International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 368-378 www.ijprajournal.com ISSN: 2456-4494

Investigation of anti-Fungal and anti-cancer activity of leaf extracts and fractions of Parthenium Hystroporus

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Date of Submission: 15-09-2024 Date of Acceptance: 25-09-2024

ABSTRACT: This study was all about checking out the anti-cancer and anti-fungal powers of Parthenium hystroporus leaves' ethanolic extract. Parthenium hystroporus ethanolic extract was tested using the well diffusion method for antifungal and anticancer effects using the MTT assay. The results have suggested that the extract could substantially decrease the number of cancer cells and severely decrease them. This means that Parthenium hystroporus leaf extractat different concentrations affects HCT-29 cancer cell lines. The antifungal profile of PH01 it was determined by finding the inhibition zone against fungal strains (Candida ijpraalbicans) via the well diffusion method. Compound PH01 had powerful effects as compared to the standard miconazole.
KEYWORD : Parthenium Hystroporus Leaves contain Anticancer ,MTT assay. Antifungal activity, well diffusion method.

I. INTRODUCTION

The term "cancer" goes way back to the days of Hipp, a Greek physician hailed as the "Father of Medicine." Back in his time (460–370 BC), he used words like tumors can be referred to as tumors and carcinosone for one for sores and one that is not ulcer forming growths. These words came from greek, where they meant something like a crab. Well, it's because those cancerous creatures with their finger-like extensions kind of looked like crabs. Fast forward to times with 25 BC–50 AD, Celsus who changed that greekterm into "crab" from the latin "cancer" next ,in his dissioof tumors galen (c. 130–200 AD) utilized the Greek word oncos, indicating swelling.[1].

The number of lives taken by cancer has been on the rise, with a 17% increase in deaths from 2005 to 2015. We really need more research to find new anticancer drugs on top of what we already havebut what is cancer exactly? It's when our body cells start growing out of control due to messed-up cell regulation, forming malignant tumors that can spread like wildfire. Did you know they found evidence of cancer in dinosaur and human bones dating back to ancient times?When it comes to fighting cancer, we've got all sorts of drugs, like alkylating agents and antimetabolites, handy[2]. Some come from natural sources or hormones too. And let's not forget those miracle drugs.Chemotherapy often gets linked with anticancer treatments, but it's really just using chemical compounds to treat all kinds ofsickness[3]

Signs and symptoms

about the signs and symptoms of cancer. The thing is, these signs can vary depending on where in your body the problem is brewing.

Now, some basic signs to keep an eye out for not just for cancer, but in general include

- finding a lump or thickening under your skin
- skin changes like color shifts, unhealed wounds, bowel or bladder habit changes, persistent coughing or breathing
- night sweats or fevers for no apparent reason, and unusual bleeding or bruising[5,6,7].

Prevention from Cancer

- steer clear of things like tobacco and radiation that can cause it.
- Making lifestyle changes
- getting vaccinated against hepatitis B and HPV can help lower your risk.[8,9].

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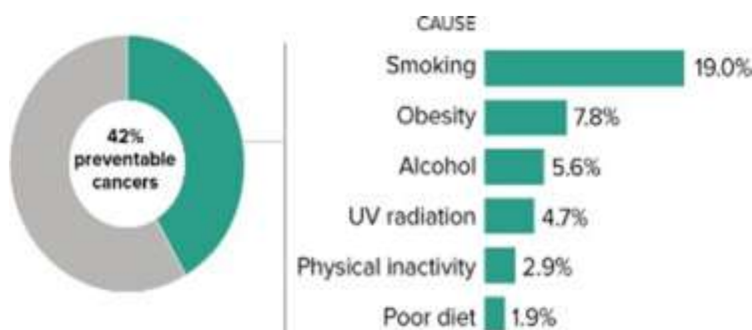

Figure No: 01 Prevention from Cancer

Causes of Cancer:

- Tobacco
- Obesity
- Age Factor[9,10]

