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INVITRO ANTICANCER ACTIVITIES OF PTEROCARPUS MARSUPIUM EXTRACTS AGAINST HUMAN CANCER CELL LINES

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Abstract:

There is evidence of herbs having been used in the treatment of various diseases. Hence Pterocarpus marsupium selected for the present investigation on in vitro anticancer activity. Hence these plant extracts may have clinical and therapeutic proposition in the most life threaten disease like cancer and further studies are required to investigate these plant samples as antineoplastic agents. Therefore, it is anticipated that plants can provide potential bioactive compounds for the development of new 'leads' to combat cancer diseases.

Key words: Invitro, Anticancer Activities, Pterocarpus Marsupium Extracts, Human Cancer Cell Lines

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INTRODUCTION:

Malignant growth is one of the significant human sicknesses and causes huge affliction and financial misfortune around the world. Chemotherapy is one of the techniques for treating disease. Notwithstanding, the chemotherapeutic medications are profoundly harmful and make wrecking side impacts. Different new techniques are being created to control and treat a few human tumors [1]. More than 60% of anticancer medications accessible in the market are of regular beginning. Normal items are additionally the lead particles for the majority of the medications that are being used [2]. Accordingly, the phytochemicals present in a few home grown items and plants might can possibly go about as preventive or helpful specialists against different human malignant growth [1]. The expanded ubiquity of home grown solutions for disease treatment maybe can be ascribed to the conviction that natural medications give benefit over that of allopathy prescriptions while being less poisonous [3]. Since the ordinary treatments make decimating side impacts, there is a persistent requirement for search of new natural fixes of disease.

Pterocarpus marsupium plant having a place with family Fabaceae has been utilized in India and its neighboring nations because of its different organic exercises from old times. All pieces of P. marsupium is utilized as a crude medication for homegrown cure against a few human illnesses. It has been comprehensively utilized in Homeopathic, Ayurvedic and Unani frameworks of medicine.[4-5] It is a deciduous tree, by and large known as Malabar or Indian Kino tree (Table 1).[6-7] It is available particularly in Western Ghats regions, Karnataka-Kerala districts and found in Madhya Pradesh, Bihar, Gujarat and Orissa.[8] Generally, the plant item are being utilized as cooling, outer application as anthelminthic, migraine, antipyretic, mitigating, Spanish fly, in mental distortions, biliousness and ulcers.[9] Phytochemical studies have uncovered that the plant contains terpenoids, aurone and isoflavonoids glycosides and related phenolic compounds, lupenol, epicatechin, β-sitosterol.[10] P. marsupium is the exceptionally rich wellsprings of flavonoid and polyphenolic compounds.[11] Its heartwood has calming, astringent, anesthetic and antidiabetic properties and furthermore waterfall, hypertriglyceridemia, cardiotonic, hepatoprotective action and as a particular inhibitor of COX-2.[12-15]

Spices are favored on the grounds that they produce no unfavorable outcome concerning their prevalence and remedial utility. There is proof of spices having been utilized in the treatment of different illnesses. Subsequently Pterocarpus marsupium chose for the current examination on in vitro anticancer movement.

METHODOLOGY:

Preparation of extracts of *Pterocarpus marsupium*

The powdered Material (1kg) was sequentially extracted using chloroform, ethanol and aqueous solution in Soxhlet apparatus. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation.

Brine Shrimp Lethality Bioassay:

The cytotoxic capability of concentrate of Pterocarpus not entirely settled by Brackish water shrimp lethality bioassay. Momentarily, eggs of saline solution shrimp Artemia salina were brought forth in a compartment loaded up with airbubbled counterfeit ocean water which was arranged utilizing 10 g of a business salt combination (GEX Inc., Osaka, Japan) and 500 ml of refined water. Following 36-48 hours, the phototropic shrimps were gathered and utilized for bioassay. To the vials containing various groupings of concentrates in ocean water (1, 10, 25, 50 and 100 µg/ml), 25 shrimps were added and the vials were brooded at 25oC and the enduring shrimps were counted following 24 hours. TheLC50 upsides of concentrates more noteworthy than1000 µg/ml were viewed as latent (non-harmful). Potassium dichromate was utilized as reference standard.

