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Research Article

PHYTOCHEMICAL SCREENING, GAS CHROMATOGRAPHY-MASS SPECTROMETRY, AND *IN VITRO* ANTIOXIDANT POTENTIAL OF *DRYOPTERIS MARGINALIS*

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ABSTRACT
The leaves of the plant *Dryopteris marginalis* collected from Kumaon District, Nainital India, were analyzed for the presence of different phytochemicals. The aim of this study is to screen the biologically active compounds; alkaloids, glycosides, tannins, flavonoids, steroids, saponins, phenolic compounds, fat and oils, reducing and non-reducing sugar and amino acids. Phytochemical screening indicated the presence of flavonoids, phenolic compounds, steroids, saponins, alkaloids, amino acids, volatile oils, reducing and hexose sugar. The phytochemical composition of the leaves of the plants indicates their medicinal properties.

Keywords: Breast cancer, Antioxidants, *Dryopteris marginalis*, Pteridophytes.

1. INTRODUCTION

Medicinal plants have its origins in ancient cultures, which are used to treat different diseases and enhance general health and wellbeing. Photochemical are naturally occurring chemical compounds in plants. Since the middle of the 19th century different phytochemicals have been isolated and characterized. Some of these are used as active ingredients in modern medicine for the treatment of some diseases [1]. Many of these have several activities such as antioxidative, antimutagenic and anticarcinogenic [2]. Some of the important phytochemical compounds includes alkaloids, glycosides, tannins, flavonoids, steroids, saponins, phenolic compounds, fat and oils, reducing and non-reducing sugar and amino acids etc. which are distributed in different parts of the plants [3]. *Dryopteris marginalis* (Dryopteridaceae) is an evergreen fern throughout its range commonly known as marginal shield or marginal wood fern. Marginal wood fern is often grows best in dappled to light shade with low to medium moisture. It prefers acidic soils but is adaptable to a wide range of soil conditions [4]. Asia's temperate climates are homes to the 250 species that make up the Dryopteris genus. In the United States and Canada, the current distributions of 14 Dryopteris species point to a Pan-Tertiary migration, possibly in conjunction with Pleistocene interglacial's. Researchers investigated the relationships between these taxa, including polyploidy, hybridization, Pleistocene glacial episodes that may have fostered diversification, and the current phytogeographic distribution of Dryopteris in North America.

Table 1: *Dryopteris marginalis*

Species	*Dryopteris marginalis*
Family	Dryopteridaceae
Class	Polypodiopsida
Division	Polypodiophyta
Kingdom	Plantae
Clade	Tracheophytes
Genus	Dryopteris
Order	Polypodiales
Other Details	
Growth Habitat	Tropical, Sub-Tropical/ Monsoonal
Plant growth form	Herbaceous Plant
Mode of Nutrition	Autotrophic
Collection	Kumaon, Nainital

The marginal shield fern, *Dryopteris marginalis*, is an evergreen that usually grow in a non-spreading, vase-shaped clump to an altitude of 1.5 to 2 foot (infrequently to 3 foot). This fern is a category of wild plant that prefers the protected crevice of rocky ledges and bluffs. Features 15-20" long, deeply cut, leathery, grayish-green fronds. The accepted name for sori comes from their position at the limitations or edges of the pinnule undersides. Its rhizome grows into an upright crown. The winter landscape is made interesting by the occurrence of evergreen fronds.

It has been determined that breast cancer is a neglected disease in comparison to other numerically more frequent health issues for a significant number of women who have just received a diagnosis worldwide. It has also been referred to as an orphan illness since there is a lack of highly specialized understanding regarding tumor features and the essential host biology required to provide basic medical care. With the exception of dietary advice, current international cancer planning and policy initiatives are unrelated to breast cancer. However, there has been reported success with mortality declines in several developed countries [5].

2. MATERIAL, METHODS AND RESULTS

2.1. Plant material

Dryopteris marginalis leaves were collected from Kumaon District, Nainital India, during the month of August, 2022. The plant was identified by Dr. Sunita Garg, former chief scientist, head, RHMD, CSIR, NISCAIR, Delhi, and a voucher specimen was deposited in the raw material herbarium and museum, Delhi (RHMD).

2.2. Extraction and Fractionation

The shaded dried and crushed leaves of *Dryopteris marginalis* was subjected to maceration with non-polar to polar solvents. The extract was then concentrated by Rota-vapor under reduced pressure at temperature below 50°C.

