ENIGMATIC INDUCTION OF CYTOMIXIS IN ALLIUM CEPA ROOT MERISTEM BY AGLAIA EDULIS ROXB. LEAF EXTRACT AND ITS PHYTOCHEMICAL RATIONALE

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ABSTRACT

Objective: The present study aims to analyze the potential of A. edulis Roxb. leaf extract to induce cytological aberrations in Allium cepa root meristem and to determine the phytoconstituents in the extract.

Methods: Cytotoxicity evaluation of the leaf methanolic extract was done using Allium cepa assay using various concentrations. Volatile phytoconstituents in the extract were determined using gas chromatography–mass spectrometry analysis.

Results: Considerable number of cytomictic cells along with other aberrations was observed. The occurrence of cytomixis was found to be dose dependent where it ranged from 6.58±0.35 to 29.45±0.45. The percentage of cytomictic cells among the total aberrant cells was observed between 35.19±1.67 and 77.39±1.39. The phytochemical analysis of the plant extract revealed the presence of active secondary metabolites.

Conclusion: The synergistic action of the active compounds might have triggered the phenomenon of cytomixis which, in turn, could be exploited for the production of polyploids.

Keywords: A. edulis, Cytomixis, Cytological aberrations, Secondary metabolites, Gas chromatography–mass spectrometry, Polyploidy.

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INTRODUCTION

Aglaia edulis is a tropical evergreen tree, belonging to the family Meliaceae. It is widely distributed in the tropical forests of Asian countries yet its utility remains underexplored. Some indigenous communities have been reported to use the plant for several purposes. The fruit and aril are used, as they are edible and its pericarp is taken against diarrhea. Wood is used for light construction work locally. A. edulis is a mid-canopy tree that can grow up to 30 m tall. The genus Aglaia is the only known source of the group of compounds, commonly called rocaglate or rocaglamide derivatives also known as flavaglines. These unique plant compounds have been reported by several researchers for its potential antimicrobial, anthelmintic, and insecticidal properties, as well as for its cytotoxic effect against human cancer cell lines [1].

Cytomixis, migration of cytoplasmic or nuclear materials among adjacent cells, is a complex phenomenon well reported in the microspore cells [2-6]. Cytomixis was observed in different plant groups including pteridophyta, gymnosperms, monocots, and dicots. More specifically, the process was mainly reported in hybrids, aneuploids, polyploids, and mutant individuals [7-10] and that too during the process of microsporogenesis. However, it is also reported in the somatic cells of root and shoot tips [11] and tapetal cells [12] but is less common.

The process of cytomixis involves the migration of cytoplasm along with the nuclear material of a cell, to its adjacent cell through a connection known as cytomictic channel. Gates [13] assigned the term cytomixis to this cytological event in meiocytes of Oenothera gigas L. This process of inter-pollen mother cell (PMC) transfer of chromatin material was first described in the microsporangia of gymnosperms by Arnoldy [14]. Later, Kornicke [15] reported it during the microsporogenesis in Crocus vernus (L.). The phenomenon has been described for the PMCsofa wide range of flowering plants [16]. On the other hand, cytomic migration has also been reported in other tissues, such as shoot apex of arboreal plants [17], in the proembryos of graminaceous plants [18], and in the vegetative tissues of anther [19]. Cytomixis was also observed to be of high frequency in transgenic tobacco [20]. Reports also show that herbicides [21] and chemicals [22] might induce cytomictic migration. Some cytotoxic phytoconstituents might also be able to induce cytomixis on meristematic tissues.

The purpose of this work is to investigate the chemical composition and potential of the leaf extract of A. edulis to induce cytotoxicity, particularly cytomixis in Allium cepa root meristem. This is the novel report on the cytotoxic effect of A. edulis on A. cepa root meristem.

METHODS

Preparation of extracts

The leaves of the plant A. edulis were collected from the Kurichiar hills of Wayanad district, Kerala, India. The plant was authenticated at the Department of Botany, University of Calicut, Kerala, India (CALI no. 123757). The leaves were dried in the shade and powdered using an electric blender and stored in a moisture-free atmosphere. Soxhlet extraction of the dried material was done for 6 h using methanol as solvent and was evaporated to dryness under reduced pressure.

Treatment of root meristem with the extract

A. cepa bulbs procured from TNAU, Coimbatore, were used for the present study. This was grown in autoclaved moist sand until the root acquires a length of approximately 10 mm. The bulbs were treated with the various concentrations of leaf extracts of A. edulis. The concentrations used were 0.5%, 0.75%, 1%, 2%, and 3% of the leaf extract in distilled water for 24 h. Mitotic squash preparations were done using the modified protocol [23] of Chazotte. The root tips of 20 mm length were excised at the peak mitotic period and fixed in alcohol:acetic acid (3:1) mixture followed by hydrolysis in 1 N HCl (Merck Pvt. Ltd., India). The fixed roots were incubated in phosphate-buffered saline (PBS) for 15 min and stained using 4’-6-diamidino-2—phenyl indole (DAPI) (HiMedia Laboratories Pvt. Ltd., India) of 0.1 mg/mL concentration in dark for 30 min. This was followed by washing in PBS and the squashed root...
meristem was mounted in glycerol. Observations were done using Leica DM6 B system microscope at ×40/0.80 magnification and fluorescent imaging was done using Leica DFC 450C camera with the acquisition software Leica LASX.

