Cytotoxic studies and phytochemical screening of *Pogostemon quadrifolius* (benth)

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Abstract

Objectives: *Pogostemon quadrifolius* (Benth.) is a medicinal aromatic shrub in family Lamiaceae which is used as folk medicine in India and reported to possess anti-proliferative and anti-oxidant property. Present study aims to evaluate the cytotoxic potential of the plant in a simple plant model system and chemical profiling of the crude extract by spectroscopic studies.

Methodology: Cytotoxicity of aqueous and other two solvent extracts; methanol and hexane was evaluated *in vivo* by *Allium* chromosome aberration assay. Cytotoxicity of the extracts was evaluated by calculating mitotic index (MI) and occurrence of chromosome aberration (CA) in root meristematic cells of *Allium*. Phytochemical analysis of two solvent extracts; methanol and hexane was evaluated by UV-Vis spectroscopy and FT-IR.

Results: Plant extracts showed significant toxicity in meristamatic cells of *Allium* in the higher concentration (100µg/ml). All the extracts had a significant effect on MI and induced CA thus revealed significant effect on the genetic material by inducing a spectrum of clastogenic as well as non-clastogenic CA. Among the tested extracts, hexane extract showed higher toxicity in terms of induction of CA and decreasing MI. Phytochemical screening of both solvent extracts showed a wide spectrum of bioactive compounds like phenolics, alkenes, alcohols, aromatic compounds *etc*.

Conclusion: Cytotoxicity of extract apprises the necessity of further studies in other *in vivo* systems. Spectroscopic analyses indicated the presence of bioactive compounds. Further isolation and characterization of the extract may lead to some pharmacologically important compounds that might pave a new avenue to drug research.

Keywords: *Pogostemon quadrifolius*, *Allium cepa* assay, Chromosome aberration, Mitotic index

1. Introduction

For the past few decades, herbal medicines are getting attention worldwide and large number of quality research is going on in different parts to identify the therapeutic benefits of medicinal plants. Western Ghats is a rich source of many endemic aromatic medicinal plants which are rich in essential oils of commercial and biological activities. Medicinal plants cover a major segment of flora which includes aromatic herbs, the prime source of raw materials used in the pharmaceutical, fragrance, cosmetic, flavour and perfumery industries. Even though much advances is happening in synthetic drug research, researches are on the way to develop more safe and potent plant derived medicaments (Harvey *et al*., 2008; Meena *et al*., 2009) [10, 14]. Although modern medicine is now available over the globe, people in most of the countries rely on medicinal plants and traditional system of medicines like Ayurveda, Unani, Sidda and many other indigenous and folk practices for different ailments [Sucher and Carles., 2008.] [19].

Nowadays, herbal medicines are now attracting attention as potential sources of anticancer agents. A single plant processed in different formulations can be used to cure a wide range of diseases. The universality and efficiency of traditional medicine/medicinal herbs is evident in their continued use and dependence up till the present day by a significant portion of the world's population (Mathews *et al.*, 1999) [13].

Pogostemon quadrifolius (Benth.) is a medicinal aromatic shrub in family Lamiaceae which distributed in India, Bangladesh and Myanmar (Bhatti and Ingrouille, 1997; Lansdown, 2011) [3, 11]. The plant is used as folk medicine in India for the treatment against chicken pox worms and also as a blood purifier (Biswas *et al*., 2010; Padal and Chandrasekhar, 2013; Padal *et al*., 2013; Padal and Raju, 2013; Raju *et al*., 2014) [2, 15, 15, 16, 17]. The essential oil of the plant was reported to exhibits mosquito larvicidal and antimicrobial property (Thoppil *et al*., 2003; Trivedi, 2006) [21]. Antiproliferative property of *P. quadrifolius* (Benth.) leaf extracts on different cell lines were also studies and it was found that a novel compound(Z)-ethylidene-4,6-dimethoxycoumaran-3-one is responsible for the apoptotic potential of the extract (Cheriyamundath *et al*., 2015; Klika *et al*., 2014) [5, 12]. The plant was found to have anti-oxidant potential as depicted from DPPH radical scavenging property (Cheriyamundath *et al*., 2015) [6].

