Original Research Article

IN VITRO ANTIMITOTIC ACTIVITY AND MOLECULAR DOCKING STUDIES OF ISOLATED COMPOUNDS FROM *RHYNCHOSIA BEDDOMEI*

ABSTRACT

The goal of this work was to use an exquisite combination of phytopharmacological and modern computational tools to examine the anticancer potential of *Rhynchosia beddomei*. The methanolic extract of *Rhynchosia beddomei* was screened for *in vitro* antimitotic activity like *Allium cepa* root tip assay. Molecular docking was carried out between the Bcl-2 Receptor, VEGFR-2 and bioactive compounds like apigenein, vitexin, isovitexin, quercetin, vicenin, orientin, rutin etc. Extract of *Rhynchosia beddomei* showed significant antimitotic activity, by decreasing rate of mitosis in comparison to water. Methotrexate (0.1 mg/mL) was used as a standard and shows highest antimitotic activity. Thus, the selected plant displayed significant antimitotic activity by showing good inhibition. Vitexin, rutin and lucenin have demonstrated remarkable binding affinity towards Bcl-2 and biochanin, isovitexin, orientin and apigenin have demonstrated remarkable binding affinity towards VEGFR-2 respectively. Molecular dynamic simulation studies have further confirmed the finding of docking analysis, suggesting that Bcl-2 and VEGFR-2 can act as an attractive molecular target for vitexin, rutin, biochanin, isovitexin, orientin and apigenin respectively.

Keywords: *Rhynchosia beddomei*, Antimitotic activity, Molecular docking, Bcl-2 and VEGFR-2.

1. Introduction

"Ayurveda, a traditional Indian medical practice using plant drugs has been successful from very early times in using these natural drugs and preventing or suppressing various tumours with different lines of treatment" [1]. " In India, people of different ethnic groups inhabiting various terrains, possess their own distinct culture, religious rites, food habit and a rich knowledge of traditional medicine" [2]. They practice herbal medicine to cure a variety of diseases. Natural products, especially plants have been used in the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient times and an impressive number of modern drugs have been developed from them. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.

"Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth" [3]. "Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind" [4].

According to Cragg and Newman [5] "over 50 % of the drugs in clinical trials for anticancer properties were isolated from natural sources or are related to them". "Several natural products of plant origin have potential value as chemotherapeutic agents. The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious drugs, drugs from natural origin have a share of 60 % and 75 % respectively" [6].

"Deaths due to cancer are projected to continuously increase, and it has been estimated that there will be 11.5 million deaths due to cancer in the year 2030" [7]. "Traditional treatments for cancer like chemotherapy, radiotherapy and surgery provide only partial and transient relief. Also, the above treatments and synthetic anticancer drugs are costly and beyond the reach of the general public. Hence, alternative herbal remedies that are commonly available and comparatively economical are to be explored" [8]. Plant drugs have been used to treat cancer for a long time in both traditional and modern societies since the discovery of anticancer potentials. A great number of *in vitro* and *in vivo* methods have been developed to measure the efficiency of natural anticancer compounds either as pure compounds or as plant extracts.

Rhynchosia beddomei Baker (*R. beddomei*) is a viscous hairy under shrub, belongs to the family of Fabaceae and widely distributed in Deccan and Carnatic regions of South India. A few plants of this genus were used in traditional medicine for the treatment of various ailments such as antibacterial, antidiabetic, abortifacients, healing of wounds, hepatoprotective, remedial of boils, rheumatic pains and skin infections.

Allium cepa bioassay is an authentic, rapid, easy, and inexpensive method for the detection of antimitotic effects of various compounds [9]. This assay has also been used for recognizing the cytotoxic, antiproliferative, and mutagenic potentials of different plant-derived anticancer compounds [10]. To the best of our knowledge antimitotic activity of aqueous extract of *Rhynchosia beddomei* whole plant and *in silico* studies not been studied yet. So, the present study was designed to investigate the antimitotic of aqueous extract of *Rhynchosia beddomei* in *A. cepa* root apical meristem cells.

