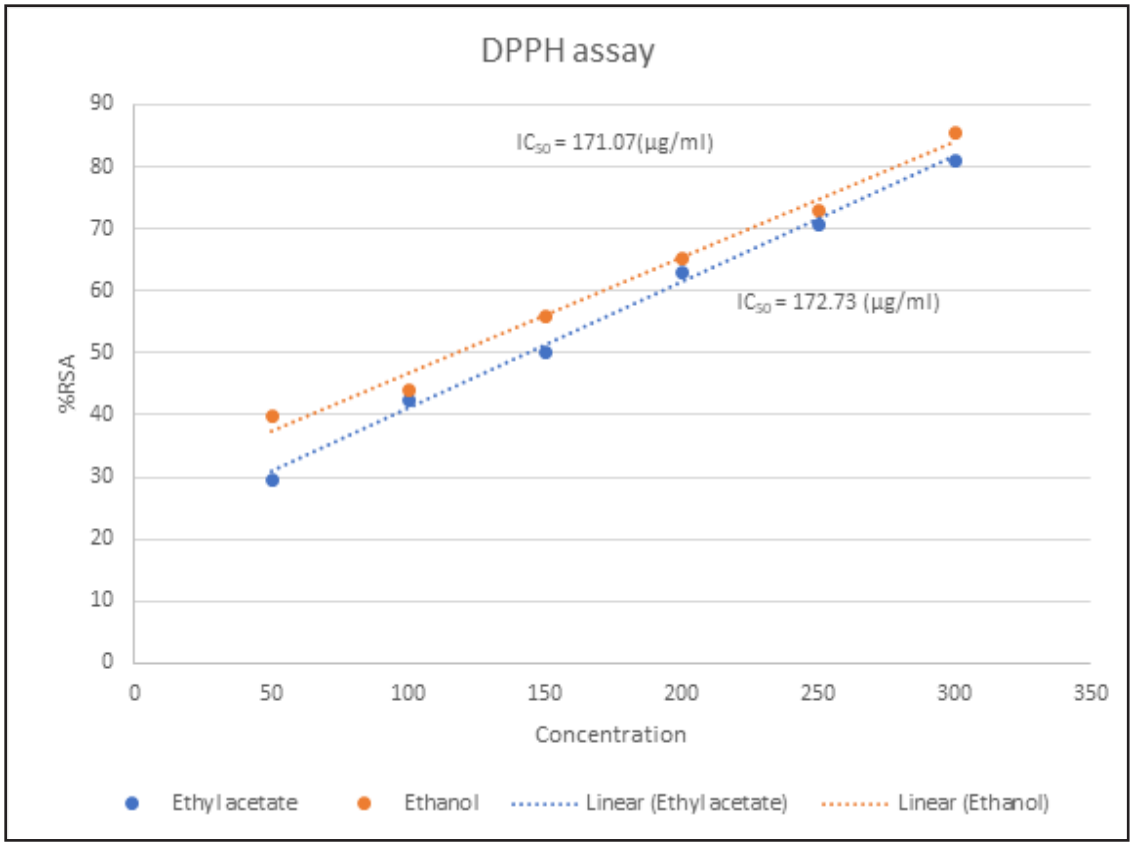

Figure 6. DPPH scavenging activity with their IC_{50} values.