2. Materials and methods

Preparation of extract: Plant materials from field were collected from the campus, identified by Dr A. K. Pradeep, Assistant Professor, dept. of Botany, University of Calicut. Methanol: water (70:30), petroleum ether, chloroform and Hexane extract of shade dried leaves were procured using Soxhlet apparatus and concentrated and kept at 4 ^{o}C. Later it was suspended in 2% DMSO for *in vivo* analysis. Onion bulbs were purchased freshly from local markets. Old roots and dry scales were removed and allowed to germinate in garden soil. Then the root meristems were exposed to various extract concentrations at its peak mitotic time for 3hrs.

Mitotic squash preparation: Mitotic squash preparation of the root tips were done with improved techniques (Sharma A.K., *et al*., 1990).

The root tip cells were fixed, stained, and examined using a compound microscope. The treated roots were rinsed in distilled water and cut into segments of 1-2 cm length from the tips and fixed in carnoy's fluid for 1hr at room

temperature. Then the specimens were transferred into cases containing 70% ethanol and sealed with stretch film and kept at +4 °C until use. The root tips were hydrolyzed in 1 N HCl for 3 min at room temperature and stained with 2% aceto-carmine solution for 1 1/2 hrs.

Selection of control: 2% DMSO was taken as negative control.

Cytological study: Five preparations were arranged for every group in mitotic index and phase index application. Specimens were transferred from dye to a microscope slide and a drop of acetic acid (45%) was added. Root tips (1-2 mm) were cut into tiny pieces and covered with a cover-glass. The cells were subsequently squashed by knocking with a blunt end of a pencil and pressing slightly down with the thumb. Excess liquid was sucked up by a piece of blotting paper. Mitotic index was calculated as percentage of dividing cells. Slides were scanned to investigate the different stages of mitosis. Approximately 5000 cells were scanned for each group of onion. Each experiment was repeated five times and at least five micro slides were prepared for each parameter (Fiskesjo 1985). The slides were scored for mitotic index (MI), percentage of abnormal cell (AC) and micronuclei (MN) in Magnus MLX series microscope and photographs were taken using camera.

Statistical analysis: The data of Mitotic index (MI) and percentage of abnormal cell (AC) are represented in percentage mean ± SE, and their level of significance was calculated by Student's *t* test. Chromosome aberration types and its percentage (Tables 1 & 2) were significant at $p < 0.05$ using Student's t test. The analysis was performed using SPSS 17.0.

3. Results and discussion

The results of the *Allium cepa* root growth response to MI by different solvent extract of *Pogostemon quadrifolius* is presented in Table 1. Negative control showed high MI which explains it least toxicity on root tip cells. From the table it was observed that Mi was highest in methanol extract. Aqueous extract showed significantly ($p \leq 0.05$).higher toxicity than methanol extract. This shows the least toxicity of methanol extract. Hexane extract showed a very low MI. This shows the mitodepressive potential of hexane extract in the *in vivo* plant system.

Table 1: Effect of different extract on mitotic index of *Allium* root tip cells

	Types extracts(100µg/ml)	MI(mean ± SE)
I h	Aqueous	80.9700±1.17246
	Methanol	79.1300±0.78048
	Petroleum ether	67.9480±2.58678
	Hexane	61.4780±3.90845
	negative control(DMSO)	88.6660±1.36627

All the values in the same column were significant ($p \leq 0.05$).

Fig 1: *Pogostemon quadrifolius* habit

Different type of aberrations, seen in different solvent extracts of *P. quadrifolius* extract is shown in fig 2. In prophase, single and double lesions (fig 2.g) were common. In metaphase, scattering was more frequent. Sticky metaphase (fig 2c), misorientations, diagonal metaphase (fig 2e), ball metaphase (fig 2 h) etc were also seen. In anaphase, sticky anaphase (fig 2a &c), diagonal anaphase, misorientation, hyper condensation etc were common. In telophase, lesions and hyper condensation were prominent.

