OPEN ACCESS INTERNATIONAL JOURNAL OF SCIENCE & ENGINEERING

IN VITRO ANTICANCER ACTIVITY OF *BIDENS BITERNATA* (LOUR.) MERR. & SHERIFF – AN ETHNO MEDICINAL PLANT OF KERALA

Pradeesh S.
Department of Botany, Christian College, Kattakada, Thiruvananthapuram - 695 572, Kerala.
pradeeshnair10@gmail.com

Abstract: Bidens biternata **(Lour.) Merr. & Sheriff, belongs to the family Asteraceae, is an erect annual herb, up to 1 m height, a wide spread weed of cultivated areas. This plant is common, particularly in Western Ghats regions of Kerala state. It is used as a leafy vegetable by Paniya, Chetti, Kani and Kattunaayika tribes of Waynadu Districts in Kerala and also to cure hepatitis, cold, cough, dysentery, asthma etc. Phytochemical constituents are responsible for medicinal activity of ethno medicinal plant *B. biternata*. In the present study, crude methanol extract and isolated bioactive compound of *B. biternata* leaves was analyzed for its *in vitro* anticancer activity. The anticancer activity was evaluated by using different cell lines like L929, H9C2 and A375. In the MTT assay of crude methanol extract, the percentage of viability of the normal cell line L929 were found to be 64.2%, 53.2% and 49.1% when crude sample was applied in a concentration of 100 µg ml^{-1}, 500 µg ml^{-1} and 1000 µg ml^{-1}respectively. In quercetin the percentage of cell viability of L929 were found to be 51.04%, 47.28% and 36.27%. The percentage of cell viability of H9C2 cell line were found to be 90.86%, 64.39% and 40.26% and for isolated compound quercetin, 86.17%, 58.42% and 28.15% for sample concentration 100 µg ml^{-1}, 500 µg ml^{-1} and 1000 µg ml^{-1}respectively. In the MTT assay of crude methanol extract, the percentage of viability of the A375 cell line were found to be 94.17%, 70.14% and 44.58% and isolated compound quercetin, 92.14%, 68.34% and 38.26%. The results revealed that the isolated compound quercetin (3, 3', 4', 5, 7-pentahydroxy flavones) of *B. biternata* possessed high anticancer activity so could be used as an effective therapeutic agent against cancer disorders.**

Keywords: *Bidens biternata, L929, H9C2, A375, MTT assay.*

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I INTRODUCTION

Plant drugs have a long history in both traditional and modern societies as herbal remedies or crude drugs. A large number of new drugs derived from plant secondary metabolites have been applied in the treatment and/or prevention of cancer (Parkin *et al*., 2001). Herbal medicines in the treatment of cancer as complementary and alternative therapy are increasingly accepted with growing scientific evidences of biochemical and clinical traits. Some of the anticancer drugs discovered from herbal medicine have been used in clinical setting as conventional anticancer drugs (Parkin *et al*., 2001). Recently, research continuously focuses on clues from traditional knowledge of herbal medicines to develop new anticancer drugs as single pure compounds. On the other hand, standardized extracts or fractions with anticancer effects or with adjuvant therapy in cancer treatment coming from single or mixed herbs are also accepted in the forms of dietary supplements and botanical drug products (Yibin *et al*., 2010). Significant progress has been made in cancer chemotherapy, a considerable portion of which can be attributed to plant-derived drugs (Conforti *et al*., 2008). Drug discovery from plants still provides important new drug leads, many of which are approved or undergo trials for clinical uses against cancer, malaria, Alzheimer disease, HIV/AIDS, pulmonary pathologies and other diseases (Conforti *et al*., 2008). The synthetic anticancer remedies are still beyond the reach of common man because of cost factor. Herbal medicines have a vital role in the prevention and treatment of cancer since medicinal herbs are commonly available and comparatively economical (Sundaram *et al*., 2011).

