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A REVIEW ON ANTICANCER ACTIVITY OF PLANTS BELONGS TO FABACEAE FAMILY

Ganga Devi. S[*1], Savithamol G M[2], Dr. Jayachandran Nair C V[3], Dr. Prasobh G R[4], Gopika Gopi[1], Jino Syam J. S[1], Soorya. S[1]

[*1] B Pharm student, Sree krishna college of pharmacy and research centre Parassala, Thiruvananthapuram, Kerala

[2] Associate Professor, Department of pharmacology, Sree krishna college of pharmacy and research centre Parassala, Thiruvananthapuram, Kerala

[3] Professor and Head of Department of Pharmacology, Sree krishna college of pharmacy and research centre Parassala, Thiruvananthapuram, Kerala

[4] Principal, Sree krishna college of pharmacy and research centre Parassala, Thiruvananthapuram, Kerala

Abstract:

Cancer is the uncontrolled growth of abnormal cells anywhere in the body. These abnormal cells are termed cancer cells, malignant cells, or tumour cells. In view of serious side effects of chemotherapy and radiation treatment for cancer, there should be immediate search for alternative and safer methods of treatment . Medicinal herbs serve as nature's gift to humans to help them pursue better health. Different phytochemicals in fabaceae family such as Flavanoids, lectins, saponins and phenolic compounds have significant anticancer potential. Therefore, these Phyto molecules are suitable candidates for the development of new anticancer agents. This review study is an aid to identify and further develop newer anticancer agents from Fabaceae family.

Key Words: Cancer, Anticancer, Pisum sativum, Phaseolus vulgaris, Cicer arietinum, Vigna ungiculata

[*]Corresponding author:

Ganga Devi. S,

Sree krishna college of pharmacy and research centre Parassala,
Thiruvananthapuram,
Kerala
E-mail: gangasankar18@gmail.com

QR code

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INTRODUCTION:

CANCER

Cancer is the uncontrolled growth of abnormal cells anywhere in the body. These abnormal cells are termed cancer cells, malignant cells, or tumour cells. These cells can infiltrate normal body tissues. Many cancers and the abnormal cells that compose the cancer tissue are further identified by the name of the tissue that the abnormal cells originated from (for example, breast cancer, lung cancer, and colorectal cancer). When damaged or unrepaired cells do not die and become cancer cells and show uncontrolled division and growth - a mass of cancer cells develop [1].

USE OF PHYTOCHEMICALS IN CANCER THERAPY

Treatment of Cancer is somewhat successful with chemo preventive agents but these treatment modalities are often accompanied by severe side effects [1, 2] . In view of serious side effects of chemotherapy and radiation treatment for cancer, there should be immediate search for alternative and safer methods of treatment[2] .

Medicinal herbs serve as nature's gift to humans to help them pursue better health. Plants and their phytoconstituents are in medicinal practices since ancient times. The search for cancer drugs from natural sources started with discovery of Podophyllotoxin in late 1960s, further lead to discoveries of vincristine, vinblastine, campothecin and taxol. Several medicinal plant species and their phytochemicals inhibit the progression and development of cancer [2].

ANTICANCER ACTIVITY IN FABACEAE FAMILY

Fabaceae family

The Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. It includes trees, shrubs, and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume) and their compound, stipulate leaves. Many legumes have characteristic flowers and fruits. The family is widely distributed, and is the thirdlargest land plant family in number of species, behind only the Orchidaceae and Asteraceae, with about 751 genera and about 19,000 known species [3] . Some of the most important commercial species include soybeans (Glycine max), garden peas (Pisum sativum), lentils (Lens culinaris), and chick pea (Cicer arietinum)

Plants belonging to Fabaceae family which contains anticancer activity

PISUM SATIVUM

P. sativum is an herbaceous annual, with a climbing hollow stem growing up to 2–3 m long. Leaves are alternate, pinnately compound, and consist of 2–3 pairs of 1.5–8 cm long large leaf-like stipules. Flowers have five green fused sepals and five white to reddish-purple petals of different sizes. Fruit grows into a pod, 2.5–10 cm long that often has a rough inner membrane. The pod is a seed container which composed by two sealed valves and splitted along the seam which connects the two valves. Seeds are round, smooth, and green color.[3]

fig I : *Pisum sativa*

MATERIALS AND METHODS:

1. Collection of Plant Material

P. sativum (Pea) seeds were thoroughly washed with tap water and dried under shadow. Then they were ground well using domestic grinder.