Statistical analysis

Data obtained from the observations were subjected to statistical analyses. One-way ANOVA was performed using Duncan’s multiple range test to determine the standard error and significance of treatments, using SPSS version 20. Data were expressed as percentage cytomicxis±standard error of mean. Cytomixis percentage out of the total aberrant cells as well as the total cells in the field was scored. p<0.05 was considered to be statistically significant.

Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis of methanolic leaf extract was carried out on a Varian model CP-3800 GC interfaced with a Varian Saturn 2200 Ion Trap Mass Spectrometer. Identification of individual components was done using NIST MS library search.

RESULTS

The cells were counted in each field and photographs were taken. These photographs were analyzed for the percentage of aberrations and this in turn was used for the calculation of the percentage of cytomicxis out of the total cells in the field as well as the percentage of cytomicxis out of the total aberrant cells. A proportionate increase in the aberration percentage as well as in the cytomicxic cell percentage according to the increasing order of the extract concentration was observed (Table 1). Although negligible aberrations were shown in the negative control (distilled water treated roots), cytomicxis was totally absent. Various stages of the phenomenon were also traced out by repeated trials and image analyses of the fixed A. cepa meristicematic cells were done (Fig. 1).

GC–MS analysis of the leaf extract revealed the presence of potential secondary metabolites in considerable quantity (Table 2). Some of the major phytoconstituents observed were [3-(dimethylamino)phenyl] methanol (14.13%), Ethyl 3-methyl-2-butenoate (13.92%), D-alpha-tocopherol (10.17%), β-methyl-α, α-diphenyl-4-morpholine butyric acid (7.82%), and O-methyl psychotrine (7.17%). γ-sitosterol, a sterol of high pharmacognostic value, was detected in a considerable amount of about 4.37%. Other potential bioactive compounds such as terpenoids (14.94%) and phenols (3.46%) were also identified.

**DISCUSSION**

The secondary metabolites present in the plant produce cytotoxic effect by disturbing the normal cell cycle [24]. Cytomixis was previously considered as a normal process occurring in the PMGs

### Table 1: Percentage of chromosomal aberrations and cytomixis induced by different concentrations of the leaf extract of *Aglaia edulis*

| Conc. (%) | % of aberration scored | % of cytomixis among total aberrant cells scored | % of cytomixis among total cells scored |
|-----------|------------------------|-----------------------------------------------|----------------------------------------|
| Control   | 1.28±1.67              | 0                                             | 0                                      |
| 0.5       | 16.61±2.65             | 35.19±1.67                                   | 6.58±0.35                             |
| 0.75      | 25.17±0.94             | 52.24±2.05                                   | 13.83±0.49                            |
| 1         | 29.42±1.26             | 53.60±1.83                                   | 15.68±0.62                            |
| 2         | 38.25±1.05             | 69.13±2.95                                   | 29.45±0.45                            |
| 3         | 42.51±3.10             | 77.39±1.39                                   | 29.45±0.20                            |

### Table 2: Phytochemical profile of *Aglaia edulis* leaf methanolic extract obtained using gas chromatography–mass spectrometry analysis