The potential of using the natural products as anticancer drugs was recognized in 1950's by U.S Natural

Cancer Institute (NCI), Since 1950 major contributions have taken for the discovery of naturally occurring anticancer drugs (Cragg and Newman, 2005). Towards the end of the 20th century, plant based products, nutraceuticals and food supplements comprising the complementary and alternative therapies have attained a big share in the drug market in the developed countries. Medicinal plants through systematic screening programs possess an important position in the drug discovery and many modern drugs have their origin in traditional medicine of different cultures. Hence, despite the advantages of the synthetic and combinatorial chemistry as well as molecular modeling, medicinal plants remain an important source of new drugs, new drug leads and new chemical entities especially in cancer prevention (Newman *et al*., 2003). Nearly 877 new chemical entities (NCEs) were introduced and among them half (49%) were natural products, semi-synthetic natural products, semisynthetic natural products analogues or synthetic compounds based on natural products. There was a considerable scientific discovery of new anticancer agents and reports from natural products (Kasahara and Hemini, 1998).

Natural products derived from plants offer a new source of bioactive molecules that may have a great impact on infectious diseases and overall human health (Cordell, 1993). Screening of various bioactive compounds from plants leads to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Kumar *et al*., 2004). Medicinal plants were subjected to various processes and admistered to the patient's as drugs. Recently ethno medicinal studies received much attention as it brings to light numerous known and unknown virtues of plant origin (Sumeet *et al*., 2012). *Bidens biternata* is used as a leafy vegetable by Paniya, Chetti, Kani and Kattunaayika tribes of Waynadu Districts in Kerala and also to cure hepatitis, cold, cough, dysentery, asthma etc. Many medicinal uses like antidiabetic, antiallergic, antipyretic, antihepatic activities of this plant have been reported by Pradeesh *et al*. (2012-2017).Though the plant and its extracts have been extensively used in the tribal medicine, information from organized search of published literature does not provide evidences for the anticancer potentiality of *B. biternata*. So the present study aimed to analyse the *in vitro* anticancer activity of crude methanolic extract and isolated bioactive compound from leaves of *B. biternata* using different cell lines like L929, H9C2 and A375.

II MATERIALS AND METHODS

Collection and Preparation of Sample

B. biternata leaves were collected fresh from Western Ghats of Kerala, shade dried, ground well using mechanical blender to fine powder and transferred to airtight containers for further analyses. Experiments were done in triplicate, graph pad Instat DTCG was employed followed by IC50 values for the calculation and comparison.

Extraction from Plant Parts

The fine powder was used for extraction by using solvents like methanol. Fifty grams of sample powder kept into the soxhlet apparatus for distillation. Methanol (300 ml) was taken in the round bottom flask. The apparatus was kept over heating mantle and heated for 8 hours at 70°C. After completing the process, extract was collected in beaker and was kept in oven at 37°C-40°C. The crude concentrated extract was again weighed and used for further analysis.

The crude methanolic leaf extract was used for the isolation of bioactive compound from *B. biternata*. The anticancer activity was analyzed *in vitro* initially with crude methanolic extract of *B. biternata* leaves and later with isolated compound. Column chromatography and Thin Layer Chromatography (TLC) were used for separation of compounds followed by UV, IR, mass and NMR spectroscopic analysis, for the characterization of phytochemical from methanolic leaf extract of *B. biternata*. Anticancer activity of crude methanol extract and isolated compound of *B. biternata* leaves was tested using different cell lines.

Anticancer properties in methanol extract and isolated compound of *B. biternata* leaves

L929 (normal fibroblast cell line), H9C2 (cardiomyoblast cell line) and A375 (human malignant melanoma) cell lines were purchased from NCCS Pune, were maintained in Dulbecco's modified eagle's media and grown to confluency at 37°C and 5% CO_2 in a humidified atmosphere in a CO_2 incubator. The cells were trypsinized (500 µl of 0.025% Trypsin in PBS/EDTA solution) for 2 minutes and passaged to T flasks in complete aseptic conditions and incubated. 100 µg, 500 µg and 1000 µg of sample from a stock concentration of 100 mg/ml was added to 80% confluent and incubated for 24 hours. The anti-proliferative effects of *B. biternata* crude methanolic extract and isolated compound was determined by MTT cell viability assay.