Anti-Fungal Activity

Fungal infections pose a serious public health concern. Patients with underlying conditions, including COVID-19, are at heightened risk of life-threatening mycoses and mortality from these infections. These infections can manifest in various forms, ranging from superficial to systemic, with varying severity. Opportunistic fungi like Candida species are part of the human microbiome and can cause infections in immunocompromised individuals, such as those with HIV, cancer patients undergoing chemotherapy, and recipients of immunosuppressive drugs. Additionally, healthcare-associated fungal infections caused by pathogens like Candida, Aspergillus, Fusarium, Mucorales, and molds can occur in patients with underlying diseases. Certain geographic regions also experience endemic, life-threatening mycoses, including blastomycosis, coccidioidomycosis, histoplasmosis, talaromycosis, paracoccidioidomycosis, and sporotrichosis.[10]

To address this public health concern, the Centers for Disease Control and Prevention (CDC) have designated September 20-24, 2021 as Fungal Disease Awareness Week, aiming to enhance public understanding of fungal infections and emphasize the importance of early detection in mitigating their debilitating effects.[10,11]

Signsand symptoms

Skin reactions:Itchy skin, burning sensation, rash, blisters.

Allergic reactions: may cause swelling of the face, neck, or tongue, difficulty breathing[12]

Prevention from Anti fungal[13]

- After cleansing, completely towel off your skin, being careful between skin folds.
- Steer clear of direct contact with individuals who have a fungal infection.
- Foot hygiene
- Antifungal medicine

Some specific antifungal medications and their side effects include[14]

- Isavuconazole: gastrointestinal adverse effects are more severe than with other azoles
- Miconazole: this can result in pruritis, burning or stinging at the application site, and contact dermatitis.

II. REVIEW OF LITERATURE

TABLE NO.01 SCIENTIFICCLASSIFICATION[15]

Kingdom	Plantae
subkingdom	Green plant (viridiplantae)
Infrakingdom	Group of plant (Streptophyta)
Division	vascular plants (Tracheophyta)
subdivision	seed plants (Embryophyta)
Order	Asterales
Family	Asteraceae
Genus	Parthenium
Species	P. Hystroporus

TABLE NO.02 CommonNames[15]

English	Carrot Weed
Hindi	Gajarghas
Kannada	Congress Gida
Marath	Gajargavat
Telugu	Congress Gaddi

Figure No. 02: Parthenium Hystroporus Plant

History

Up to 80% of people globally still receive their main medical treatment from traditional medicine, especially in developing countries. This is because it is less harmful and has a higher level of societal acceptance and appropriateness. As plants serve so widely to treat a wide range of conditions, a great deal of research has been done on their biological activity for medicinal purpose.

The smelled , upright branches ,annual short-lived annual herb parthenium hystroporus has a deep Taproot.It grows quickly and reproduces through seeds.it can reach a Hight of 30 to 90 cm in the typical neotropical area, but in exotic settings, it may grow as Hight as 1.5 or even 2.5 meter.the

seedling rapidly grows into a basal rosette of 8-2 cm long by 4-8 cm wide, pale green, pubescent leaves with deep lobed margins. Long-term persistence of this rosette stage is possible under adverse circumstances like water or cold stress. The stem elongates and produces alternating rows of smaller, narrower,and less divided leaves.[7,15].

Medicinal importance[7,15].

1. Skin inflamation
2. Urine track infection
3. Rheumatic pain

Chemical[16] **ingredients**

Common phytoconstituents include daucosterol, sitosterol, triterpenoid sitosterol, flavonoids, steroids, glycosides, and stigmasterol-3-O-glucopyranosid. Parthenin, 16-dihydroxyolean-12-en-28-oic acid, oleanolic acid, 3-oxo-16-hydroxyolean-12-en-28-oic acid, the oleanolic acid derivative 28-O--D-glucopyranoside, and dichrocepholide D were the six determined terpenoids. Three-O-methylquercetin is a benzened derivative of a flavonoid.

Table No. 03 Chemical composition of P. hystroporus leaf ethanol extract

Sr no.	RT	Compounds name	Molecular formula	Molecular weight	Peak area(%)
1	12.289	Caryophyllene	$C_{15}H_{24}$	204.35	10.37
2	13.386	β-Cubebene	$C_{15}H_{24}$	204.35	11.98
3	20.774	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	11.22
4	21.850	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47	8.74
5	23.622	Phytol	$C_{20}H_{40}O_2$	296.5	38.68
6	23.880	Methyl stearate	$C_{19}H_{38}O_2$	-	9.01
7	9.98	9,12,15-Octadecatrienoic acid, ethyl ester, (z,z,z)	$C_{20}H_{34}O_2$	306.48	9.98
8	60.12	Stigmasterol 3	$C_{13}H_{50}O_2$	454.74	82.38
9	60.12	Stigmasterol-3-o-glycopyronosid	$C_{35}H_{58}O_6$	578.8	