***In vitro* Anticancer activity**

Cell lines and culture conditions

HeLa (Human cervical cancer cell line) and PC3 (Human prostate cancer cell line) cell lines were procured from NCCS, Pune, India. Cells were grown in Minimum Essential Medium Eagle (Gibco, UK) supplemented with 10% heat inactivated fetal bovine serum (Gibco, UK), 29 µg/ml L-glutamine, and 40 µg/ml Gentamicin. Cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

Antiproliferative activity

The antiproliferative action of plant separates was estimated utilizing MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) measure (Promega, USA). The examine identifies the decrease of MTT by mitochondrial dehydrogenase to blue formazan item, which mirrors the typical capability of mitochondria and cell reasonability [38].

Dramatically developing cells were washed and cultivated at 17000 cells/well (in 200 µl of development medium) in 96 well microplates (Nunc, Denmark). After 24 h brooding, a fractional monolayer was framed then the media was eliminated and 200 µl of the medium containing the plant extricate (at first disintegrated in DMSO) were added

and yet again hatched for 48 h. Then, at that point, 100 µl of the medium were suctioned and 15 µl of the MTT arrangement were added to the excess medium (100 µl) in each well. After 4 h contact with the MTT arrangement, blue precious stones were framed. 100 µl of the stop arrangement were added and brooded further for 1h. Decreased MTT was measured at 550 nm utilizing a microplate peruser (Das, Italy). Control bunches got a similar measure of DMSO (0.1%).Untreated cells were utilized as a negative control while, cells treated with vincristine sulfate were utilized as a positive control at the accompanying focuses 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100 nM.

IC50 values were determined as the focuses that show half restraint of multiplication on any tried cell line.

Stock arrangements of the plant remove were disintegrated in (DMSO) then, at that point, weakened with the medium and disinfected utilizing 0.2 µm film channels. The last weakening of concentrates utilized for treating the cells contained not over 0.1% DMSO. Information were accounted for as the normal of three duplicates. The antiproliferative impact of the tried not entirely settled by contrasting the optical thickness of the treated cells against the optical thickness of the control (untreated cells).

DNA fragmentation assay

To decide the concentrates prompt apoptosis in SiHa cells, DNA discontinuity measure by agarose gel electrophoresis was performed . The cells (1 x 106) were cultivated in 60 mm tissues culture dish treated regardless of medication and brooded for 48 h. Cells were collected by centrifugation and lysed in ice for 30 min by the expansion of 20 µl lysis support contains 20 mM EDTA, 100 mM Tris (pH 8.0), and 0.8% (w/v) sodium lauryl sarcosine. The lysates was processed with RNase A (2 µl, 5 mg/ml) and proteinase K (20 µl, 10 mg/µl) at 37˚C for 1 h and 2 h, separately. Absolute lysates were stacked onto 1.5% agarose gel stained with 0.5 µg/ml ethidium bromide and isolated at 50 mV. DNA pieces were imagined by the Gel Doc 100 framework (Bio-Rad; Hercules, CA).

Statistical Analysis

All experiments were repeated at least three times. At least quadruplicate cultures were scored for an experimental point. All values were expressed as mean ± S.E.M. The Student's one tail t-test was applied for statistical treatment of the results; $p < 0.05$ were considered as the statistically significant value.

RESULTS:

The preliminary phytochemical screening of *Pterocarpus marsupium*and *H. indicum* leaf extracts showed presence of steroids and sterols, triterpenoids, alkaloids, flavonoids, saponins, tannins and phenolic substances, gums and mucilages, carbohydrates, and proteins, respectively, in different extracts (Table 2).

Brine Shrimp Lethality Bioassay

The result of cytotoxic potential of ethanol extract of *Pterocarpus marsupium*and *H. indicum* in terms of mortality of brine shrimps (%) is presented in Figure 1. The degree of lethality was directly proportional to the concentration of the extracts. The percentage mortality of shrimps was recorded higher in case of *Pterocarpus marsupium*LC_{50} were 50 and 25 µg/ml of chloroform and ethanol extract respectively (Figure 1) than that of *H. indicum* LC_{50} were 25 and 25 µg/ml of chloroform and ethanol extract respectively (Figure 2). Extract of *H. indicum* showed more lethality when compared with the reference control i.e., potassium dichromate (LC_{50} 32.77µg/ml). Highest mortality (100%) was observed at concentration 100 µg/ml of both the extracts. The aqueous extracts of both the plats didn't show any mortality in brine shrimp assay.