2. Preparation of Extracts

About 250 gm of dried, fine seed powder of P. sativum was loaded into the Soxhlet column connected with the round bottom flask constituted 500 ml of ethylacetate solvent and extracted at the appropriate temperature of solvent. The Soxhlet apparatus was continued for extraction up to 2 cycles for 48 hrs. Then, extract was concentrated to dryness and the residues were obtained. The residues were stored into a pre-weighed sample container for further use.

3. Phytochemical Screening of P. sativum seed Extract

The ethyl acetate extract of P. sativum seed were used for qualitative screening of phytochemicals such as alkaloids, carbohydrates, flavonoids, cardiac glycosides proteins, phenols, saponins, steroids, tannins and terpenoids by standard biochemical procedure.[4]

4. GC-MS Analysis

GC-MS analysis was performed to identify the bioactive compounds in the P. sativum ethyl acetate extract. GCMS analysis was performed using a JEOL

GC MATE II instrument employing the following conditions: Front inet temperature 220°C; Column HP 5Ms; Helium gas (99.99%) was utilized as carrier gas at a constant flow rate of 1ml/min. In the case of oven temperature it was 50 to 250°C @ 10°C/min. The ion chamber temperature and GC interface temperature was 250°C. Mass analysis was done with the help of Quadruple Double Focusing Analyzer: For detection, the Photon Multiplier tube was used. At 70eV, mass spectra were taken. The necessary data were gathered by the full-scan spectra within the scan range of 50-600 amu. Percentage peak area was nothing but the percent composition of constituents of the extract.[5]

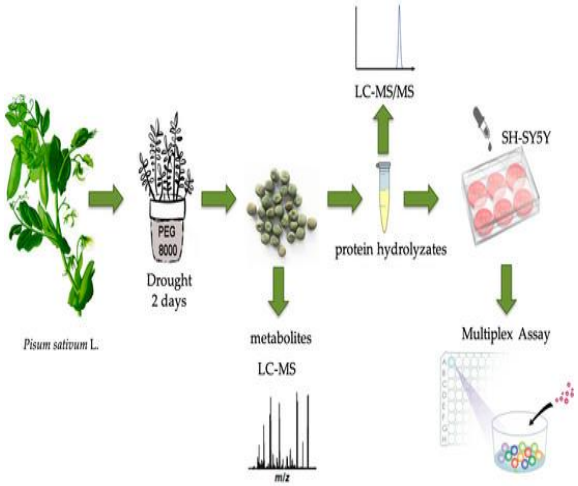

Fig 2 : GC-MS Analysis

5. Identification of Components

On the basis of GC retention time, chemical compounds in ethyl acetate extract were identified. The mass spectra were matched with the standard mass spectra available in libraries. By employing National Institute Standard and Technology's data base having more than pattern and Wiley spectra libraries, mass spectrum GC-MS interpretation was made. Components were compared with the known spectrum compounds which were stored in NIST library. The compound name, weight of molecule and its formula and compound structure of the test materials were ascertained from NIST and Pubchem libraries.[5]

6. Procurement of Cell Lines

Cell lines of human breast cancer (MCF-7) were obtained from National Center for Cell Science (NCCS), Pune, India. These cells were stored in DMEM medium supplemented with 10 % FBS (Sigma-Aldrich, St.Louis, Mo, USA). Penicillin at 100 µg/mL and streptomycin at 100 µg/mL were used as antibiotics (Himedia, Mumbai, India). At humidified atmosphere the culture was kept at 5% CO2 level in a CO2 incubator at 37◦C .[6]

PRELIMINARY PHYTOCHEMICAL SCREENING

The results showed the presence of secondary metabolites such as alkaloids,cardiac glycosides, flavanoids, phenols, saponins, phytosterols,tannin and triterpenoids in ethyl acetate extract of p.sativum.Flavanoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity.[6, 7]

PHASEOLUS VULGARIS

Cytotoxic activity of a black bean (phaseolus vulgaris l.) Extract and its flavonoid fraction in both in vitro and in vivo models of lymphomas.