METHODOLOGY

Table No.04: List of Chemicals and Reagents

SI.NO	Chemicalsandreagents
1.	Ethanol
2.	Dil. HCL
3.	Dragondroff'sReagent
4.	AlphaNaphthol
5.	Conc.SulphuricAcid
6.	FerricChloride
7.	Chloroform
8.	GlacialAceticAcid
9.	Conc.H2SO4
10.	NinhydrinReagent
11.	Wagner'sReagent
12.	TricholoroaceticAcid (TCA)
13.	Magnesium
14.	AceticAnhydride
15	DMEM(Dulbecco'sModifiedEagleMedium)
16	DMSO

Table No. 05: List of Apparatus and Equipment's

SI.NO	Apparatus/equipment's
1.	SoxhletApparatus
2.	RotaryFlashEvaporator
3.	Desiccator
4.	WaterBath
5.	Incubator
6.	Refrigerator
7.	BiologicalSafetyCabinet
8.	Cell Culture
9.	Cell Counter
11.	Pipettes,SyringesAndNeedles
12.	Cells
13.	3-[4,5-dimethylthiazol-2-yl] MTT (3-Tetrazolium bromide-2,5-diphenyl
14.	PH Meter
15.	CentrifugeMachine
16.	T-20 Flask
17	well diffusion media

Determination of acute toxicity

The leaves of Parthenium hystroporus were gathered and cautiously washed to get rid of any dirt before being listed in the herbarium.

1. The cleaned leaves were subsequently allowed to dry for approximately three to four days on the paper sheets. The leaves were then dried and pulverized in a grinder.
2. Soxhlet apparatus extraction of solvents: ethanol-based alcoholic extraction.
3. Fracted using a solvent incorporating chloroform.

Collection of plant

The mature leaves of plant Parthenium Hystroporuswere gathered, after identification and authentication by Dr. Ramachandra Naik, who is a Professor and Head of the Botanical department at KCP Science College in Vijayapura, Karnataka.

Extract fabrication:

The plant leaves were air-dried at ambient conditions, then ground into a gritty dust.This gritty dust has placed to an ethanol extraction process using a Soxhlet apprataus. The resulting extract was subsequently centered using a rotating evaporator. The total yield of the crude extract was 93.04%, which was stored in a closed container and maintained frozen at a heat below 10 °C for further research.