MTT Cell viability assay

MTT assay is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase as per the methodology of Arung *et al*. (2009). The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with isopropanol and thereby released formazan. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.The cell culture suspension with methanol extract and isolated compound from *B. biternata* at concentration of 100

µg/ml, 500 µg/ml and 1000 µg/ml from a stock of 100 mg/ml, was washed with 1x PBS and then added with 200 µl MTT solution to the culture (MTT-5 mg/volume dissolved in PBS), followed by incubation for three hours at 37°C. Media was removed, washed with 1x PBS and 300 µl of DMSO was added. Incubated at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for two minutes to precipitate cell debris. OD was read at 540 nm using DMSO as blank. Percentage viability was calculated using the formula

Percentage cell viability = [OD of test/OD of control] x100

III RESULTS AND DISCUSSION

***In vitro* anticancer properties of methanolic extract of *B. biternata* and isolated compound, quercetin Cytotoxic effect of *B. biternata* in normal cell line L929**

L929 is a normal fibroblast cell line. Doxorubicin is used as standard anticancer compound. In the MTT assay of crude methanol extract, the percentage of viability of the normal cell line L929 were found to be 64.2%, 53.2% and 49.1% when crude sample was applied in a concentration of 100 µg ml^{-1}, 500 µg ml^{-1} and 1000 µg ml^{-1}respectively (Fig 1 and Plate 1). In quercetin the percentage of cell viability of L929 were found to be 51.04%, 47.28% and 36.27% for sample concentration 100 µg ml^{-1}, 500 µg ml^{-1} and 1000 µg ml^{-1} respectively ("Fig. 1" and Plate 1). Increasing the concentration of the methanol extract/isolated compound quercetin of *B. biternata*, the viability of normal cell line L929 was decreased but not drastically. The methanol extract and isolated compound quercetin showed a concentration depended cytotoxic activity to L929 cells. The methanolic leaf extract and isolated compound, quercetin from *B. biternata* have very low cytotoxic activity compared to standard (doxorubicin) and *Tecomaria capensis* (100 µg-34.27%, 200 µg-38.05%) belongs to Bignoniaceae (Tamil *et al*., 2013). The IC_{50}values for methanol extract and quercetin were compared to the standard (>100 µg/ml).

Antiproliferative activity of methanolic extract and isolated compound, quercetin from *B. biternata* in H9C2 and A375 cell lines

The H9C2 cell line (Cardiomyoblast cell line) was established from embryonic BD1X rat (*Rattus norvegicus*) heart tissue. It exhibits myoblast morphology as well as possess many skeletal muscle properties. This cell line could be useful for studying the heart tissue of mammals and in a variety of specialized research related to molecular and cellular biology. H9C2 expresses the acetylcholine receptor and is useful in scientific research as a transfection host (Li *et al*., 2009). Doxorubicin is used as standard anticancer compound. In the MTT assay of crude methanol extract, the percentage of cell viability of H9C2 cell line were found to be 90.86%, 64.39% and 40.26% (IC_{50}= 38 µg/ml) as shown in "Fig. 2" and Plate 2 and for isolated compound quercetin, 86.17%, 58.42% and 28.15% (IC_{50}= 35.4 µg/ml) for sample concentration 100 µg ml^{-1}, 500 µg ml^{-1} and 1000 µg ml^{-1}respectively ("Fig. 2" and Plate 3).

A375 cell line was used as a model of skin melanoma malignant cells. Melanoma is a skin cancer that arises from the malignant transformation of melanocytes. Epidemiological studies showed that the incidence of melanoma is increasing at a rate faster than that of any other cancers worldwide (Stratigos *et al*., 2007). Early stage melanomais confined to epidermis and is curable, metastasized melanoma has an unfavorable prognosis, where the overall survival for patients with metastatic melanoma ranges from 4.7 to 11 months, with a survival rate of 8.5 months (Sun and Schuchter, 2001). This poor prognosis is due to the lack of effective treatment options (Atallah and Flaherty, 2005).

In the MTT assay of crude methanol extract, the percentage of viability of the A375 cell line were found to be 94.17%, 70.14% and 44.58% (IC_{50}= 25.3 µg/ml) as given in "Fig. 3" and Plate 4 and isolated compound quercetin, 92.14%, 68.34% and 38.26% (IC_{50}= 20.9 µg/ml) for sample concentration 100 µg ml^{-1}, 500 µg ml^{-1} and 1000 µg ml^{-1} respectively ("Fig. 3" and Plate 5). The percentage cell viability of both H9C2 and A375 cell lines in isolated compound quercetin could be compared to standard drug (doxorubicin, IC_{50}= 23.2 µg/ml). The result of the analysis of antiproliferative activity showed that high amount of activity in H9C2 cell lines compared to A375 cell line.