2. MATERIALS AND METHODS

2.1 Plant material and preparation of extract

R. beddomei whole plant was collected from Seshachalam hills and authenticated by Dr. K. Madhava Chetty, Assistant Professor in Department of Botany, Sri Venkateshwara University, Tirupati, Chittoor district, Andhra Pradesh. The crude plant material was dried under shade and powdered mechanically to coarse powder. The coarsely powdered plant material (500g) was subjected to extraction with methanol using simple distillation [11].

2.2 In vitro Antimitotic activity

The antimitotic activity of *Rhynchosia beddomei* were screened by using Allium cepa root meristematic cells using methotrexate as a standard [12].

2.2.1 Antimitotic activity

The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screening of drug with antimitotic activity. Cells of this region undergo repeated divisions, known as meristematic region, which is similar to cancer division in human. Hence *Allium cepa* meristematic cells can be used for preliminary screening of drug with anticancer activity. Procedure followed for carrying out antimitotic mentioned in following steps [13].

2.2.1.1 Roots development:

The experiment was planned as per the standard protocol. Onions were descaled and placed on glass cups which were filled with distilled water, kept in incubator at 24˚ for 72 hrs then 2-3 cm roots were allow to germinate. These roots were used for further process.

2.2.1.2 Sample Preparation for Treatment:

Sample: Extracts of *Rhynchosia beddomei* (10mg/ml),

Standard: Methotrexate (0.1mg/ml)

Control: Distilled water

2.2.1.3 Treatment: Developed roots were kept in different extracts for 3 hr at 18ºC.

Fixation: Then root tips were cut and place in fixing solution caronys fixative for 24 hr at room temperature.

Composition of caronys fixative; Glacial acetic acid 25 ml and Ethanol 75 ml

2.2.1.4 Squash preparation:

2.2.1.4.1 Hydrolyzation:

These roots were hydrolyzed with 1N HCl, by keeping it with HCl in oven at 60º for 10 min.

2.2.1.4.2 Staining:

Transferred root tips into 2% acetocarmine stain for 20 min.

2.2.1.4.3 Slide Preparation:

Taken a stain root, cut it from the tip (which get dark stain) where meristematic cells were present. Then place cover slip on it and observed under microscope at 40 X objective and cells were counted. Mitotic Index was determined using following formula

$$\textbf{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

2.3 MOLECULAR DOCKING STUDIES

2.3.1 Protein-ligand interactions

Docking stimulations predicts the binding orientation of drug candidates to their protein targets. Mcule was used for generating docking simulation studies.

2.3.2 Docking simulations on Bcl-2 and VEGFR-2

Cancer related issues are linked to the BCL-2 family of proteins. B-cell lymphocyte-2 (Bcl-2) is an antiapoptotic protein, which is an important member of Bcl-2 family. B-cell lymphoma (Bcl-2) is the key target to stimulate apoptosis process in cancer cells. There is some evidence that metastasis of many solid tumours is attributed to overexpression of VEGFR-2. It was reported that tumour growth can be efficiently inhibited by blocking of angiogenesis. Hence, VEGFR-2 inhibition is an efficient approach to obtain effective anticancer agents. BCL-2 and VEGFR-2 inhibitors are known cancer target with several potential inhibitors under validation. Therefore, it is of interest to design and develop new compounds from plant source with improved binding features with the BCL-2 and VEGFR-2 protein. Hence, we report the molecular docking analysis of Bcl-2 and VEGFR-2 with phytocompounds [14].

2.3.3 Ligand preparation

The 2D ligands sketched in Mcule docking in the ligand imported side.

2.3.4 Protein preparation

The x-ray crystallised structure of BCL-2 (PDB ID:2W3L) and VEGFR-2 protein (PDB ID: 2OH4) were retrieved from RCSB protein bank. Attributes of SBD site sphere are obtained from discovery studio visualizer.