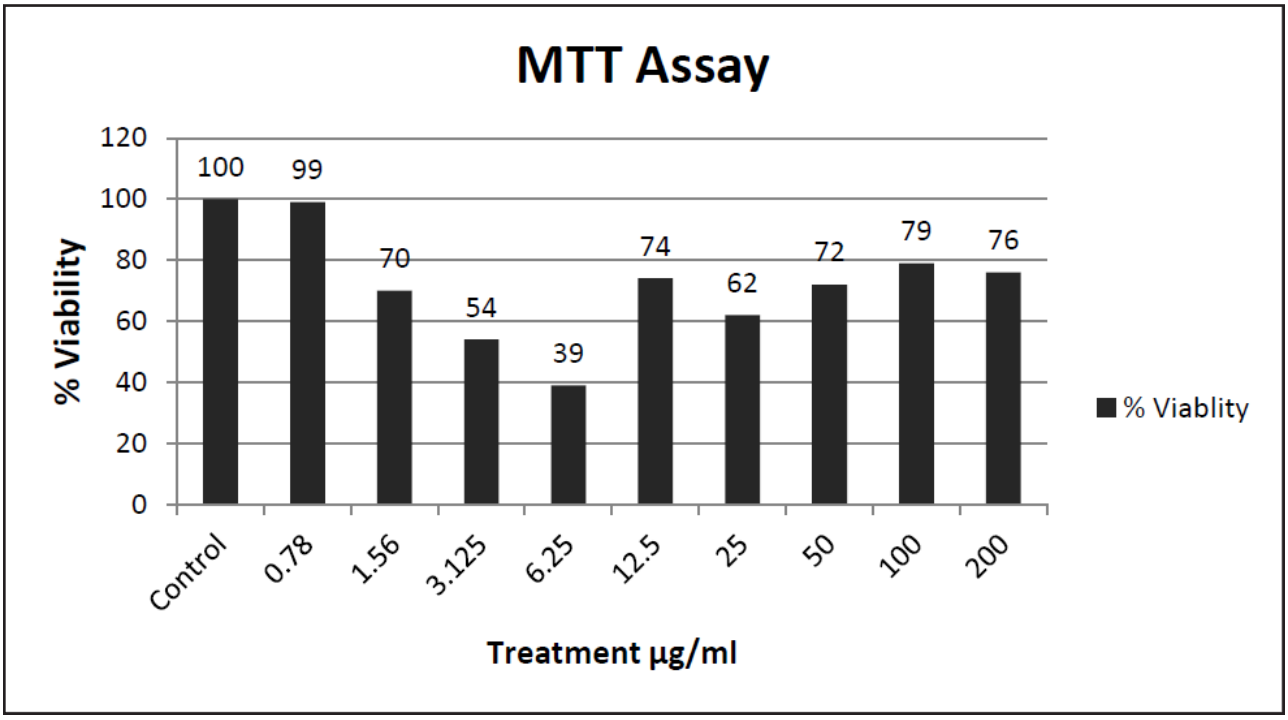

Figure 7. Cytotoxic activity of ethyl acetate extract against normal cell line (J774A).

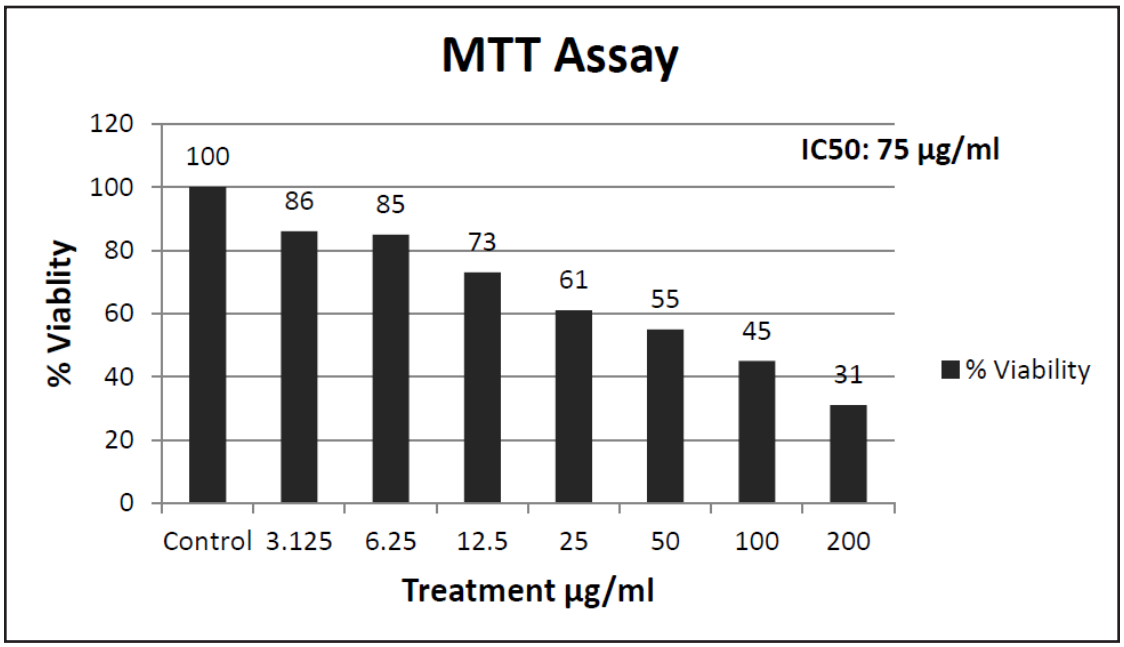

Figure 8. Cytotoxic activity of ethyl acetate extract against cancer cell line (HeLa).