IV CONCLUSION

Quercetin reported to have anticarcinogenic activity against cancer cell types including, breast, leukemia, colon, ovary, squamous cell, endometrial and gastric cells. Quercetin exerts its antitumor effects through mechanisms such as apoptosis induction, anti-angiogenesis, prevention of cell metastasis and arrest of cell growth proliferation (Zhang *et al*., 2013).

Quercetin, one of the flavonoids in fruits and vegetables reported to have powerful antioxidant activity (Tong-un *et al*., 2010). Quercetin is included in flavonoids present in plants that are used as foods. Quercetin act as antioxidants, scavenges oxygen radicals, inhibits xanthine oxidase and lipid peroxidation. It also inhibits oxidation of low density lipoprotein (LDL) cholesterol, probably by inhibiting LDL oxidation itself, by protecting Vitamin-E in LDL from being oxidised or by regenerating oxidized Vitamin-E. It possessess useful pharmacological effects due to its strong antioxidant effects such as prevention of circulatory disease, antiinflammatory effects and anticancer effects (Rice-evans and Miller, 1998). The anticancer effects

of quercetin could be associated with the mechanism that supressed mutations by eliminating free radicals as per Edenharder and Grunhage (2003). Quercetin belongs to the group of flavonoids that are largely effective as antioxidant, antiinflammatory, hepatoprotective and anticancer agents (Sharada *et al*., 2013).

The isolated compound quercetin had more *in vitro* anticancer activities on various cell lines than crude methanolic extract of *B. biternata*.

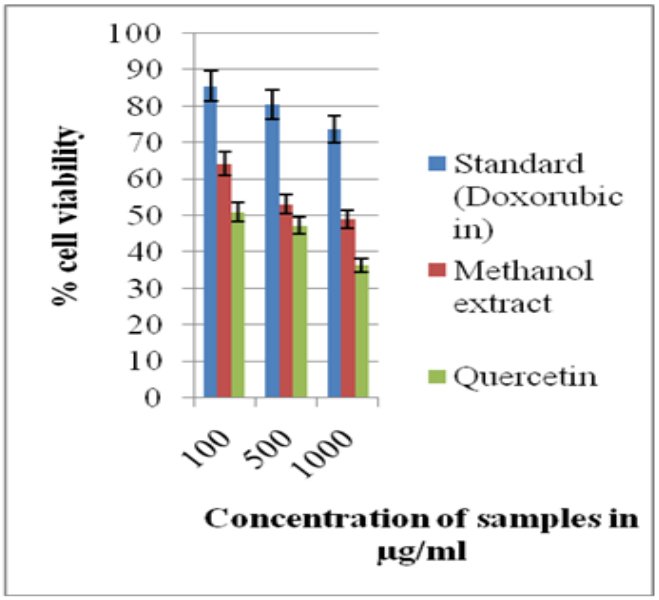


Figure 1 Cytotoxic effect of methanol extract and quercetin on L929 cell line

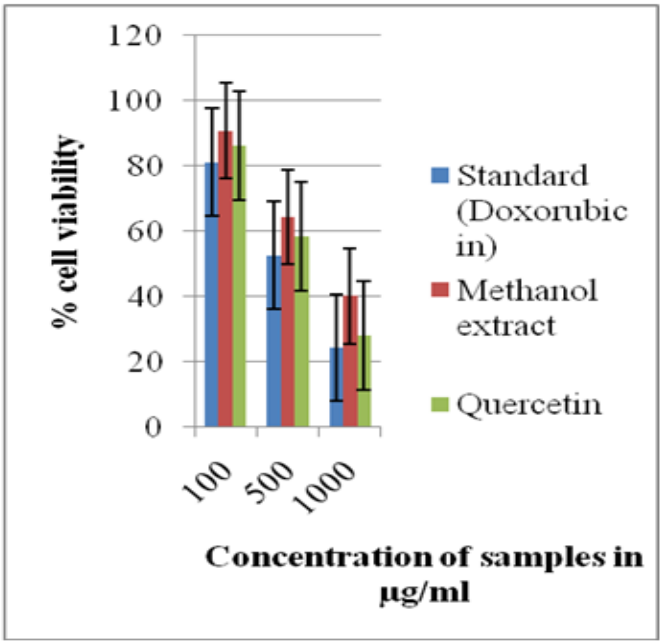


Figure 2 Antiproliferative activities of methanolic extract and quercetin of *Bidens biternata* in H9C2 cell lines