2.3.5 Ligand docking and scoring

Protein ligand interactions were stimulated though flexible glide-ligand docking with mCULE Docking allowed. The compounds docked displays a docking score

2.3.6 Visualization and analysis

The resulting docking poses were visualized through discovery studio visualizer. The ligand interactions were visualized to know the binding interactions between ligands and protein. The best docked structures were chosen using glide score function. The more negative the score the more favourable the binding. Additionally, the docked ligand poses were visualized and the different ligand receptor interactions were studied.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Evaluation of Antimitotic activity *Allium cepa* root tip assay

The antimitotic activity of methanolic extract of *Rhynchosia beddomei* whole plant was carried out by using Allium cepa root tip assay

Table 1. Antimitotic activity on allium cepa root tip cell using allium cepa root tip assay

S.NO	Different Solutions Used for Treatment	% Non Dividing cells	% Dividing cells	Mitotic Index (MI)%
1	Water(control)	38	62	62 ± 1.26
2	Methotrexate (0.1 mg/ml positive control)	61	39	39 ± 0.91
3	Aqueous extract (10 mg/ml)	45	55	55 ± 1.87

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The *Allium cepa* root tips were treated with water (control), Methotrexate and aqueous plant extract (*Rhynchosia Beddomei*) and observed under the compound microscope. The obtained results are mentioned above.

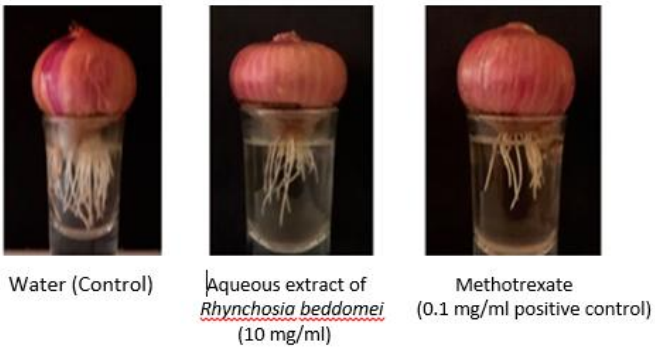


Fig. 1. Antimitotic activity of *Rhynchosia beddomei* using *Allium cepa* root tip assay