2.3. Phytochemical screening

The chemical tests were performed on the pet ether, n-Hexane, chloroform, and ethanolic extracts of *Dryopteris marginalis* plant leaves using standard procedure to identify the constituents as described by sofowora [6], Trease and Evans [7] and Harborne [8].

2.4. Phytochemical screening

The photochemical screening of the ethanolic extract of the plant revealed the presence of alkaloid, sterols, saponins, and flavanoids (Table 3). The total weight % yield of the crude extract of pet ether, n-hexane, chloroform, and ethanol of *Dryopteris marginalis* is shown in Table 2. The MTT test was used to determine how ethanolic extracts of *Dryopteris marginalis* and Paclitaxel (Standard) affected the development of MCF-7 cell lines. With higher concentrations of both the plant extract and the standard-control drug paclitaxel, the number of viable cells decreased.

Loss of moisture content is important but rate of drying is the deciding factor, which provides the information for the preparation of the drug. The loss on drying value of the plant is estimated 4.4 ±0.1which signify that drug is properly dried. The results of the Ash values represent the purity of the drug, *i.e.,* the presence or absence of extraneous substances like metallic salt or silica in the raw material. The silicates and silica, which include both physiological ash and non-physiological ash, carbonates, phosphates, and silicates often, make up the total ash [9]. The ash value of the crude drug is 7 ± 0.02%. Acid insoluble ash is a sign of contamination with silicious materials, such as sand and earth. Comparing this value to the total ash value of the same sample will help distinguish between contaminants and variations in the natural ash of drug [10]. The total acid insoluble ash value of the crude drug is 4.5±0.17% w/w which signifies that the crude drug less contamination with silicious matter. Water soluble ash value is 5.9±0.3 % and total alcohol soluble ash value is 5.2±0.6 % the ash value of the drug is in the limits which represent the purity and quality of the crude drug. In this study various parameters such as Total Ash value, Total moisture content, Total acid, water insoluble value was evaluated and studied in ethanolic extract it helps in the authentication, preparation of monograph and identification of both plants.

Table 2: Percent yield of leaves of *Dryopteris marginalis*

Part	pet ether	n-hexane	chloroform	ethanol
Leaves	1.8%	6.38%	8.24%	23.21%

Table 3: Phytochemicalscreening of the crude extracts of *Dryopteris marginalis*

Sl. No.	Chemical Tests	pet ether	n-hexane	chloroform	ethanol
1.	ALKALOIDS				
	Mayer's reagent	-ve	+ve	-ve	-ve
	Dragendroff's reagent	-ve	-ve	-ve	+ve
	Wagner's reagent	+ve	-ve	-ve	-ve
2.	GLYCOSIDES				
	Killer-Killani test	-ve	-ve	-ve	-ve
	Sodium nitropruside test	-ve	-ve	-ve	-ve
	Borntrager test	-ve	-ve	-ve	-ve
3.	CARBOHYDRATES				
	Molisch's reagent	-ve	-ve	-ve	-ve
	Fehling solution	-ve	-ve	-ve	-ve
4.	STEROLS				
	Liebermann- Burchard's test	-ve	-ve	-ve	+ve
	Salkowski test	-ve	-ve	-ve	+ve
	Hesses reaction	-ve	-ve	-ve	+ve
	Hersch reaction	-ve	-ve	-ve	+ve
5	SAPONINS				
	Foam test	-ve	-ve	-ve	+ve
	Sodium bicarbonate test	-ve	-ve	-ve	+ve
6	PHENOLIC COMPOUNDS & TANNINS				
	Ferric chloride solution				
	Lead acetate solution	-ve	-ve	-ve	-ve
		-ve	-ve	-ve	-ve
7	FLAVANOIDS				
	Shinoda/Pew test	-ve	-ve	-ve	+ve
	Ammonia test	-ve	-ve	-ve	+ve

Table 4: Loss on drying results

Wt. of drug (gm)	Wt. of empty china dish (gm)(W2)	Wt. of empty China dish (gm) + Wt. of drug (gm)	Wt. of empty China dish (gm) + Wt. of drug (gm) after drying (W3)	Drug %	Mean ±SEM
5	50.00	55.00	50.23	4.6%	
5	50.21	55.21	50.43	4.4%	4.4 ±0.1
5	49.98	54.98	50.20	4.4%	