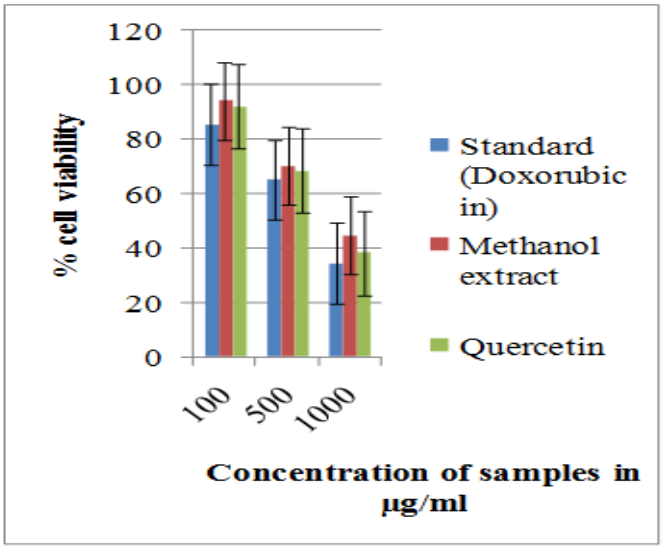


Figure 3 Antiproliferative activities of methanol extract and quercetin of *Bidens biternata* in A375 cell line

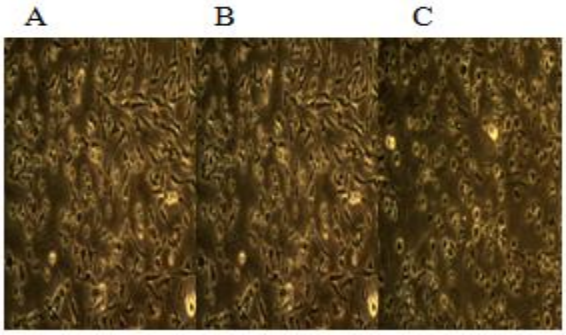
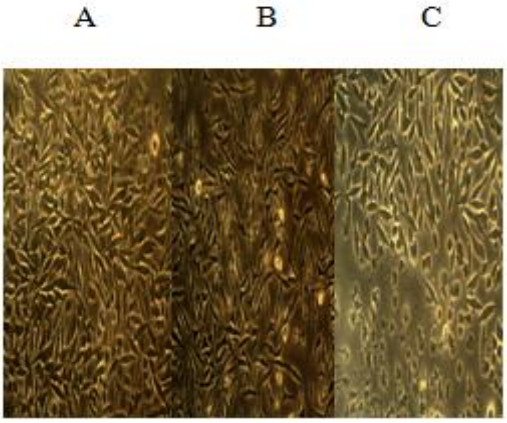


Plate 1 Cytotoxic effect of methanol extract and quercetin on normal cell line L929