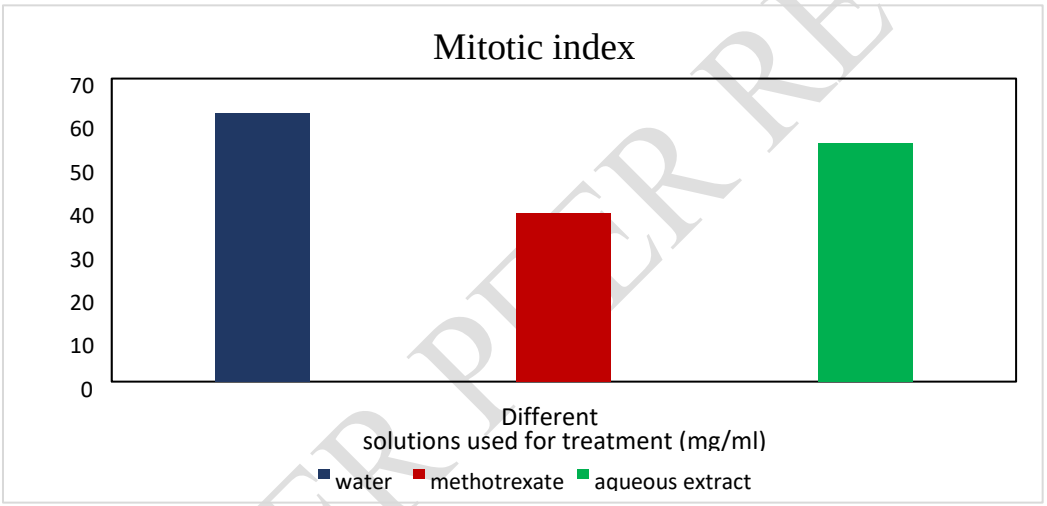


Fig. 2. Mitotic index of *Rhynchosia beddomei*

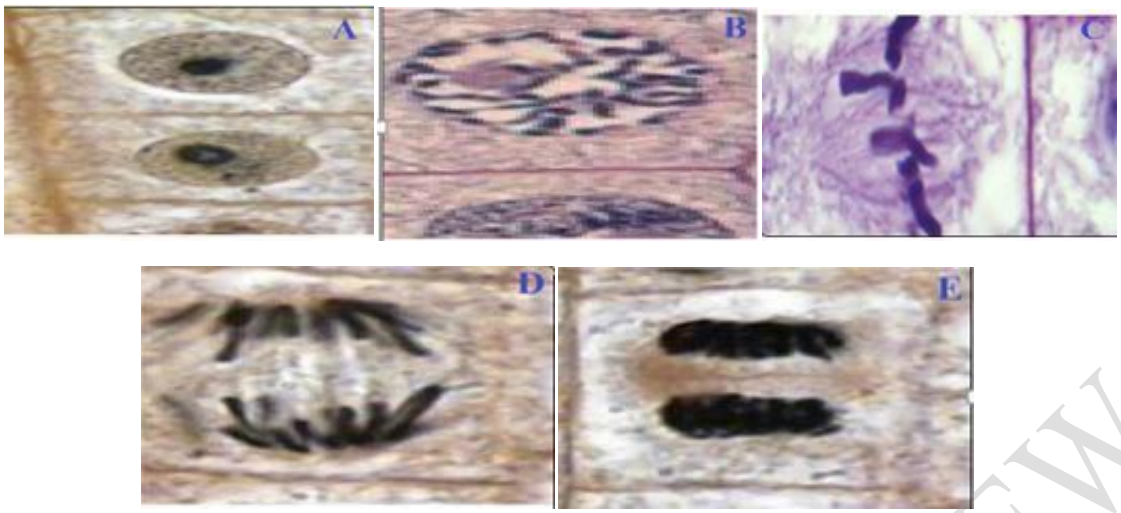
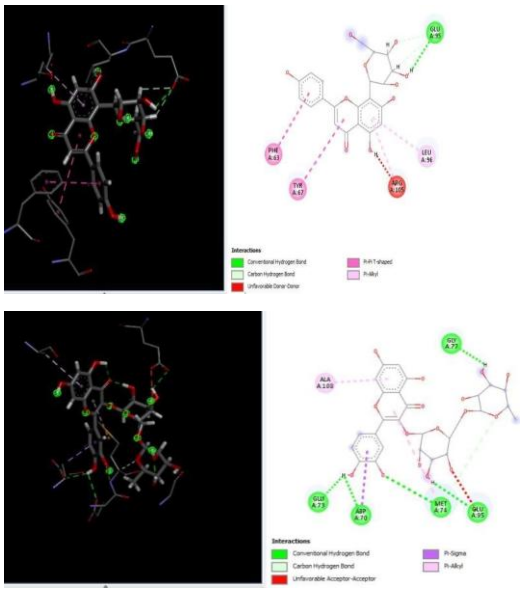


Fig. 3. Stages of onion mitotic cell division. A: interphase; B: prophase; C: metaphase; D: anaphase and E: telophase

The mitotic index of water, methotrexate and plant extract treated Allium cepa root tip cells are given in Table 1. Extract of *Rhynchosia beddomei* showed significant antimitotic activity by decreasing rate of mitosis in comparison to water. Methotrexate (0.1 mg/mL) was used as a standard and shows highest antimitotic activity. Thus, the selected plant displayed significant antimitotic activity by showing good inhibition which indicates its use as a potent antimitotic agent.

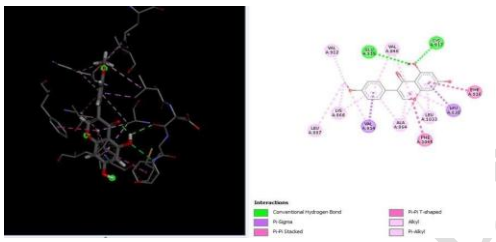
3.2 Molecular Docking Studies

Initially the proteins were downloaded from PDB was prepared by removing extra chains. Attributes of spheres are prepared and noted. Later molecules drawn in molecular and ligand preparation was created. Proteins are uploaded with sphere attributes and the structures were docked against 2W3L and 2OH4 proteins. Docking indicated that some of our compounds have good binding ability with both Bcl-2 and VEGFR-2 proteins. Following are the ligand interactions of compounds present in *Rhynchosia beddomei* whole plant with 2W3L and 2OH4 proteins.