Table 5: Total ash value results

Wt. of drug	Wt. of crucible	Wt. of crucible + wt. of ash (g)	Wt. of crucible after ignition (g)	Wt. of ash	Total ash (%)
5	44.62	49.62	44.98	0.36	
5	46.23	51.23	46.58	0.35	7 ± 0.02
5	43.02	48.02	43.36	0.34	

Table 6: Total acid insoluble value

S.NO.	Wt. of drug	Wt. of Crucible	Wt. of Crucible+ Wt. of drug	Wt. of Crucible after ignition (g)	Wt. of ash	Total ash (%)	Mean ± SEM
1.	5	45.00	50.00	45.23	0.23	4.6	4.5±0.17
2.	5	45.36	50.36	45.57	0.21	4.2	
3.	5	45.21	50.21	45.48	0.24	4.8	

Table 7: Total alcohol soluble ash

S.NO.	Wt. of drug	Wt. of Crucible	Wt. of Crucible+ Wt. of drug	Wt. of Crucible after ignition (g)	Wt. of ash	Total ash (%)	Mean ± SEM
1.	5	45.16	50.16	45.42	0.26	5.2	5.2±0.6
2.	5	45.48	50.48	45.76	0.28	5.6	
3.	5	45.05	50.05	45.30	0.25	5.0	

Table 8: Total water- soluble ash

S.NO.	Wt. of drug	Wt. of Crucible	Wt. of Crucible+ Wt. of drug	Wt. of Crucible after ignition (g)	Wt. of ash	Total ash (%)	Mean ± SEM
1.	5	46.04	51.04	46.33	0.29	5.8%	5.9±0.3
2.	5	46.19	51.19	46.48	0.29	5.8%	
3.	5	46.13	51.13	46.44	0.31	6.2%	

2.5. GC-MS analysis report

GC-MS techniques provide valuable and crucial information about ethanolic extracts containing multiple compounds and provide a new platform for understanding bioactive compounds in their discovery as targeted therapies for the treatment of various diseases. Activity prediction of the various compounds is done with help of the web-based software (way2 drug) which predict the presence of different pharmacological activity in various compound.

Phytoconstituents [n-Dodecyl pyridinium chloride**,**2,4-Di-tert-butylphenol**,**trans-2-methyl-4-npentylthiane, S, S-dioxide**,** Methyl 12,13- tetradecadienoate**,** Benzene propanoic acid, 3,5-bis (1,1dimethylethyl)-4-hydroxy-**,** ethyl ester, trans-2,4-Dimethylthiane**,**S,Sdioxid, 2-Pentene, 3- (chloroethylboryl)-2- (chlorodimethylsilyl)-**,** (E)- , Arsenous acid, tris (trimethylsilyl) ester] are identified in the ethanolic extract of the *Dryopteris marginlis* through GC-MS , structure of the compounds and structure based smile code is obtained by using Chem Draw software.

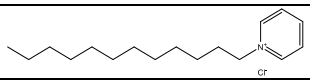
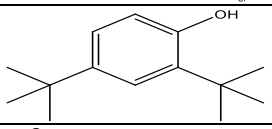
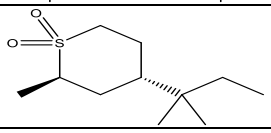
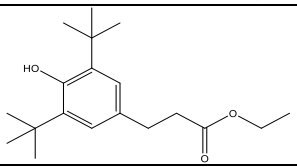
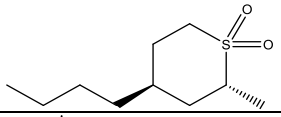
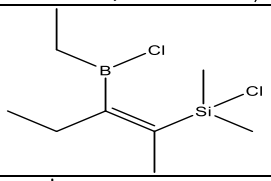
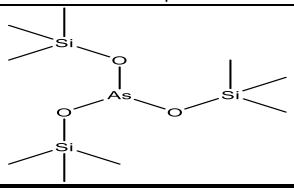
2.6. *In-silico* studies of ethanolic extract of *Dryopteris marginalis*