MATERIALS AND METHODS

Preparation of the Black Bean Extract and Flavanoid Extract

The Black Bean Extract was prepared as described by Gutiérrez-Uribe et al.10, whereas the Flavanoid Fraction was obtained by dissolving 2 g of dried BBE in 20 mL of methanol followed by sonication for 5 min, then 20 mL of tri-distilled water was added and homogenized by sonication for 5 more min. The blend was centrifuged at 800 g for 5 min and the supernatant was recovered. The flavonoids were purified using a C18 Solid Phase Extraction cartridge 20 cc/5 g followed by a washing step with 10 mL of 25% MeOH, and the flavonoid-rich fraction was recovered by eluting 10 mL of 60% MeOH. The flavonoid solution was then dried by lyophilization.[8]

fig 3 : *Phaseolous vulgaris*

Cell culture

The DLBCL-derived cell line (OCI-Ly7) was kindly provided by Professor Ricardo Dalla Favera from Columbia University, (New York, NY). NIH-3T3 and VERO cells were obtained from ATCC (CRL-1658 and CCL-81). Cells were cultured in IMDM, (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, (Invitrogen, Carlsbad, CA), 1% pen-strep; and maintained at 37°C in 5% of CO2 atmosphere.

Cytotoxicity assays

OCI-Ly7 cells were cultured in a white opaque 96-well plate (Corning, Pittston, PA), at a density of 2 × 104 cells/well. Cells were exposed to different concentrations of BBE, FF or CTX, used as positive control. After 24 h, cellular viability was monitored using CellTiter-Blue reagent. To evaluate the effect over normal cells, NIH-3T3 and VERO cell lines were exposed to the extracts under the same conditions.[9]

Apoptosis and cell cycle assay

Cells were plated in a 12-well plate at a density of 2 × 105 cells/well and exposed to either BBE or FF using the IC50 obtained in the cytotoxicity assays. Following an incubation of 24 h, cells were trypsinized and washed with cold phosphate-buffered saline (PBS); the number of apoptotic cells was determined using the annexin V (AV)-FITC/PI apoptosis assay kit. Fluorescence intensities for both AF and PI were obtained and analyzed in FACSDiva software (BD Biosciences). Double positive (AF and PI) or single positive (AF) were set as apoptotic cells. For the cell cycle analysis, the Cell CycleTEST™ plus DNA reagent kit BD was used. The percentage of cells in each cycle was determined using the ModFit LT 3.2, Verity Software House (Topsham, ME). All experiments were run in triplicate.

Mice strain and housing conditions

The protocol was approved by the Institutional Committee for laboratory animal welfare, and the animals were treated under institutional guidelines for the care and use of animals. Male C.B-17/IcrHsdPrkdc SCID mice, 6–8 weeks old, were obtained and housed at sterile conditions in the clean room conditioned with positive pressure air flow. Environmental conditions were: 21°C ± 2°C and 55% ± 15% relative humidity and light/dark cycles of 12 h. Mice were held individually in sterile ventilated cages. Before the experiments, mice were kept under standard animal housing conditions for 2 weeks with free access to sterile water and food.Sterile water was supplemented with trimethoprim and sulfamethoxazole as a prophylactic antibiotic as part of the protocol for avoiding opportunistic infections. At the end of the experimental protocol, animals used as negative controls underwent anesthesia and euthanasia with a mixture of Ketamine (100 mg/mL and Xylacine (20 mg/mL, 25 mL). The doses were 200 mg of ketamine and 16 mg of xylacine per kg of body weight administered into the anterolateral region of any caudal limb muscle, followed by cervical dislocation once the mice presented absence of motor reflexes and periorbital signs.[10]