1. Preliminary phytochemical analysis
2. Cancer preventive activity
3. Anti- Fungal Activity

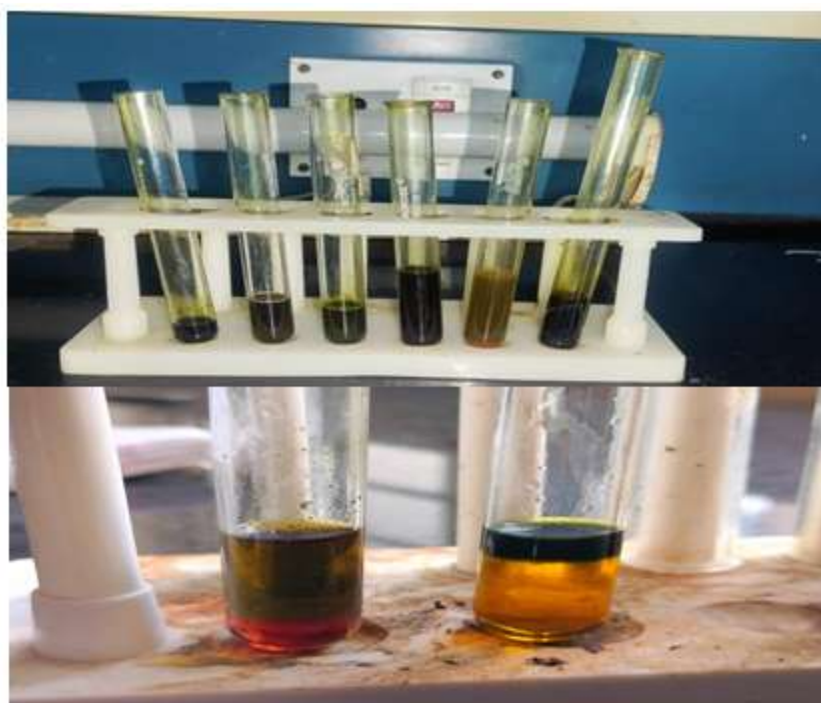

Figure No: 03 Phytochemical evaluation: anti-cancer activity

Cancer preventive Activity

- **Methodology for MTT Assays[17]**

The MTT technique is a good technique for determining the impact of various substances in vitro proliferation of casual or cancerous cell lines. The test is carried out on polystyrene microtiter plate with 96 and a flat bottom. suspended cells are in the adding the proper growing media to the replicated wells. Before introducing the test compounds or agents, it is best to start to start by filling the necessary number of wells with the cells. and allow them to settle and attach (for adherent cell lines) while the test substances are being prepared.

The drugs or other chemicals to be evaluated are then added to each set of replicate wells at predetermined concentrations. It is important that the test agents are adequately dissolved, as protein-based agents like antibodies and cytokines are not affected by aqueous solubility,

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 368-378 www.ijprajournal.com ISSN: 2456-4494

whereas some small -molecule drugs havelow solubility when water.These are often split in solvents like dimethyl formamide (DMF), with DMSO being chose as it is less harmful to cultured cells. Since the compound of concern typically exhibit Chemicals should be dissolved at level with biological activity below the micromolar level. ranging from 10–100 mM to create a stock solution that is 1000–5000 times more concentrated.[17]

The range about amounts to be tested is determined based on prior knowledge toward the substance and the cell line(s) being used. If no information is available, a higher start has to serve as chosen, it came at least five tries to try to address a 100-fold limit, typically using threefold or higher repeated dilutions. Once the changing variety of inhibitory activity has been established, a tighter range for concentration can examined by making twofold dilutions starting from the maximum concentration with a minimum volume of two ml of the optimal tissue culture media.

Later preparing the first tube by including appropriate amount of test agent in its stock solution and thoroughly mixing it, 1 mL is added to the second tube from the first tube.after that tube 2 is combined,and 1ml from it is put into tube 3, etc. until the desired quantity of tube is reached.

Cells are allowed to develop in an incubator (typically 37 °C with supplemented CO2). The number of cells to add to each well is determined by the duration of the test. An incubation time of 5-7 days is appropriate for evaluating the antiproliferative effects of a wide spectrum of substances.

Untreated cells that serve as control should be allowed to proliferate until the degree of cellular confluence is 70-90% the dynamic range of this experiment. This parameter should be maintained on a daily basis by viewing plates with an inverted microscope.

Processing Adherent Cell Plates for the MTT Assay

When the cells in the untreated wells have reached 70–90% confluence, add 5mg/ml of MTT to the plate. the procedure for MTT add and plate harvesting differs based on the populations or cell lines, have gave or non-adherent patterns of growth. Specifically, the distinction lies in which trypsin medication is required to detach the cells from the culture flask surface. The following approach can be used for cell types thatadhere,such fibroblast like and epithelial cells.

1. Prepare an MTT stock solution on PBS. Sterile conditions are not necessary for the assay's last stage.
2. Take out the microplate from the incubator gently shake the majority of the culture by placing it over a container media, and blot the residual medium on paper towels.
3. Dilute the MTT stock solution 1:10 in tissue culture medium (RPMI-1640-based) to make a working solution, then pour 100µL of this mixture into every well using a repeating pipette.
4. Incubate the plate for a minimum of four hours to enable the MTT to fully convert.
5. To pellet the precipitated formazan dye, centrifuge the plate then invert and blot the plate in order to drain most of the liquid.
6. Make the MTT product (formazan) soluble in each well with 100µL of DMSO. To accelerate dye solubilization, return the plate to the incubator set at 37°Cgently 5-10times tap the plate minutes. Solubilization is normally finished in 30 minutes. Alternatively, apply an acid-isopropanol solvent to adhering cell typesadding an equal volume (100µL) to each well. In this case, the plate read through pages 5-15 minutes after the acid-isopropanol solution is added, as the if the solution is let to settle ,any leftover protein may precipitate sit for longer.