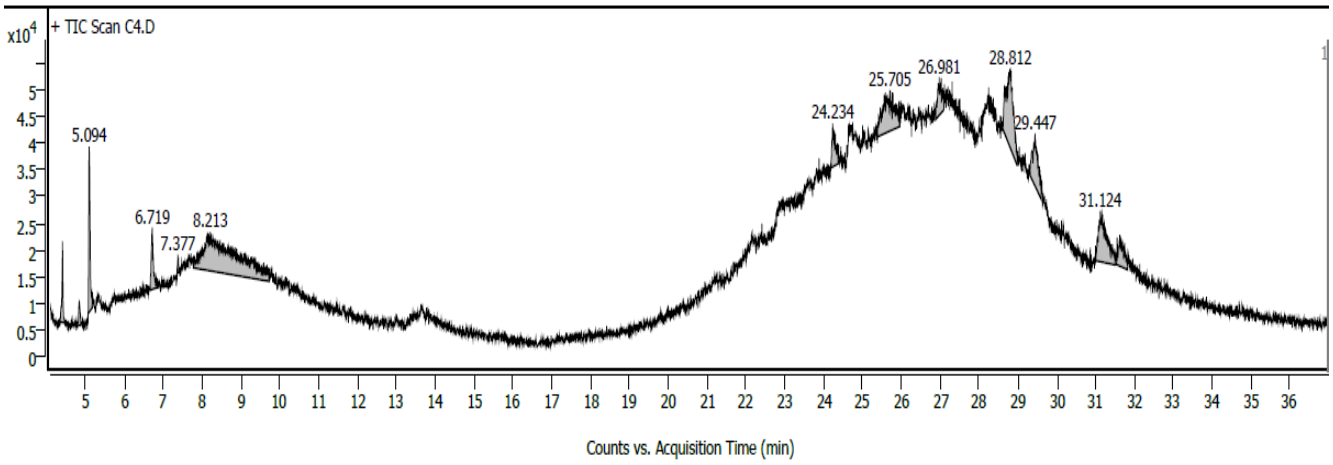
2.6.1. Docking

Interaction and binding affinity of the molecules are founded using Moldock. Compounds founded in the ethanolic extract have been shown anticancer bioactivity. Investigation of the several compounds from these plants shows the inhibitory action on the protein 3PTG, 3TL5, 3MVM, and 5ITD which involved in the cancer. Binding affinity scores obtained by the docking of 3PTG, 3TL5, 3MVM, and 5ITD on the reference ligands shows in Table 11. Higher the binding scores the molecule considers as the more effective. Binding affinity of the compounds lies between 4.2 k Cal/mol to 8 Kcal/Mol. Molecule no. 2,6, and 19 have affinity of -4.2, -8 and -5.7 for protein 3E8N likewise same molecule have affinity of -4.8 , -7 and -6 for the 3MVM, for other protein 3 PTG and 5ITD docking score is -4.4, -6.5, -5.7, -5.3, 7.9, and -7.5 respectively so this result suggests that molecule no. 6 i.e., Melezitose is more potent anticancer activity in compare to other molecules found in the ethanolic extract.

In the above figure TRAF2 and TAK1 pathways where demonstrated to show the extra and intra cellular pathway which are impacted by cancer. The alteration in the pathway in the cancer is varying very frequently and changes differently in various organ and tissue which indcates the different cross pathway and complex changes [11]. For the development of the novel therapeutic approches it is very important to understand the specific mechanism and changes in the oncogenic pathway. Molecules found in the extract of Dryopteris marginalis have inhibitory action for the protein those are involved in the breast cancer.

Table 9: Phytochemical compounds present in *Dryopteris marginalis* and its structure, smile code and respective pharmacological activity

Name	Smile	Structure	Activity
n-Dodecylpyridinium chloride	CCCCCCCCCCCC[N+]1=CC=CC=C1.[Cl-]		Anti-infective
2,4-Di-tert-butylphenol	OC1=CC=C(C(C)(C)C)C=C1C(C)(C)C		Lipoprotein lipase inhibitor
trans-2-methyl-4-npentylthiane, S,S-dioxide	CCC([C@@H](CC1)C[C@@H](C)S1(=O)=O)(C)C		Calcium regulator
Methyl 12,13-tetradecadienoate	C=C=CCCCCCCCCCCC(OC)=O		Antieczematic
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, ethyl ester	O=C(OCC)CCC1=CC(C(C)(C)C)=C(O)C(C(C)(C)C)=C1		Anti-inflammatory
trans-2,4-Dimethylthiane, S,Sdioxid	CCCC[C@@H](CC1)C[C@@H](C)S1(=O)=O		Lipoprotein lipase inhibitor
2-Pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (E)-	C/C([Si](C)(Cl)C)=C(B(Cl)CC)\CC		CYP3A2 substrate
Arsenous acid, tris(trimethylsilyl) ester	C[Si](O[As](O[Si](C)(C)C)O[Si](C)(C)C)(C)C		Antineoplastic