Xenografting model and treatment

To generate the animal model, a suspension of lymphoma cells (1 × 107 cells suspended in 300 µL of media) was administrated intraperitoneally as previously described by Schimdt et al.20. Cells used for injection were maintained in log phase with viability around 98%. The animal model for lymphoma was characterized by histopathological analysis using hematoxylin and eosin staining, along with immunohistochemistry for CD79a and BCL-6 to confirm the tumor type. Following the characterizations, mice underwent treatment and were divided into four groups: The placebo group which received only PBS, one group receiving the FF (15 mg/mouse/day, using the rodent pellets as the vehicle), another group receiving the BBE (20 mg/mouse/day, using the rodent pellets as the vehicle), and the control group which received CTX (i.p. At 150 mg/kg of body weight); the administration schedule was one injection every 3 days for a total of 7 applications21. Body weight and mice behavior were monitored during the experiment. After animal death, tumors were extracted and underwent pathological analysis. The survival time was registered since tumor implantation.[11]

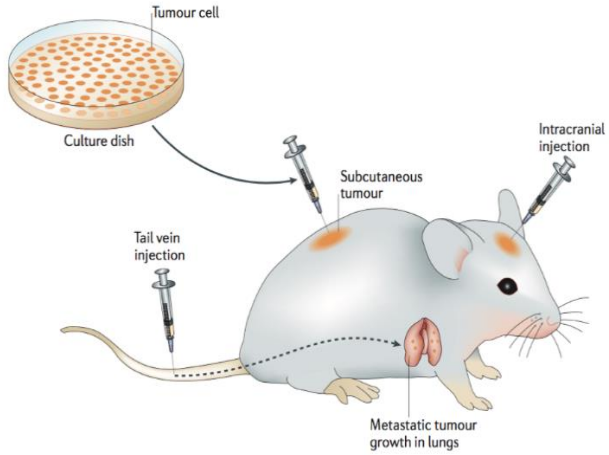

Fig 3 : Xenografting model

Statistical analysis

Matlab® 2010 software was used for the statistical analysis. All in vitro assays were performed in triplicate reporting data as a mean and standard deviation. IC50 was calculated by a variation of the Hill equation22. For apoptosis and cell cycle, statistical differences were assessed using the two-tailed t-test to test the null hypothesis of no difference between populations. The differences between tumor volumes and weights were evaluated through ANOVA analyses. In the animal model, statistical differences between treatments from Kaplan–Meier curves were evaluated using the log-rank test (SPSS Statistics 19 was used in this test). $p \leq 0.05$ was considered significant.[12]

Characterizations of flavonoids content

The chromatographic analysis of FF identified three glycosylated flavonoids: myricetin 3-O-glucoside at a concentration of 11.29 mg/g, while quercetin 3-O galactoside and kaempferol 3-O glucoside were present at concentrations of 97.68 and 1.00 mg/g, respectively.

Finally, in this study, we found that both BBE and FF from P. vulgaris L. Had cytotoxic properties comparable to cyclophosphamide, a widely-used molecule for cancer therapy. In addition, these extracts seemed to be specific for tumor cells, since they were almost innocuous to non-cancer cells (NIH-3T3 and VERO cells). Another important observation was that the administration of the extracts in a xenografting model of lymphoma increased the SR, similar to that was observed with cyclophosphamide treatment.[13]

CICER ARIETINUM L

Chickpea (Cicer arietinum L.) of Fabaceae family is the third most vital food legume of the world. Chickpea seeds are predominantly consumed due to its protein composition. One of the seed proteins albumin is reported to possess medicinal properties including antioxidant, antihyperlipidemic and antiproliferative. It is a source of functional peptides too.[14]