***In Vitro* Anticancer Activity**

Antiproliferative activity

Human prostate cancer cell line (PC-3) and Human cervical cancer cell line (HeLa) were exposed to chloroform and ethanol extract of *Pterocarpus marsupium* and *H. indicum* for24 h and cytotoxicity was determined with the MTT assay. The Percentage cancer cell inhibition profiles were found to be concentration dependent (Figures 3-6). The maximum concentration used in the study was 500 mg/ml. HeLa cell lines, when subjected to different concentrations of plant extracts displayed weak inhibition of 31.25%. It was observed from figures 3-6 that a gradual increase in percentage inhibition was observed in all the cases.

The extracts of *Pterocarpus marsupium* exhibited significant cytotoxic activity against PC3 cell line with IC_{50} values of 70.23 mg/ml and 79.02 mg/ml for chloroform and ethanol fraction respectively (Figure 3), where good cytotoxicity were shown against HeLa cells with IC_{50} values of 48.13 mg/ml ad 59.87 mg/ml for chloroform and ethanol fraction respectively (Figure 5). Similarly, the extracts of *H. indicum* exhibited IC_{50} of 67.23 mg/ml

and 72.23 mg/ml for chloroform and ethanol fraction respectively (Figure 4), where good cytotoxicity were shown against HeLa cell line with IC_{50} values of 58.43 mg/ml ad 64.23 mg/ml for chloroform and ethanol fraction respectively (Figure 6). Whereas, aqueous extracts of both the plants were found to be non toxic in both the cell lines.

DNA fragmentation assay

Induction of apoptosis on HeLa and PC3 cells by *Pterocarpus marsupium*and *H. indicum* extracts was validated by DNA fragmentation analysis using gel electrophoresis technique. The DNA bands obtained from both extract-treated HeLa and PC3 produced ladder pattern as observed from Lane 2 to 7 (Figure 7). A ladder formation was used to indicate that the DNA has undergone fragmentation, and each fragment corresponded to aband in the ladder.

Table 1: Successive extraction of Chloroform, ethanol ad aqueous extracts of *Pterocarpus marsupium*

S.No.	Extracts	Percentage yield ofextracts of *Pterocarpus marsupium* w/w
1.	Chloroform	4.23
2.	Ethanol	5.35
3.	Aqueous	13.8

Table 2: Phytochemical screening of Chloroform, ethanol ad aqueous extracts of Pterocarpus marsupium

Tests	*Pterocarpus marsupium*		
	CE	EE	AE
Alkaloids	+	+	-
Carbohydrates	-	-	+
Glycosides	-	-	-
Gums and mucilages	-	-	+
Proteins and amino acids	-	-	-
Tannins and phenolic compounds	-	+	-
Steroids and sterols	+	+	-
Triterpenoids	+	-	-
Saponins	-	-	+
Flavonoids	-	-	+

(+) indicates presence and **(-)** indicates absence of phytochemicals

CE – Chloroform extract, **EE** – Ethanol extract, **AE** – Aqueous extract

Table 3: Brine shrimp lethality bioassay of *Pterocarpus marsupium*

Conc. (µg/ml)	LogC	% Mortality (*Pterocarpus marsupium)*		
		Chloroform extract	Ethanol extract	Aqueous extract
200	2.301	100	90	100
100	2.000	80	80	100
50	1.699	50	70	100
25	1.398	45	50	90
10	1.010	40	40	80
5	0.750	30	30	70
3	0.450	20	20	70
1	0.150	10	10	60