75 µg/ml. Additional studies are essential to describe the extracts and explicate their action mechanism (Figures 7 and 8).

In addition, many compounds present in ethyl acetate extracts act as anticancer agents and contribute to the overall cytotoxic effect. Additional studies are needed to characterize the extracts and explicate the mechanism of action.

4. Discussion

Many plant species are the basis of phytotherapeutic products. The existing knowledge about the studied plant is very limited. It is a report about the antioxidant and cytotoxic effects of *T. dolicophyllum*. A qualitative and quantitative analysis of plant extract was performed to observe the presence of various bioactive phytochemicals.

Bioactive compounds such as carbohydrates, steroids, glycosides, cardiac glycosides, tannins, phlobatannins, alkaloids, and flavonoids were existing abundantly in nearly all plant extracts. Amino acids, proteins, phenols, and polyphenols were found in ethyl acetate and ethanolic extracts. Flavonoid and alkaloid compounds are involved in the defense against cardiovascular diseases[52]. From the phytochemical analysis of plant extracts, ethyl acetate, and ethanolic extracts showed good results which lead to antioxidant and anticancer activity.

The RPA was based on the reduction of Fe^{3+} to Fe^{2+} measured at 700 nm. The phosphomolybdenum assay was based on the reduction of phosphomolybdate measured at 765 nm. The RPA and phosphomolybdenum assay of both the ethyl acetate and ethanolic extracts increased with an increase in concentration. FRAP is a redox reaction that uses iron as the oxidant. FRAP value of the ethyl acetate extract is 273.45 µM/g Fe (II) and the ethanolic extract is 144.15 µM/g Fe (II), while the FRAP value of the standards (quercetin and ascorbic acid are 752.1 µM/g Fe (II) and 900.54 µM/g Fe (II), respectively. DPPH of plant extracts of *T. dolicophyllum* were tested at 50, 100, 150, 200, 250, and 300 µg/mL concentrations. A lower IC_{50} value links to a higher antioxidant activity and the values for DPPH of ethyl acetate extract with 172.73(µg/ml) and ethanolic extract with 171.07(µg/ml) IC_{50} values.

Ethyl acetate extract shows better results than ethanolic extract. So, ethyl acetate extract leads to the further examination of anticancer properties.

The evaluation of cytotoxic activity is based on the reduction of the MTT assay. The cytotoxic activity of *T. dolicophyllum* was assessed against HeLa cancerous and J774A normal cell lines. The ethyl acetate extract of *T. dolicophyllum* showed its activity after 24 hours of incubation with HeLa cells. On increasing the concentration % viability decreases in HeLa cells. In living cells, the number of active mitochondria is proportional

to the reduction of MTT[53,54]. The ethyl acetate extract from *T. dolicophyllum* exhibits the most potent activity with an IC_{50} value of 75 µg/ml. Many compounds present in ethyl acetate extracts act as anticancer and contribute to the cytotoxic effect. The plant is capable of showing its application in the pharmaceutical industry.

5. Conclusion

The *Tanacetum* genus has been used as a medicinal plant for centuries but complete phytochemical analysis, and pharmacological activities are essential to approve their therapeutic value. Phytochemical investigations allowed the identification of many biologically active compounds. Both extracts exhibited antioxidant activity. Ethyl acetate extract shows better antioxidant activity than ethanolic extract which is then investigated for MTT assay. Ethyl acetate extract showed high cytotoxicity on HeLa and the J774A cell line. In HeLa cells, its cytotoxicity decreases by increasing the concentration. The results show that the analyzed species could be the hopeful sources of active components in the pharmaceuticals.

6. Acknowledgement

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