Fig 4 : *Cicer arietinum*

MATERIALS AND METHOD

Chickpea seed extract preparation

Chickpea seed powder suspended in distilled water (1:10 w/v) was stirred for 8 h at 4 °C (pH 8.0), followed by centrifugation at 10,000×g for 15 min. The pH of the supernatant was adjusted to 4.5 and stirred for 30 min in cold and re-centrifuged at 10,000×g for 15 min (Yust et al. 2003). Protein concentration was estimated through Bradford's method and later lyophilized and stored at − 20 °C for further use. Synthesis of bioactive peptides, ultrafltration and purifcation by FPLC Initially, chickpea seed extract was subjected to digestion using six diferent proteases viz. alcalase, favorozyme, chymotrypsin, trypsin, pepsin and papain. The protein hydrolysate/peptides obtained through digestion was subsequently tested for their antiproliferative activity against above mentioned cell lines. Alcalase generated peptides gave signifcant activity out of six proteases and therefore, further fractionated using ultrafltration membranes of diferent molecular weight cut-ofs (30, 10, 5 and 3 kDa). The 3 kDa peptide fraction revealed highest antiproliferative activity and for that reason subjected to FPLC system equipped with a HiTrap Q XL column. Unbound proteins were washed with the Tris bufer (pH 8). A linear salt gradient was used to elute the bound peptides. Two peaks were obtained. The peak fraction showing highest antiproliferative activity was lyophilized and used for further analysis.[15]

Western blot assay

The Ishikawa cell line was nurtured for 48 h with a medium holding chickpea peptide (250 and 500 µg). RIPA bufer containing 50 mM Tris HCl (pH 8.0)+150 mM NaCl+1% Triton X-100 + 0.1% SDS + 0.5% deoxycholic acid, was mixed with protease and phosphatase inhibitor and used for extracting proteins for western blotting. The membrane was probed with appropriate primary antibodies against caspase-3, Bcl-2, Bax and β-actin and visualized by Pierce ECL plus western blotting kit.

Antiproliferative assay

Sulforhodamine B (SRB) based cell viability assay on human cancer cell lines was performed to analyze the cytotoxic impact of chickpea protein. SRB assay primarily uses the potential of the sulforhodamine B dye to bind electrostatically to amino acid residues of TCA-fxed cells (Skehan et al. 1990). The efect (inhibition of cell proliferation) of chickpea protein concentrations (5–500 µg/ml) on six diferent human cancer cell lines, MCF7, MDA-MB-231, have been examined using SRB assay.

Statistical analysis

All the tests were performed in triplicates and represented as mean ± SD. Statistical analysis of the data was done using Graph pad prism (version 5) software. Chickpea peptide concentration giving 50% inhibition was calculated by non-linear regression analysis to generate the curve and IC50 value. Dunnett's compare test was applied with 95% confdence with diference considered as signifcant at $p<0.05$. [15]

From this study it is clear that, chickpea seed peptides harbor pharmacological activities. Foods containing chickpea peptides can be supplemented in diets to prevent the occurrence pre-cancerous lesions. A discrete cytotoxic activity shown that chickpea peptide induced apoptosis through down-regulation of Bcl-2 and upregulation of Bax directed activation of caspase-3. However, more in-depth and meticulous in vivo studies on the anticancer potential of chickpea peptides shall bring greater insights into the mechanisms involved.[15]

VIGNA UNGICULATA

Cowpea (Vigna unguiculata) is one of the most popular legume seeds. It has similar physical and chemical properties as common beans (Phaseolus vulgaris), except for its fat content. Cowpeas contain around 9–10% fat, whereas common beans have only 0.5–1.5% lipids.[16]

Fig 5 : *Vigna ungiculata*

Materials and methods

Cowpeas were hand-cleaned to remove foreign material and splits. Test or volumetric weight and 1000 seed weight were determined. Test weight, expressed as kg/hl, was determined according to method 14-40 of the AACC (2000) using the Winchester Bushel Meter and thousand-seed weight by randomly selecting and weighing 100 seeds and the resulting weight multiplied by 10. Clean cowpeas (5 kg) were decorticated in order to obtain seed coats and cotyledons. The milling procedure consisted of first drying cowpeas at 60 C in a convection oven during 3 h. Upon equilibration at room temperature, the seeds were mechanically decorticated for 6 min in a PRL mill equipped with a set of carborundum abrasive disks. Seed coats were separated from cotyledons by air aspiration and sifting through a sieve with 2 mm diameter orifices.[16]

Determination of total phenolics and flavonoids

The total phenolic contents of extracts were determined using the Folin–Ciocalteu method. Gallic acid was used as a standard and total phenols expressed as mg gallic acid equiv. in 100 g (dry weight). Free and bound flavonoids were determined by the colorimetric method. Flavonoid concentration was expressed as mg quercetin equiv./100 g. 2.5. Determination of antioxidant capacity The antioxidant capacity was determined using the ORAC method and the ABTS method). [17]