Cell Viability Assay[17]

HCT-29 colon cancer the culture medium used for the cells was DMEM with 10% FBS in a T-20 flask until they reached approximately 80% confluency. Using trypsin EDTA, the cells were subsequently separated from the flask. The detached cells were counted and seeded into 96-well plates. After that the cells were exposed to different extract concentrations anda vehicle/DMSO control for around 24 hours.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 368-378 www.ijprajournal.com ISSN: 2456-4494

Following the removal of the medium, phosphate buffered saline 9PBS0 was used to wash the cells. An MTT solution with a concentration of each well received 0.5mg/ml, and the plate was then darkly incubated for 4 hours at 37°C. The plate was jointed at 180 rpm for 10 minutes, use a plate reader to determine the optical density at 570nm (Tecan). The test was conducted once more twice, and the data was used to plot a graph.

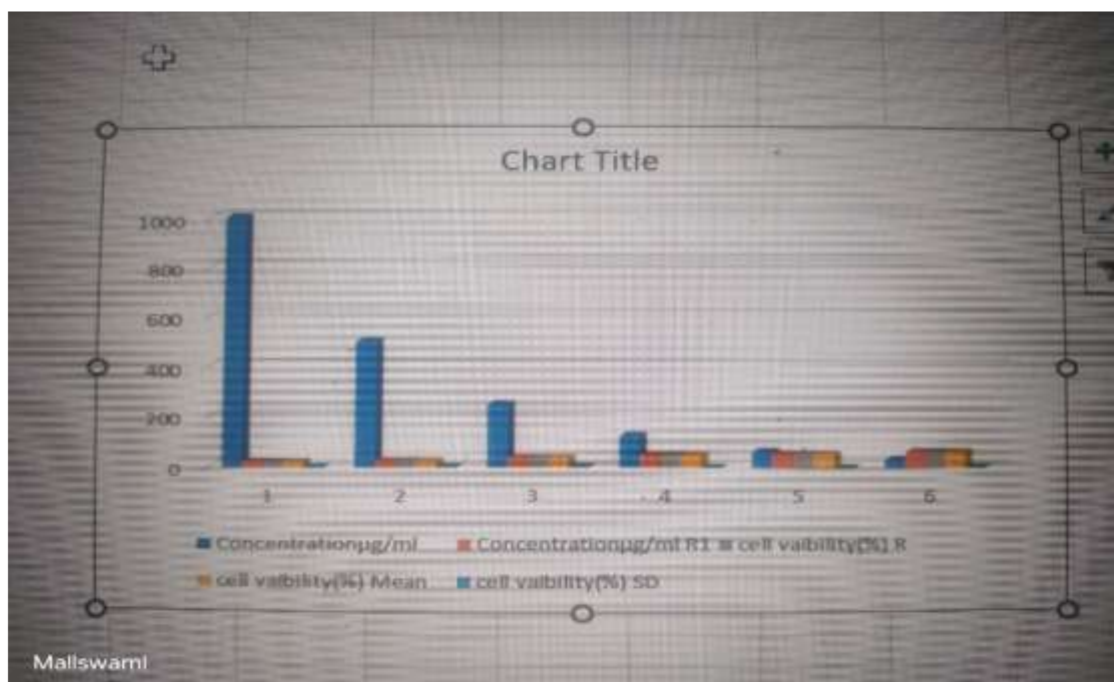

Figure No.04 cell vaibility in Chart

Anti-fungal Activity[14]

1. The vaccine microorganism made using the bacterial cultures. 15ml of Saubroadin sterile cleaned petri dishes agar medium was added, and the plates were let to cool and set.
2. 100µl of the medium was evenly covered with the pipetted fungal strain broth and allowed to dry completely using a spreading rod
3. 6mm diameter wells were drilled using a sterile cork borerresolutions of thecompounds (100µl/ml)were prepared in DMSO and 100µl of prepared test solutionsand standard to the well was introduced the petri dishes were cultured at37°C for 24 h.
4. Miconazole 1mgwas ready as the control positive and DMSO was used as the negative one.
5. The area of limits diameter were used to judge the antimicrobial properties every test were done in triplicate.