(A)

Chromatogram Peaks

Peak	Start	RT	End	Height	Area	Area %
1	4.350	4.408	4.459	15199	27637	6.70
2	4.805	4.837	4.877	4438	11031	2.67
3	5.067	5.094	5.174	31109	74983	18.17
4	6.668	6.719	6.827	11636	41847	10.14
5	7.356	7.377	7.400	3392	4643	1.13
6	7.784	8.213	9.723	7361	412679	100.00
7	24.194	24.234	24.377	8213	43914	10.64
8	25.393	25.705	25.957	7710	145209	35.19
9	26.889	26.981	27.101	7100	48205	11.68
10	28.618	28.812	28.989	15095	185340	44.91
11	29.321	29.447	29.602	9865	92227	22.35
12	31.012	31.124	31.507	9561	137210	33.25
13	31.541	31.627	31.822	5827	53853	13.05

(B)

Fig. 1: (A) Detected phytoconstituents in the ethanolic extract of *Dryopteris marginalis* by GC-MS; (B) Retention time and peak data of the phytoconstituents found in ethanolic extract.

Table 10: Docking score of Ligand and Protein involved in cancer

Receptor PDB ID	Ligands	Binding Affinity(ΔG) en Kcal/mol)
3E8N	3e8n__uff_E=125.11	-4.8
	3e8n__uff_E=147.44	**-4.2**
	3e8n__uff_E=363.47	-4.7
	3e8n__uff_E=447.68	-5.2
	3e8n__uff_E=1039.88	-6.1
	3e8n__uff_E=739.39	**-8**
	3e8n__uff_E=55.65	-4.9
	3e8n__uff_E=1022.27	-6.2
	3e8n__uff_E=315.11	-4.7
	3e8n__uff_E=62.37	-4.9
	3e8n__uff_E=625.19	-4.9
	3e8n__uff_E=112.28	-4.7
	3e8n__uff_E=199.61	-5.8
	3e8n__uff_E=695.98	-5.1
	3e8n__uff_E=87.69	-4.7
	3e8n__uff_E=100.87	-4.8
	3e8n__uff_E=304.65	**-5.7**
	3e8n__uff_E=625.52	-4.9
3MVM	3mvm__uff_E=125.11	-4.2
	3mvm__uff_E=147.44	**-4.8**
	3mvm__uff_E=363.47	-4.8
	3mvm__uff_E=447.68	-4.6
	3mvm__uff_E=1039.88	-6.7
	3mvm__uff_E=739.39	**-7**
	3mvm__uff_E=55.65	-4.4
	3mvm__uff_E=1022.27	-6.6
	3mvm__uff_E=315.11	-4.9
	3mvm__uff_E=62.37	-4.5
	3mvm__uff_E=625.19	-5.1
	3mvm__uff_E=112.28	-4.8
	3mvm__uff_E=199.61	-5.5
	3mvm__uff_E=695.98	-5.3
	3mvm__uff_E=87.69	-4
	3mvm__uff_E=100.87	-4
	3mvm__uff_E=304.65	**-6**
	3mvm__uff_E=625.52	-5.1
3PTG	3ptg__uff_E=125.11	-3.7
	3ptg__uff_E=147.44	**-4.4**
	3ptg__uff_E=363.47	-4.8
	3ptg__uff_E=447.68	-4.3