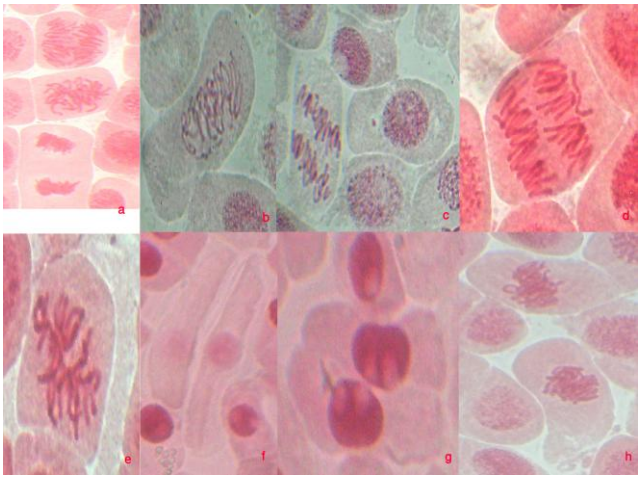

Fig. 2: Allium root tip cells showing chromosome aberrations by the treatment of *Pogostemon* solvent extracts a. Cell with polyploidy and sticky metaphase b. Cell with striated metaphase c. sticky polyploidy anaphase & cells with MN d. Polypoidy e. Diagonal metaphase f. Ghost cell & Giant cells g. Double lesion h. Ball metaphase

Percentage of chromosome aberrations induced by different solvent extract in the study is shown in fig 3. Control group showed the least aberration where hexane extract induced highest percentage of CA.

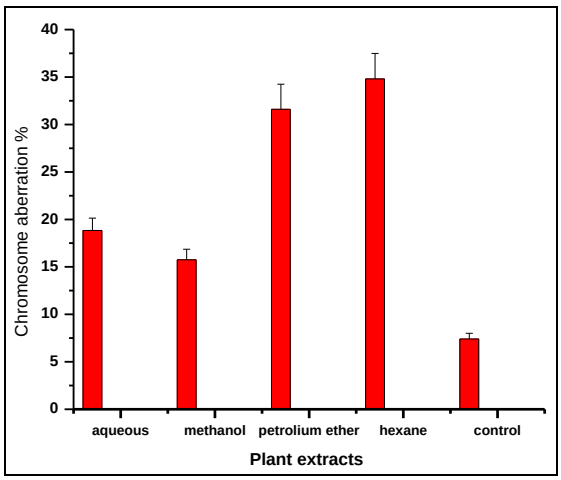

Fig 3: Incidence of chromosome aberrations (%) in different plant extract used in the study

The results of this study showed that *P. quadrifolius* aqueous and three solvent extracts elicit anti-mitotic activity and clastogenic events in *Allium cepa*.

Antimitotic effects under the present investigation were manifested as mitotic inhibition, mitotic arrest and various spindle abnormalities. In prophase, major aberrations in all extracts were single, double and multiple nuclear lesions. Chromosome lesions were due to the interruption in DNA replication in cell which had just finished the phase of synthesis, when affected by analogues (Taylor *et al*. 1962) [20]. Misorientaions were the most frequent non clastogenic aberration observed in almost solvent of *P. quadrifolius* which may be due to changes in the polarity of spindle assembly and the spindle interruption. Fragments, gaps, bridges in different phases were observed in hexane extract of *P. quadrifolius*. The process of chromosome fragmentation results in loss of viability, but is apparently non-apoptotic and further differs from cellular death defined by mitotic catastrophe (Christine *et al*. 2007) [7]. Chromosome fragmentation represents an efficient means of induced cell death and is a clinically relevant biomarker of mitotic cell death (Steven *et al*. 2005) [18].