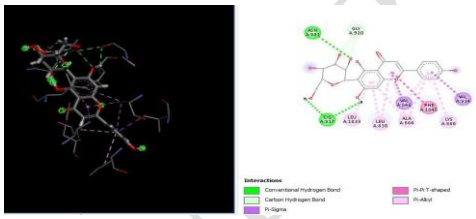


Vitexin -9.3 Rutin -7.8

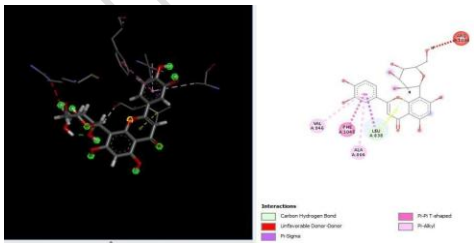
Fig. 4. Docking poses of phytocompounds with BCL-2 protein



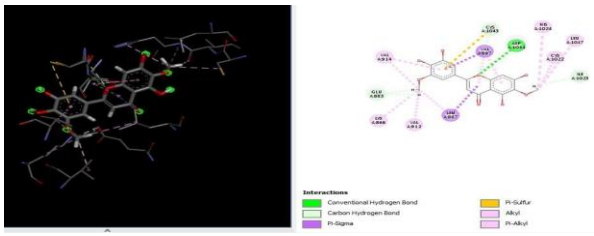
Biochanin -9.5



Iso Vitexin -8.3



Orientin -7.5



5,7,3’,4’-tetrahydroxy 6-c- β -D-glucopyrynosyl flavone -7.4

Fig. 5. Docking poses of phytocompounds with VEGFR-2 protein

Table 2. BCL-2 protein score of phytoconstituents from *Rhynchosia beddomei* whole plant

COMPOUNDS	BCL-2 PROTEIN SCORE
Vitexin	-9.3
Rutin	-7.8
Lucenin	-7.0
Apigenin	-6.9
Vicenin	-6.9
Orientin	-6.9
Iso orientin	-6.8
Iso vitexin	-6.8
Rhynchosin	-6.6
Quercetin-7-o-methyl ether	-6.5
Biochanin	-6.4
5,7,3’,4’-tetra hydroxyl-6-c-β-D- glucopyrynosyl flavone	-6.4
D-pinitol	-3.7
D-inositol	-3.7

Table 3: VEGFR-2 protein score of phytoconstituents from *Rhynchosia beddomei*

COMPOUNDS	VEGFR-2 PROTEIN SCORE
Biochanin	-9.5
Iso Vitexin	-8.3
Orientin	-7.5

5,7,3',4'-tetrahydroxy 6-c-β- Dglucopyrynosyl flavone	-7.4
Apigenin	-7.2
Rutin	-7.2
Vicenin	-7.1
Rhynchosin	-7.0
Vitexin	-6.8
D-Pinitol	-5.9
D-inositol	-5.8
Quercetin-7-O-methyl ether	-2.3
Lucenin	-1.6
Iso Orientin	-0.7

The more negative the score the more favourable the binding.

"The *Rhynchosia beddomei* plant shows wide spectrum of medicinal activities. The plant contains various phytochemical compounds having diverse chemical structure and nature. In the present study, attempts were made to investigate antimitotic activity of aqueous extracts of *Rhynchosia beddomei* in 10 mg/ml concentration by using *Allium cepa* roots tip assay. In *Allium cepa* root meristem model, commonly known as *Allium* assay, root meristematic cells are used for screening of drugs with antimitotic activity. In meristematic region, the cell division is similar to cancer cell division in humans" [15]. "Therefore, these meristematic cells can be evaluated for screening of drugs with potential antimitotic activity. *Allium* assay is considered a rapid, highly sensitive and reproducible bioassay for detecting cytotoxicity and genotoxicity" [16]. The root growth inhibition and antimitotic effects provide the indication of genotoxicity. The good genotoxic assay performance of *Allium cepa* as a plant system has been attributed to the easily studied karyo type of plant and the ability to correlate outcomes of assays with those of mammalian cells in the course toxic evaluations.