Figure 1: Brine shrimp lethality bioassay of *Pterocarpus marsupium*

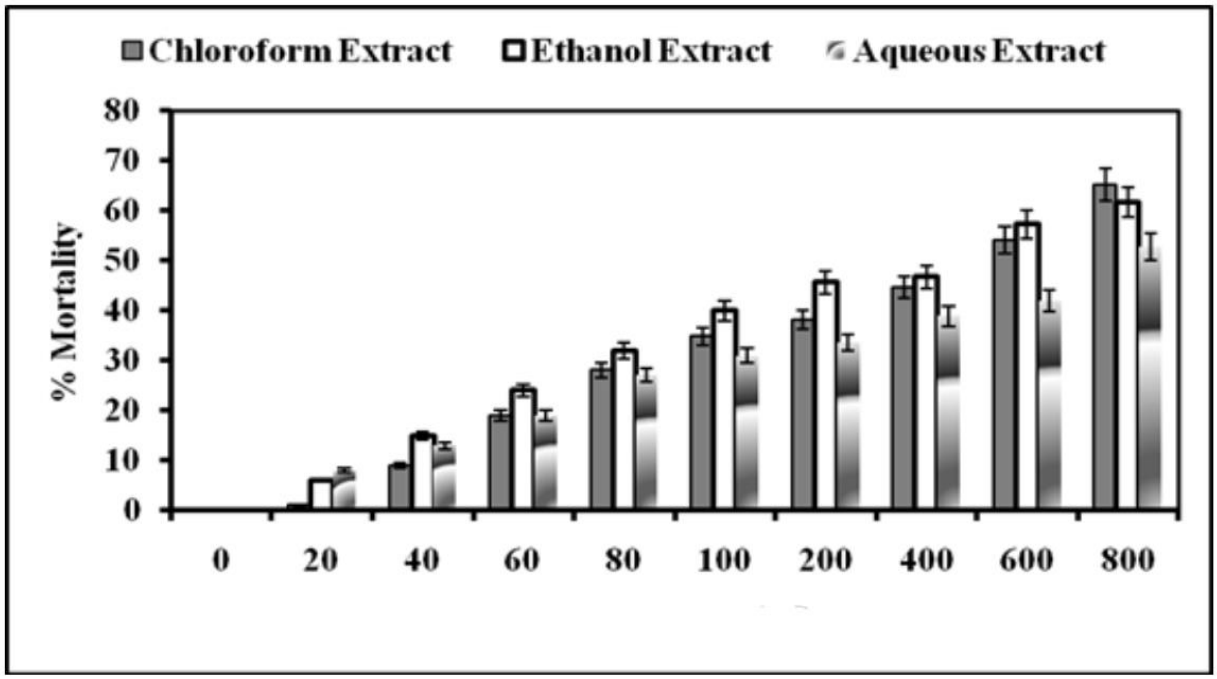


Figure 3: Antiproliferative activity of extracts of *Pterocarpus marsupium* in PC3 cell line