a) Methanol extract

A –100 µg ml^{-1} Sample, B –500 µg ml^{-1} Sample, C – 1000 ml^{-1} Sample



b) Quercetin

A –100 µg ml^{-1} Sample, B –500 µg ml^{-1} Sample, C – 1000 ml^{-1} Sample

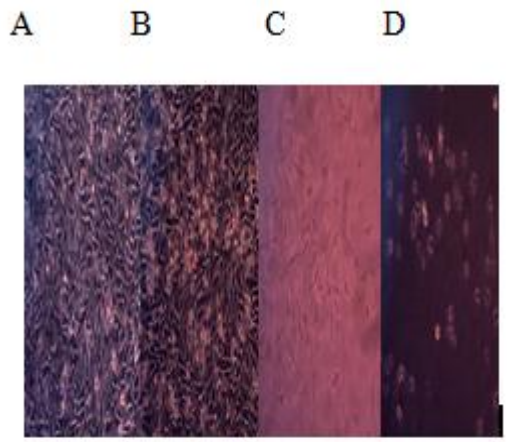


Plate 2 Antiproliferative activities of methanol extract in H9C2 cell line

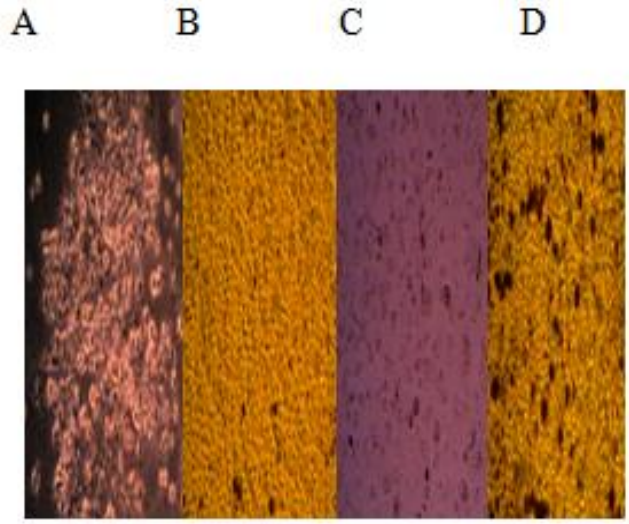


Plate 3 Antiproliferative activities of isolated compound quercetin in H9C2 cell line

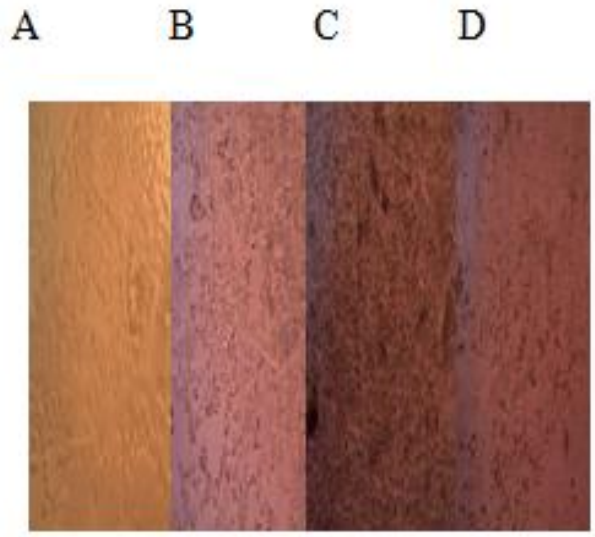


Plate 4 Antiproliferative activities of methanol extract in A375 cell lines

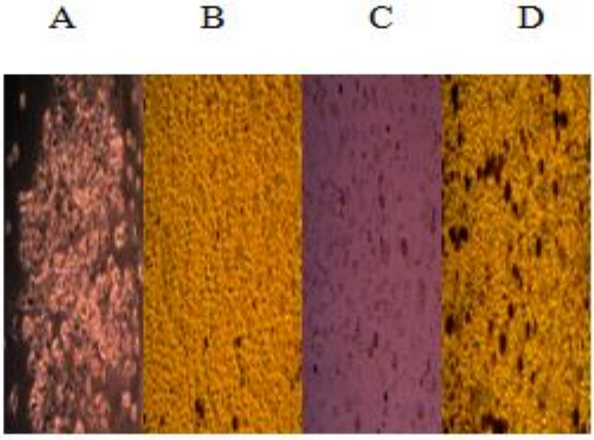


Plate 5 Antiproliferative activities of isolated compound quercetin in A375 cell line

A – Standard, B - 100 µg ml^{-1} Sample, C - 500 µg ml^{-1} Sample, D - 1000 µgml^{-1} S

ACKNOWLEDGEMENT

The author is grateful to the Kerala State Council for Science Technology and Environment (KSCSTE), Pattom, Thiruvananthapuram for funding this research work.

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BIOGRAPHY

Dr. Pradeesh. S has graduated from Christian College, Kattakada, Thiruvananthapuram and post graduation from University College, Palayam, Thiruvananthapuram, under University of Kerala. He procured his Doctoral Degree in Tissue culture and Phytochemistry from University of Kerala (under the guidance of **Dr. T. S. Swapna**, Associate Professor, Department of Botany, University of Kerala, Karyavattom, Thiruvananthapuram). He has also worked as Botanical Assistant at Botanical Survey of India (BSI) Southern Regional Centre, Coimbatore, Tamil Nadu. Currently he is working as Guest Lecturer at Dept. of Botany, Christian College, Kattakada, Thiruvananthapuram, Kerala. He has published over 20 research papers in International and National Journals and Conferences