In *Allium cepa* assay, aqueous extract of *Rhynchosia beddomei* (10 mg/ml) was found to exhibit anti-mitotic action on *Allium cepa* root meristematic cell and it was indicated by decreased mitotic index after treatment. From result, it was concluded that 10 mg/ml concentration of aqueous extracts of *Rhynchosia beddomei* plant inhibits cell division in "*Allium cepa* assays and suggests that the plant may exhibit inhibitory influence on abnormal cell growth as like in cancer. Though the present study validates the traditional

use of extract in the treatment of cancer, further studies in cancer cell lines is necessary. The antimitotic effect is the important *in vitro* assay for the screening of anticancer compounds. In the present study mitotic index of extracts clearly indicates the efficiency in the inhibition of growth of cancer cells either by affecting microtubules or encouraging microtubule formation, and thus stopping the microtubules from being broken down. This makes the cells become so clogged with microtubules that they cannot continue to grow and divide. As a result of this cells arrest in mitosis and eventually, die by apoptosis" [17].

The molecular docking in this study shows a vital role in predicting molecular interactions of phytocompounds with targeted proteins. This application is widely used in the pharmaceutical industry as a powerful tool, particularly in the analysis of structure–activity relationship. The analysis of molecular docking outputs, such as binding affinity, are frequently applied in the determination of potential ligands. Molecular docking also has the ability to predict small molecule ligands binding toward appropriate target binding site.

Vitexin, rutin and lucenin have demonstrated remarkable binding affinity towards Bcl-2. Vitexin shown highest docking score -9.3 in comparison to other compound topwards Bcl-2 [18]. Biochanin, isovitexin, orientin ,7,3',4'-tetrahydroxy 6-c-β- D-glucopyrynosyl flavone, apigenin, rutin, vicenin and rhynchosin have demonstrated remarkable binding affinity towards VEGFR-2. Biochanin shown highest docking score -9.5 in comparison to another compound towards VEGFR-2. Thus, indicating that these compounds are potent inhibitor of the Bcl-2 antiapoptotic family of proteins and VEGFR-2 proteins [19].

"Phytochemically successive chloroform extract (SCH), successive ethanol extract (SEE) of *T. indicum* were rich in triterpenoid and polyphenolic constituents. The major polyphenolic and triterpenoid in SEE were identified as gallic acid, catechin and β-sitosterol. Polyphenol and triterpenoids are well known for their anticancer activity. Our plant also possesses triterpenoids which might be responsible for anticancer activity. These molecules might act as cancer-blocking agents, preventing initiation of the carcinogenic process and as cancer-suppressing agents, inhibiting cancer promotion and progression" [20].

Overall results explain us that methanolic extract of *Rhynchosia beddomei* has proven *in-vitro* antimitotic activity. The application of molecular docking studies for the compounds that are present in methanolic extract of *Rhynchosia beddomei* with proteins 2W3L and 2OH4 are considered very useful and proven anticancer activity. Our findings conclude that all phytocompounds are possibly able to act as potential inhibitors for the targeted Bcl-2 and VEGFR-2 proteins, supported by the high binding affinities.

4. CONCLUSION

In the present study the antimitotic potential of methanolic extract of *Rhychosia beddomei* was screened for *in vitro* antimitotic activity by Allium cepa root tip assay showed decreasing rate of mitosis. Molecular docking study is carried out to explore the potentials of various phytochemicals to inhibit cancer growth. Based on our analysis reveals that bioactive compounds have the ability to bind with targeted proteins causing inhibition of growth factors which in turn hinders cancer cell proliferation. Our findings support the reported therapeutic use of this plant as an anticancer agent in the traditional system of medicine. Further experiments are needed, both *in vitro* and *in vivo* to obtain more detailed mechanisms of action.

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