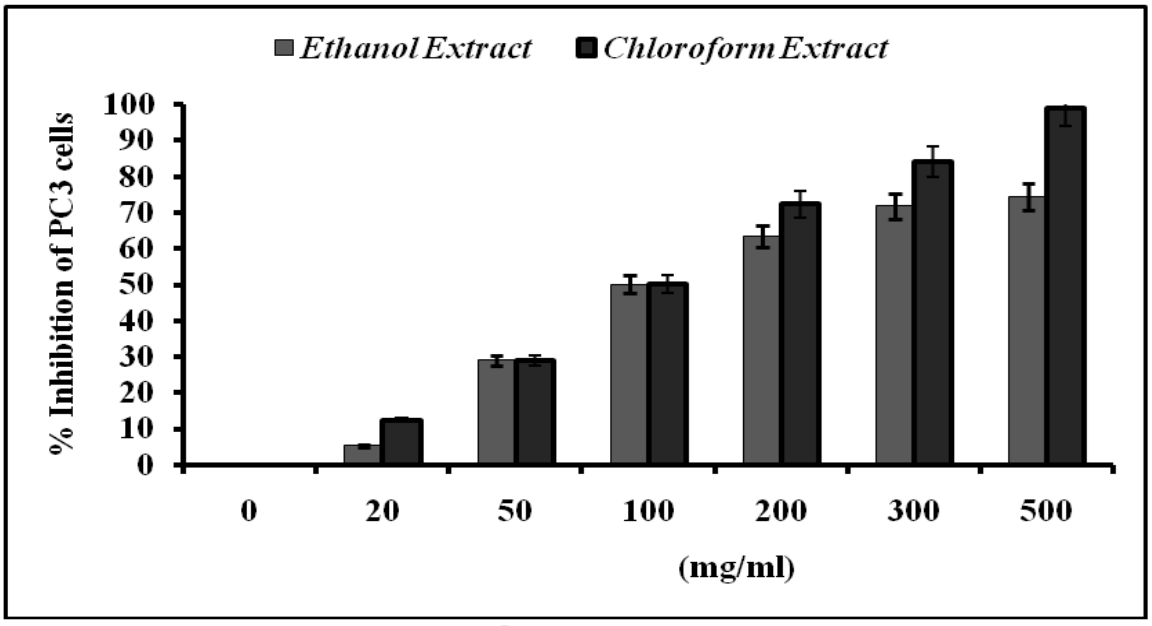
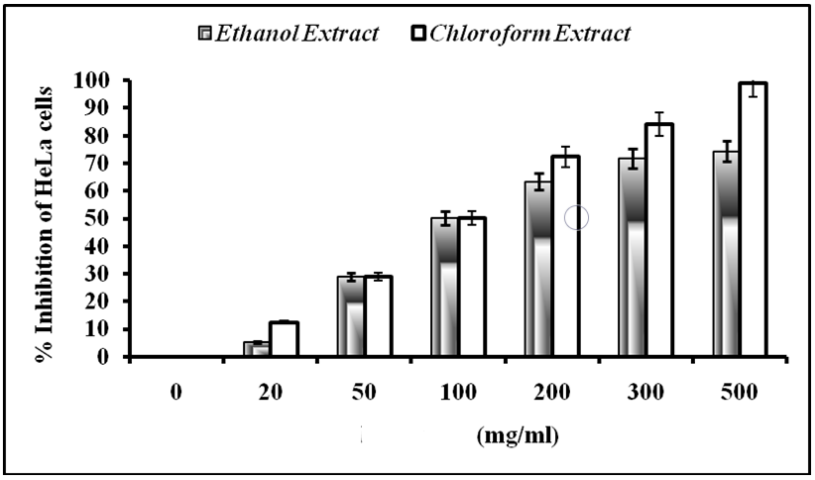
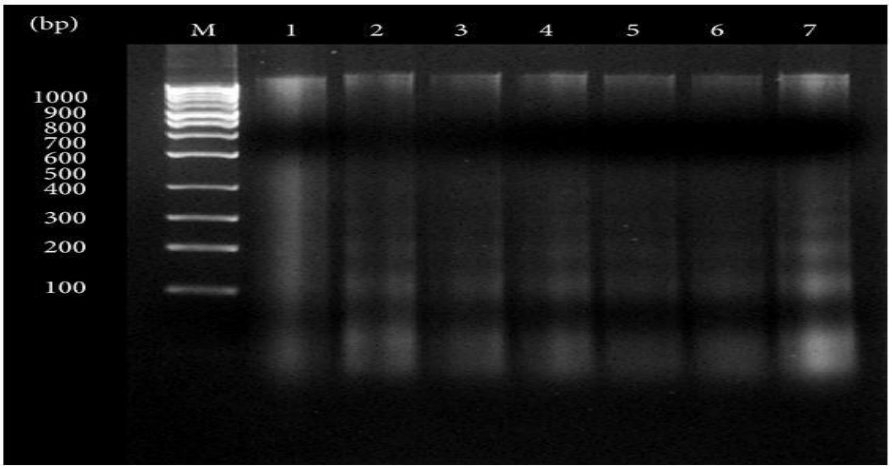
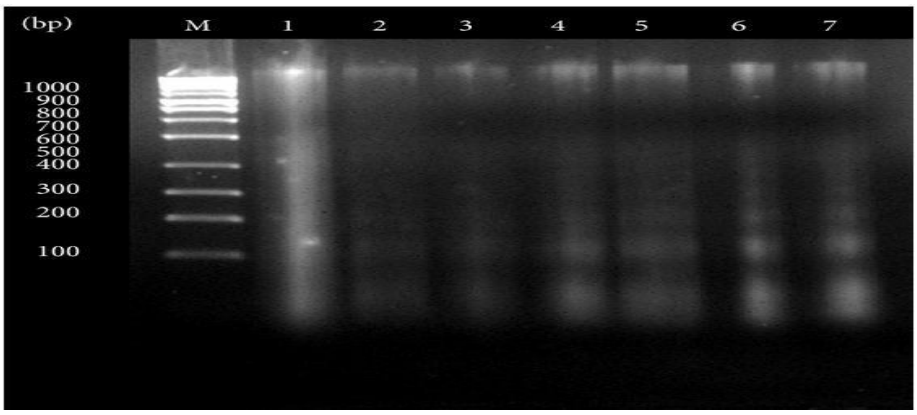