Mammary cancer cell culture

Human hormone-dependent mammary cancer cells (MCF-7) were maintained in DMEM-F12 medium containing 10% fetal bovine serum. Cells were grown at 37 C in a humidified 5% CO2 atmosphere and maintained at a cell concentration between 8 · 104 and 1 · 105 cells/cm2 by subcultivating in a ratio of 1:3 every week. The CellTiter 96 AQueous One Solution Cell Proliferation Assay was used to determine cell viability. Plates of 96-wells were prepared with 100 ll of a suspension containing 5 · 104 cells/ml of MCF-7 at least 12 h before adding the extracts. Extracts of free or bound phenolics were adjusted with cell growth medium to 376.2 mg GAE/l and 100 ll of these solutions were added to the prepared plates. After 48 h incubation, 20 ll of CellTiter were added and absorbance measured at 490 nm in a microplate reader . The concentration needed to inhibit 50% cell growth (IC50) was calculated by testing at least five sample concentrations being the highest the one that contained 188.1 mg GAE/l.[18]

In vitro mammary cancer cell inhibition by cowpea extracts

All extracts were tested at a phenolic concentration of 188.1 mg GAE/l. Free phenolics were more effective compared to bound counterpartsParticularly, bound phenolics of seed coats promoted the growth of MCF-7 cells. These compounds can promote the growth of hormone-dependent cells in vitro. Soyasapogenol B, a triterpenic saponin, inhibited growth of MDA cells at a concentration of 10 lM but did not significant affect at any concentration the proliferation of estrogen dependent MCF-7 cells. This compound has been previously reported in cowpeas. Free phenolic extracts of whole seeds, seed coats or cotyledons, tested at a dosage of 188.1 mg GAE/l, were equally effective in inhibiting mammary cancer cell growth.[19]

Statistical analysis

Total phenolics, flavonoids and HPLC characterizations were done in triplicate and results expressed as mean ± standard deviation. The bioactivities of free and bound phenolics extracts from whole cowpeas, seed coats and cotyledons were statistically compared. Statistical analysis was conducted by one-way ANOVA followed by the Tukey's HSD test at a = 0.05.[20, 21]

Results indicated that whole cowpeas are a good source of phytochemicals because inhibited in vitro cancer cell growth. Besides the presence of flavonoids and antioxidants, mainly associated to seed coats, components of the cotyledons were necessary to increase the anticancer effects. Antioxidant activity was mainly found in free phenolics and those were more bioactive.[22, 23]

DISCUSSION:

Species of the Fabaceae family are a rich source of phytochemcicals including flavanoids, lectins, saponins, alkaloids, carotenoids and phenolic acids. The phytochemicals from Fabaceae members are effective in the prevention and treatment of cancer.

From the studies, *Pisum sativa, Phaseolus vulgaris, Cicer arietinum, Vigna ungiculata* showed that the presence of secondary metabolite such as alkaloids, cardiac glycosides, phenols, saponins and other secondary metabolites play significant roles in either inhibiting cancer cell activating proteins, enzymes and signalling pathway or by activating DNA repair mechanism or bye inducing antioxidant action.

CONCLUSION:

Cancer is a devastating disease and the current treatment modalities available for cancer treatment put patients in lots of struggle due to the undesirable side effects. Chemotheraputic agents have limitations due to their toxic effect on non-targeted tissues and further impair human health. Therefore, there is an increased demand for plant derrived anticancer agents.As demonstrated above, different phytochemicals in fabaceae family such as Flavanoids, lectins, saponins and phenolic compounds have significant anticancer potential. Therefore, these Phyto molecules are suitable candidates for the development of new anticancer agents. Some of these compounds are excellent lead molecules and by making suitable pharmaceutical interventions such as structural modification, alternative formulation and effective delivery systems, the pharmacological potential can be increased. This review study is an aid to identify and further develop newer anticancer agents from Fabaceae family.

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