MN induction was significantly low in aqueous extract of *P. quadrifolius* but frequent in hexane and petroleum ether extract. The induction of MN is usually the outcome of chromosome breaks/fragments or spindle poisoning which is an anomalous disjunction of chromosomes at anaphase stage of cell cycle (Grover *et al*. 1999) [9]. The micronucleus test in interphase cells gave a much higher mutagenicity than the chromosomal aberration test in anaphase-telophase cells (Feretti *et al*. 2007) [8]. The significant and concentration dependent induction of chromosomal aberrations including micronucleus formation, in root tip cells exposed to extract indicates the genotoxic potential of both plants especially *P. quadrifolius.*

Mitotic Index (MI) level shows that experimental materials had mitodepressive effect resulting in the inhibition of cells access to mitosis. The decline of MI below 22% in comparison to negative control can cause lethal effects on the organism (Antonsiewicz 1990) [1]. The inhibition of mitotic index is attributed to be the effect of environmental chemicals on DNA/protein synthesis of the biological system (Chauhan *et al*. 1998) [4].

Root growth in *Allium cepa* like other plants is due to expansion of cells in the elongation zone of the root tip where cellular differentiation occurs. The biological processes involved in cellular expansion include water uptake, nitrogen mobilization, increased sugar synthesis and plasma and tonoplast membrane flexibility. Metabolites such as ascorbate and enzymes such as asparagine synthase and membrane ATPases have been described as promoters and mediators of these biological processes. Alterations in these biological processes including disrupted lipid biosynthesis by plants and toxins have been linked to reduced cell wall expansibility, loss of vacuolar homeostatic regulation, cellular cytotoxicity, cell necrosis and root growth inhibition. Oxidative stress characterized by depletion of the root reduced glutathione (GSH) has been demonstrated to mediate these pathologic pathways.

Therefore, the observed inhibition of root growth by *P. quadrifolius* in this study suggests that the extract contain biologically active compounds that can impair one or more other biological processes which mediate cell expansion and differentiation at the elongation region of *Allium cepa* root tip. Plants and toxins such as *Calotropis procera* and podophyllotoxins have been described to impact negatively on these processes to cause root growth inhibition/arrest. It is observed *P. quadrifolius* to cause reduction in mitotic index even in a very low concentration (This provides an indication for mitodepressive activity of *P. quadrifolius* on *Allium cepa* at these concentrations. They also suggest that cytotoxicity is linked to mitodepression of *Allium cepa* root meristem due to the extract, meaning that *P. quadrifolius* affects both meristematic and differentiated cells in the root tip of *Allium cepa*. The mitodepressive activity of folk medicines has also been reported for *O. gratissimum, Azadirachta indica, Mangifera indica, Morinda lucida* and *Cymbopogon citratus* (Akintonwa *et al.,* 2009; Oyedare *et al.,* 2009; Akinboro *et al.,* 2007*).*

In this study, chromosomal scattering and multipolar anaphase chromosomes were observed at all extracts but relatively at low concentrations suggesting that these aberrations are sub-lethal when occurred temporarily. Aberrations observed at interphase stage of cell cycle like binucleated cells and micronucleus formation was very rare. In *Allium cepa*, such inhibition arrest cell plate formation and this has been attributed to phlamogram inhibition at the early stage of telophase (Fiskesjo, 1997; Rank *et al.,* 2002; Badr *et al.,* 1987). However, complete arrest of mitosis by these plants was not observed at lower concentrations in the range tested *P. quadrifolius* in this study.

Recent researches in the phytochemical analysis and its bio activity suggest that the plant is rich in monoterpenoids, triterpenoids, sesquiterpenoids, phytosterols, flavonoids,

organic acids, lignins, glycosides, alcohols and aldehydes. Many of these compounds were reported to possess cytotoxic and antiproliferative activity. Park *et al*., 1998; Yu., 2012; Jeong *et al*., 2013)

Plant extracts showed significant toxicity in meristamatic cells of *Allium* in the tested concentration (100µg/ml). All the extracts had a significant effect on MI and induced CA and revealed significant effect on the genetic material by inducing a spectrum of clastogenic as well as non-clastogenic CA. Among the tested extracts, non-polar extracts showed higher toxicity. Hexane extract showed highest toxicity in terms of induction of CA and decreasing MI.

4. Conclusion

Antimitotic substance has been using with some success in cancer chemotherapy. Cytotoxicity of extract apprises the necessity of further studies in other *in vivo* systems. Chemical profiling of the plant extract is also necessary to understand about the active components in crude extract.

5. Reference

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