Figure 4: Antiproliferative activity of extracts of *Pterocarpus marsupium* in HeLa cell line





(a)



(b)

Figure 7: DNA band patterns of (a) HeLa and (b) PC3 cells treated with various concentrations of *Pterocarpus marsupium*. Lane 1: negative control; Lanes 2 to 3 were bands of cancer cells treated with 100 and 200 mg/mL extract

DISCUSSION:

Since the presence of individual, plants have been taken advantage of for a few purposes including restorative purposes. Plants are the essential wellspring of organically dynamic phytochemicals present in traditional medicaments. Restorative frameworks viz., Ayurveda, Unani and Sidda utilize the utilization of these plants for treatment of sicknesses. Ethnobotanical concentrates on feature the connections between different societies and the conventional utilization of plants. A few ethnic gatherings all around the world utilize various plant species for treatment of different illnesses going from gentle contaminations to deadly diseases. Frequently, these investigations are of significance and give fundamental data to improvement of logical exploration to legitimize the restorative capability of plants.

Brackish water shrimp lethality bioassay is an in vivo lethality examine that utilizes a straightforward zoologic organic entity as a helpful screen for screening, finding and observing different bioactivities of normal mixtures. This test is extremely valuable in deciding different natural exercises, for example, cytotoxic, phototoxic, pesticidal, trypanocidal, protein restraint, and particle guideline exercises. The examine can likewise be extrapolated for cell-line poisonousness and antitumor action. The technique is quick as it uses just 24 hours, reasonable and needs no extraordinary hardware. It is even straightforward in that it doesn't need aseptic circumstances to perform. The examine utilizes enormous number of living beings for approval and a somewhat limited quantity of test. This bioassay has been utilized to decide cytotoxic movement of plant separates. In our review, the ethanol concentrate of

Pterocarpus marsupium showed cytotoxic movement as confirmed by the portion subordinate mortality of salt water shrimp hatchlings. In one more review showed cytotoxic action as far as saline solution shrimp mortality of two mixtures segregated from leaves of Pterocarpus marsupium. Unrefined ethanol concentrate and dissolvable parts of bark of Pterocarpus marsupium were displayed to show checked cytotoxic impact as far as mortality of salt water shrimp hatchlings .

Apoptosis is by and large viewed as an energy-subordinate cycle requiring dynamic investment of numerous proteins and other cell macromolecules. It is because of the way that a large portion of the extreme genotoxic improvements harm the proteins (or qualities which are making those proteins) and other cell macromolecules which might be expected for apoptosis. The harm to proteins would bring about their denaturation. This denaturation would limit the harmed DNA to the atomic region giving a more honed framework to the atomic limit in necrotic cells. This sharpness in frame in necrotic cells may likewise be because of bigger measured DNA, which doesn't diffuse as it does in apoptotic cells where DNA is just about as little as 180 bases.

Check of the apoptotic action was completed in view of the example of DNA groups delivered from a gel electrophoresis. In apoptosis, cells are lysed step by step and deliberately to deliver layer bound apoptotic bodies, which was recommended to assume a significant part in smothering fiery reactions to other adjoining cells. Apoptotic bodies or cells which went through apoptosis produce a particular example of DNA

parts with the products of 200 bp because of explicit activity of initiated nucleases. These separated sections delivered groups in a stepping stool design, conversely, with the spread example created from corruption action (Figure 7). Results got in this concentrate in a way upheld the different cases made by explores on the anticancer properties of these plant. Further examinations are being done to recognize the dynamic standard of the concentrate. Subsequently, it very well may be presumed that the solid antiproliferative movement of concentrate on disease cells recommends its conceivable improvement as an anticancer specialist. The method of activity of the concentrate was by the acceptance of apoptotic movement on disease cells.

CONCLUSION:

Normal items found from restorative plants play had a significant impact in the therapy of malignant growth. The current review focuses to the likely anticancer movement of chloroform and ethanol concentrate of Pterocarpus marsupium. Further examinations to describe the dynamic standards and clarify the component of the activity of ethanol and chloroform remove are underway.

Thus these plant concentrates might have clinical and helpful recommendation in the most life undermine illness like disease and further examinations are expected to explore these plant tests as antineoplastic specialists. Hence, it is guessed that plants can give likely bioactive mixtures to the advancement of new 'prompts' battle malignant growth illnesses.

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