| S. No. | Compound name                                      | Class                  | Retention time | Area% |
|--------|----------------------------------------------------|------------------------|----------------|-------|
| 1      | 3-Hydroxyphenyl phosphoric acid                    | Organic compound       | 8.747          | 2.51  |
| 2      | Benzene acetaldehyde                               | Aldehyde              | 10.237         | 0.52  |
| 3      | Mequinol                                           | Phenolic compound      | 11.536         | 2.03  |
| 4      | Ethyl 3-methyl-2-butenoate                         | Ester                 | 12.236         | 13.92 |
| 5      | 2-Naphthyl-β-D-galactopyranoside                   | Glyceride             | 12.965         | 0.71  |
| 6      | 7-Propylquinoline                                  | Quinone               | 13.352         | 0.41  |
| 7      | 5-Hydroxypipeolic acid                            | Organic compound      | 13.525         | 1.30  |
| 8      | m-Tolualdehyde                                    | Aldehyde              | 14.318         | 2.29  |
| 9      | β-methyl-α, α-diphenyl-4-morpholine butyric acid   | Organic compound      | 14.493         | 7.82  |
| 10     | 3-[dimethylamino] phenyl] methanol                 | Alcohol               | 15.424         | 14.13 |
| 11     | Copene                                             | Sesquiterpene         | 16.113         | 1.26  |
| 12     | 1,2,3,4,4a,5,6,8a-octahydroxaphthalene             | Hydrocarbon           | 16.228         | 0.42  |
| 13     | 1-[3,6,6,Trimethyl-1,6,7,7a-tetrahydrocyclopenta [c] pyran-1-yl] | Ketone | 16.295 | 1.46 |
| 14     | 2,5-Octadecanoyl acid methyl ester                 | Ester                 | 16.456         | 0.11  |
| 15     | Isoeucalyphene                                     | Sesquiterpene         | 16.610         | 0.42  |
| 16     | Cs-isoeugenol                                      | Phenolic compound     | 16.880         | 1.43  |
| 17     | γ-Himachalene                                      | Sesquiterpene         | 17.114         | 0.35  |
| 18     | Himachala-2,4-diene                                | Sesquiterpene         | 17.231         | 1.53  |
| 19     | Aciphiylene                                        | Sesquiterpene         | 17.357         | 2.02  |
| 20     | Cedrelanol                                         | Terpene               | 17.497         | 0.83  |
| 21     | 3-Methoxyethyl-2,5,5,8a-tetramethyl-6,7,8a-tetrahydro-5H-chromene | Organic compound | 17.634 | 1.15 |
| 22     | 9-Methoxylcalamene                                 | Organic compound      | 17.745         | 0.21  |
| 23     | Giotugenni                                        | Steroid               | 18.166         | 1.79  |
| 24     | Urs-12-en-28-ol,[3-acetoxy]-3β                      | Terpenoid             | 20.396         | 1.03  |
| 25     | 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8a, 11,11,12a,14,14b-octadecahydro-2H-pipec-3-one  | Terpenoid             | 20.459         | 3.93  |
| 26     | Lambda-8 [17,14-diene-13,17-diol                   | Diterpenoid           | 20.755         | 0.39  |
| 27     | 2,5-Bismethy1-1-silacyclobutyl] p-xylene            | Organic compound      | 20.861         | 2.90  |
| 28     | Abietu-6,13-diene                                  | Diterpenoid           | 21.080         | 2.77  |
| 29     | Agatic acid                                       | Diterpenoid           | 21.213         | 0.61  |
| 30     | Bicyclo[9.3.1]pentadeca-3,7, diaryl-12-ol          | Organic compound      | 21.515         | 0.58  |
| 31     | 3β-Pregn-5-ene-3,17, 20-triol                     | Steroid               | 22.454         | 1.70  |
| 32     | O-methyl psychotrine                              | Alkaloid              | 22.749         | 7.17  |
| 33     | 2, 2, 6, 6’, 6”, 9’, 9’-OCTAMETHYL-8, 8’-BITRCYCL[5.4.0]undecan | Organic compound | 22.871 | 4.00 |
| 34     | Lupeol acetate                                    | Terpenoid             | 23.069         | 1.06  |
| 35     | D-alpha-tocopherol                                | Organic compound      | 28.897         | 10.17 |
| 36     | 4,4-dimethyl-cholesta-22, 24-dien-5-ol             | Sterol                | 30.612         | 0.70  |
| 37     | γ-Sitosterol                                       | Sterol                | 31.598         | 4.37  |
inducing ability of the plant Allium might be the responsible factors for triggering cytomictic migration in these are observed, the most prominent among them was found to be cytological aberrations including both clastogenic and non-clastogenic cell cycle by acting against spindle formation, structural and functional extracts. The secondary metabolites in the plant extract might hinder the remarkably hiked according to the increasing concentrations of the the leaf extract of Allium cepa. Further investigations are required to elucidate the pathway by which the secondary metabolites induce cytomixis.

CONCLUSION

Leaf extract of A. edulis was proved to be a source of potential bioactive compounds that might have triggered aberrations in Allium root meristem among which cytomixis was found to be the prominent one.

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AUTHORS’ CONTRIBUTIONS

AER collected the plant specimen, carried out experimental analyses, and prepared the draft manuscript. JET designed and guided the experimental analyses, edited and finalized the manuscript. Both authors read and approved the manuscript.

CONFLICTS OF INTEREST

Declared none.

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Fig. 1: Various stages of cytomixis induced by Aglaia edulis leaf extract in Allium cepa root meristem: (a) initiation of cytomictic protuberance, (b) cytomictic protuberance, (c) cytomictic channel formation, (d) initiation of cytomictic migration, (e) cytomictic exchange, (f) cytomictic migration of chromatin, (g) cytomictic fusion of nucleus after migration, (h) enucleated donor cell after cytomixis showing the remnants of the channel, (i) enucleated donor cell after cytomixis, (j and k) binucleate cells after cytomixis, Bar=10 μm.
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