III. RESULTS:

Preliminary phytochemical screening

The results obtained from the phytochemical analysis of the ethanolic extract of Parthenium hysterophorus leaves revealed the presence of various phytochemical constituents, including carbohydrates, proteins, amino acids, alkaloids, phenols, flavonoids, phytosterols, glycosides, saponins, and oils and fats.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 368-378 www.ijprajournal.com ISSN: 2456-4494

Sr .No	Phytochemical Components	Test	Ethanolic Extract Of Parthenium hystroporus	
			Alcohol	Water
1	Carbohydrates	Molisch's Test	+	+
		Benedicts test	+	+
		Fehling's Test	+	+
2	Proteins And Amino Acids	Ninhydrin's Test	+	+
		Xanthoproteic Test	+	-
3	Alkaloids	Dragendrof's Test	+	+
		Wagner's Test	+	+
		Hagers test	+	+
		Mayers test	+	-
4	Phenols	Ferric Chloride Test	+	+
		Lead Acetate Test	+	+
5	Flavonoids	Shinoda Test	+	-
		Alkaline Reagent Test	-	-
		Sodium hydroxide	+	+
6	Phytosterols	Salkowski Test	+	+
		Libermann Buchard's Test	+	+
7	Glycosides	Keller Kilani Test	-	-
		NaOH Test	-	-
		Baljet test	-	-
		3,5 dinitrobenzoic test	-	-
8	Saponins	Froth Test	+	
		Olive Oil Test	+	+
9	Gums And Mucilages	Alcohol Test	-	+
		Ruthenium Red Test	+	+
10	Oils And Fats	Spot Test	+	

Table No. 06 : Phytochemical screening of Parthenium hystroporus leaf extract

Evaluation of anti-cancer activity of test extract:
Cell Viability Assay:

The MTT test was conducted to evaluate the anticancer effects of the Parthenium Hystroporus plant extract on HCT-29 cell lines at different concentrations. As illustrated in Figure 15 and observed through the MTT assay, the extract decreased the feasibility of HCT-29 cell in a way that is dependent concentration. The on HCT-29 cell viability was found to be 28%, 33%, 41%, 58%, 78%, 85%, and 100% at extract concentrations of 310, 150, 70, 30, 20, 08, and 00 ug, respectively. The IC 50 of the extract is shown in the graph below.

International Journal of Pharmaceutical Research and Applications
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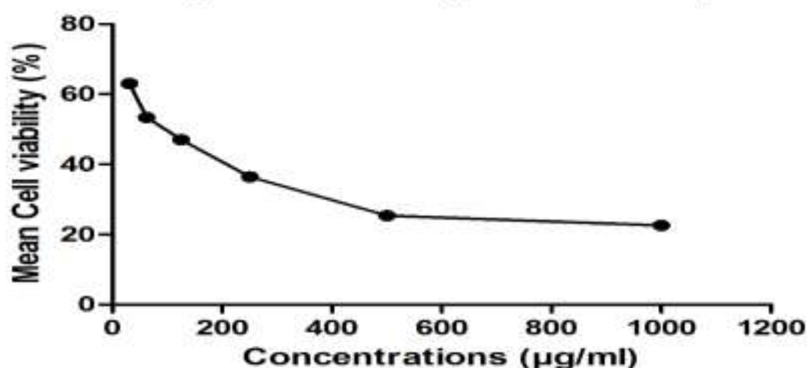

FIGURE NO .05 Evaluationof Anti -Fungalactivityoffractionated extract:

Table No.07Antifungal Activity of test samples against Candida albicans

SR.NO	SAMPLES	ZONE IN DIAMETER (mm)
1	Control	0
2	Standard (Miconazole)	24
3	PH-01	13

FIGURE NO .06 well diffusion method

IV. DISCUSSION

Parthenium hystroporusis is a tomentose shrub that is widely distributed in India, North Africa, Asia, and Australia. The plant can reach a Hight of two meters and produces small, ovoid fruits with tasteless seeds. Its leaves possess antibacterial, antioxidant, and antifungal properties.

Parthenium hystroporusis is a perennial herb to shrub, rarely a small tree, that grows abundantly along roadsides, in open fields, and in garden waste spots. The genus is distinguished from the rest of the Malvaceae family by the absence of an epicalyx, wingless mericarps, and the presence of an endoglossum. It was differentiated from the closely related uni-ovulated genus Sida by the presence of more than one ovule in a locule, and the flower in Abutilon opens in the evening, whereas the flower in Side opens in the morning[14].