	3ptg__uff_E=1039.88	-6.3
	3ptg__uff_E=739.39	**-6.5**
	3ptg__uff_E=55.65	-4.5
	3ptg__uff_E=1022.27	-6.2
	3ptg__uff_E=315.11	-4.4
	3ptg__uff_E=62.37	-3.5
	3ptg__uff_E=625.19	-4.7
	3ptg__uff_E=112.28	-4.1
	3ptg__uff_E=199.61	-4.7
	3ptg__uff_E=695.98	-5.2
	3ptg__uff_E=87.69	-3
	3ptg__uff_E=100.87	-4.3
	3ptg__uff_E=304.65	**-5.7**
	3ptg__uff_E=625.52	-5.1
5ITD	5itd__uff_E=125.11	-4.9
	5itd__uff_E=147.44	**-5.3**
	5itd__uff_E=363.47	-5.9
	5itd__uff_E=447.68	-5.7
	5itd__uff_E=1039.88	-7.9
	5itd__uff_E=739.39	**-7.9**
	5itd__uff_E=55.65	-4.8
	5itd__uff_E=1022.27	-7.9
	5itd__uff_E=315.11	-5.1
	5itd__uff_E=62.37	-5.2
	5itd__uff_E=625.19	-6.1
	5itd__uff_E=112.28	-5.7
	5itd__uff_E=199.61	-6.5
	5itd__uff_E=695.98	-6.4
	5itd__uff_E=87.69	-4.3
	5itd__uff_E=100.87	-5.2
	5itd__uff_E=304.65	**-7.5**
	5itd__uff_E=625.52	-5.9

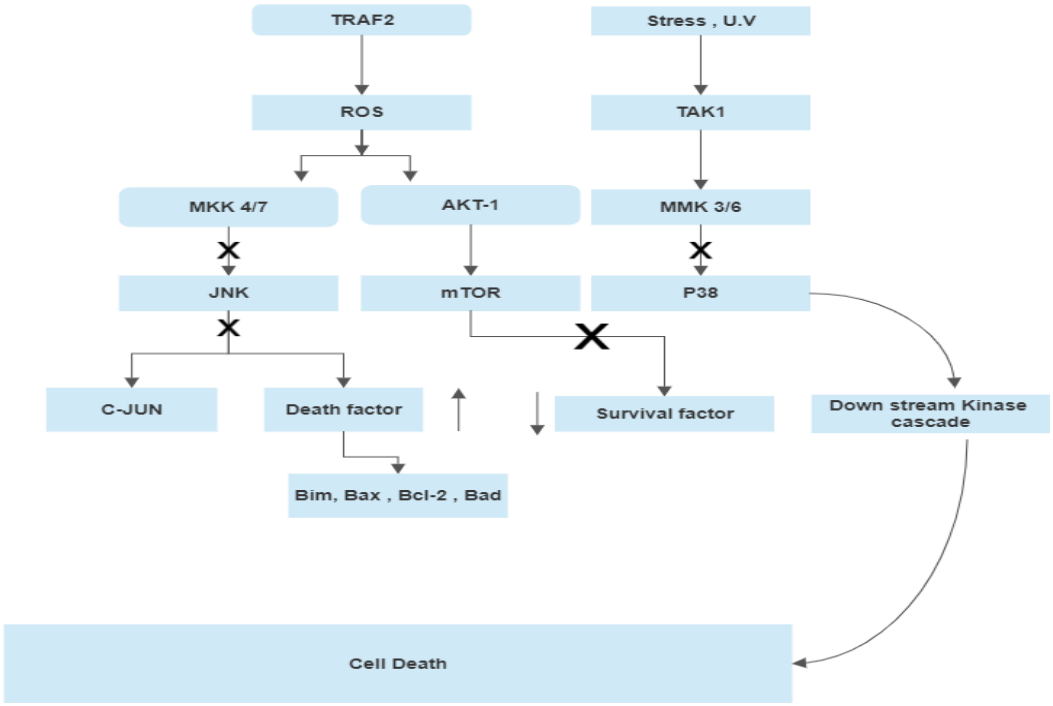


Fig. 2: Effectivness of compounds having MAPKinase, m-TOR, Pi3K, JNK pathway inhibitory activity in breast cancer

2.7. *In-vitro* activity of *Dryopteris marginalis* and Paclitaxel

In present study our main objective is to evaluate the anticancer activity of *Dryopteris marginalis* on the MCF-7 as well as effect on cell invasion and different pathway like MAPK. The result shows that ethanolic extract shows the activity in concentration and time dependent manner. Ethanolic extract of *Dryopteris marginalis* on the MCF-7 shows cytotoxic of 60µgm/ml in breast cancer cell line after the exposure of 48 hours. The result was presented in table 12.