The phytochemical examination within the ethanolic extract of Parthenium hystroporus leaves revealed alkaloid, protein, carbohydrate, and amino

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 368-378 www.ijprajournal.com ISSN: 2456-4494

acid phenols, flavonoids, phytosterols, glycosides, saponins, and oils and fats.

A MTT assay has conducted to study the anticancer effects of the plant extract of parthenium hystroporus at different concentrations on HCT-29 cell lines. The extract decreased HCT-29 cell viability in a concentration-dependent manner. The HCT-29 cell viability was found to be 28%, 33%, 41%, 58%, 78%, 85%, and 100% at 310, 150, 70, 30, 20, 08, and 00 ug of the extract, respectively[16].

Thusit can be said that parthenium plantsHystroporus has anti-fungal and anti-cancer activity due to the presence of chemical constituents such as carbohydrates, proteins, amino acids, alkaloids, phenols, flavonoids, phytosterols, glycosides, saponins, and oils and fats.

The vaccine of the microorganism was made using bacterial cultures. 15ml of Saubroadin sterile cleaned petri dishes agar medium was addedlet to cool and become solid 100µl of the fungal strain broth was pipetted out and evenly distributed over the medium using a spreading rod until it had dried out using a sterile cork borer 6mm diameter wells were bored.Resolution of the compounds (100µl/ml) were prepared in DMSO and 100µl of prepared test solutions and standard to the wells was added petri dishes were incubated37°C for 24 h. Miconazole (1mg/ml) was prepared as a positive control and As a negative control DMSO was used the zone of inhibitions diameters were used to measure the antibacterial activity all measurement were made in triplicate.

V. CONCLUSION

It is possible to conclude based on the data that the leaves of Parthenium hystroporus have an anticancer effect. Nonetheless additional research is required to identify the specific chemical constituent(s) responsible for the observed effects in this study[16,17].

The antifungal profile of PH01 was assessed by calculating the inhibition zone against fungal. strains (Candida albicans(NTCT1699) via well diffusion technique. The compounds PH01exhibitedgood activity as compared to the standard Miconazole[14].

SUMMARY

The present study aimed to investigate the anti-cancer properties of Parthenium hysterophorus leaves in experimental animal models.

The mature Parthenium hysterophorus leaves were collected after identification and authentication by Dr. Ramachandra Naik, Professor and Head of the Department of Botany at S.B. Arts and KCP Science College, Vijayapur, Karnataka.

The leaf powder was packed into a Soxhlet column and extracted with 90% ethanol. The extract was then subjected to the following studies:

1. Preliminary phytochemical investigation
2. Anti-cancer evaluation
3. Anti-fungal evaluation

The key findings are as follows:

- Preliminary phytochemical analysis of the ethanolic extract of Parthenium hysterophorus leaves (EEAPL) revealed the presence of carbohydrates, proteins, amino acids, alkaloids, phenols, flavonoids, phytosterols, glycosides, saponins, and oils and fats.
- The cytotoxic activity of EEAPL was assessed using the MTT assay for anti-cancer effects, and the results showed a dose-dependent decrease in the viability of HCT-29 cancer cells.
- The HCT-29 cell viability was found to be 28%, 33%, 41%, 58%, 78%, 85%, and 100% at EEAPL concentrations of 310, 150, 70, 30, 20, 8, and 0 µg, respectively.
- The anti-cancer efficacy of EEAPL was attributed to the presence of the identified phytochemical constituents, including carbohydrates, proteins, amino acids, alkaloids, phenols, flavonoids, phytosterols, glycosides, saponins, and oils and fats.
- The inoculum of the microorganism was made using bacterial culture in sterile petri dishes 15milliliter of saubroad agar medium were added and the plates were left to cool and harden.
- 100µl of broth of fungal until the medium was adequately dry the strain was pipetted out and distributed evenly using a spreading rod.
- A sterile cork borer was used to bore well with a diameter of 6mm resolution of the compounds (100µl/ml) were prepared in DMSO and 100µl of prepared test solutions and standard was added to the wells.
- The petri dishes were incubated for twentyfour hours at 370c DMSO was used as the negative control and miconazole(1 mg/ml) was generated as the positive control.
- Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination were performed in triplicates.

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Volume 9, Issue 5 Sep - Oct 2024, pp: 368-378 www.ijprajournal.com ISSN: 2456-4494

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