Table 11: Result shows the percentage inhibition

S. N.	Concentration	Dryopteris marginalis ethanolic extracts	Paclitaxel
		percentage growth inhibition (%)	
1	5 µg/ml	2%	5%
2	10 µg/ml	4%	13%
3	20 µg/ml	20%	26%
4	40 µg/ml	23%	41%
5	60 µg/ml	30%	50%

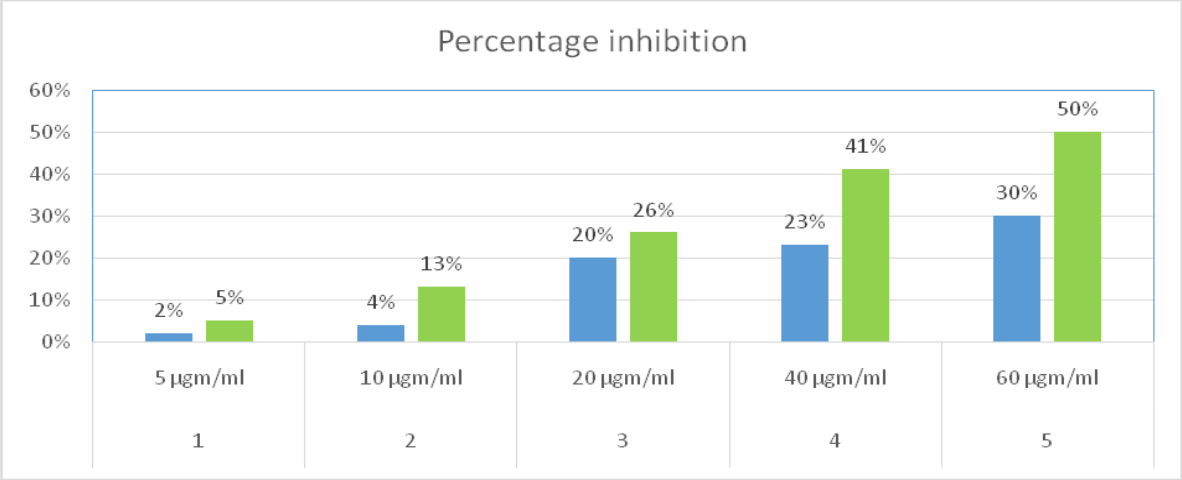


Fig. 3: Percentage growth analysis of *Dryopteris marginalis* *Paclitaxel*

3. DISCUSSION

Cases of breast cancer increases rapidly 10-fold every year. In European country 50% increase in the case of breast cancer is reported in last decade. Therapeutic approach available nowadays mainly designed based on the molecular target. Major therapeutic approaches are developed by targeting the pathways which are involved in the breast cancer. Target based therapy may be produce a good effect when given with systemic therapy so maybe it produces a good result in the treatment of cancer. We have started exploring the knowledge about the pharmacological action of the ethanolic extract of the *Dryopteris marginalis*.

On that basis I started literature review and plant are selected for this research project. Plant is collected from the Himalayan valley of Nainital, Uttarakhand and authentication was done from Council of Scientific and Industrial Research-National Institute of Science Communication and Policy Research (CSIR-NIScPR).

In this study various solvents having different polarity are used like Petroleum ether, n- hexane, Chloroform, and ethanol. Petroleum ether used to remove the Waxy material from the leaf and ethanolic extract is used for research because ethanol has an ability to extract out most of the compound. Phytochemical analysis of the ethanolic extract has been done for the estimation of the flavonoids, alkaloids saponins and glycosides etc. GC-MS analysis is carried out for the detection of the compounds in the ethanolic extract of Pteridophytes. GC- MS analysis performed in IIT Delhi/CIF facility. Result shows the presence of various compounds in the ethanolic extract.

Pharmacological studies were carried to explore the *in-vitro* anticancer activity of the ethanolic extract.

Anticancer activity observed more when compared to standard when observed against MCF-7 cell line through MTT assay.

In silico studies were done to demonstrate the inhibitory action of the compounds on the protein involved in the oncogenic pathway. Way 2 drug web-based software is used for the prediction of the pharmacological activity of the compounds.

Based on the above finding results we conclude that ethanolic extract of the *Dryopteris marginalis* have more anticancer potential. Further research is required for *Dryopteris marginalis* extracted compounds for the molecular mechanism therapy of breast cancer.

Conflict of interest

Authors declare that there is no conflict of interest

Source of funding

None declared

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