ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 3, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

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

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***Received: 21 November 2019, Revised and Accepted: 25 January 2020***

**ABSTRACT**

**Objective:** The present study aims to analyze the potential of *Aglaia 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:** *Aglaia edulis*, Cytomixis, Cytological aberrations, Secondary metabolites, Gas chromatography–mass spectrometry, Polyploidy.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i3.36429

## 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, anthelminthic, 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 PMCs of a wide range of flowering plants [16]. On the other hand, cytomictic 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 LAS X.

**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 cytomixis±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.

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

| Conc. (%) | % of aberration | % 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 |

## 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 cytomixis out of the total cells in the field as well as the percentage of cytomixis out of the total aberrant cells. A proportionate increase in the aberration percentage as well as in the cytomictic 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), cytomixis was totally absent. Various stages of the phenomenon were also traced out by repeated trials and image analyses of the fixed *A. cepa* meristematic 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 PMCs

**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. | P-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 | Glycoside | 12.965 | 0.71 |
| 6. | 7-Propylquinolinine | Quinone | 13.352 | 0.41 |
| 7. | 5-Hydroxypipecolic 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. | Copaene | Sesquiterpene | 16.113 | 1.26 |
| 12. | 1,2,3,4,4a, 5,6,8a-Octahydronaphthalene | 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-Octadecadienoic acid methyl ester | Ester | 16.456 | 0.11 |
| 15. | Isocaryophyllene | Sesquiterpene | 16.610 | 0.42 |
| 16. | Cis-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. | Aciphyllene | Sesquiterpene | 17.357 | 2.02 |
| 20. | Cedrelanol | Terpene | 17.497 | 0.83 |
| 21. | 3-methoxymethyl-2,5,5,8a-tetramethyl-6,7,8a-tetrahydro-5H-chromene | Organic compound | 17.634 | 1.15 |
| 22. | 9-Methoxycalamenene | Organic compound | 17.745 | 0.21 |
| 23. | Gitoxigenin | Steroid | 18.166 | 1.79 |
| 24. | Urs-12-en-28-al,(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, 7,8,8a, 10,11,12,12a, 14,14b-octadecahydro-2H-picen-3-one | Triterpenoid | 20.459 | 3.93 |
| 26. | Lambda-8 (17),14-diene-13,17-diol | Diterpene | 20.755 | 0.39 |
| 27. | 2,5-Bismethyl-1-silacyclobutyl)-p-xylene | Organic compound | 20.861 | 2.90 |
| 28. | Abieta-6,13-diene | Diterpene | 21.080 | 2.77 |
| 29. | Agathic acid | Diterpenoid | 21.213 | 0.61 |
| 30. | Bicyclo[9.3.1]pentadeca-3,7, dien-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', 6', 9, 9'-Octamethyl-8, 8'-bitricyclo[5.4.0]undecan | Organic compound | 22.871 | 4.00 |
| 34. | Lupeol acetate | Triterpenoid | 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 |

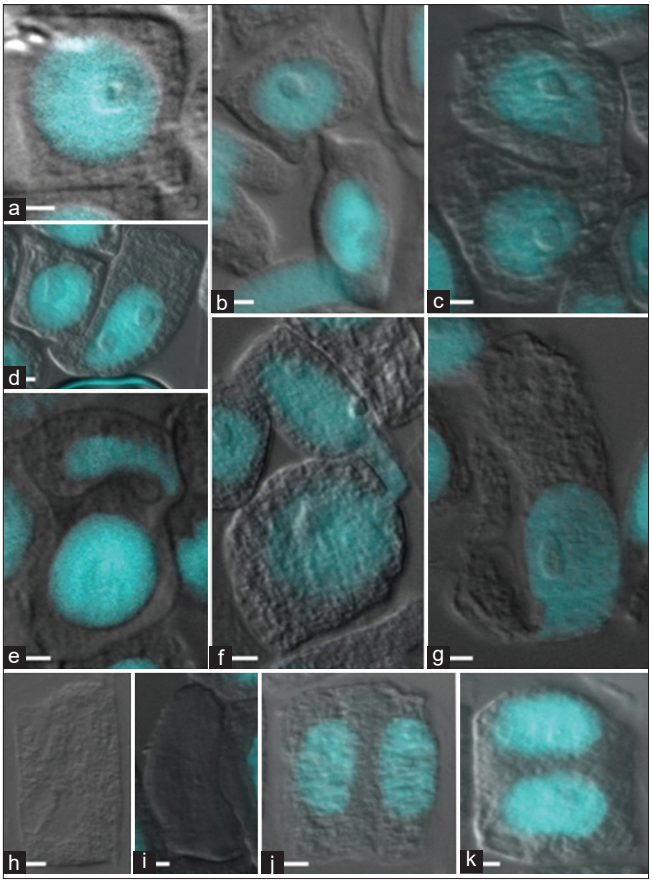


**Fig. 1: Various stages of cytomixis induced by *Aglaia edulis* leaf extract in *Allium cepa* root meristem: (a) initiation of cytomictic protuberance, (b) cytomictic protuberance, (c) cytomictic channel formation, (d) initiation of cytomictic migration, (e) cytomictic exchange, (f) cytomictic migration 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**

only. Recent research revealed that cytomixis might arise in the somatic cells too due to several reasons, namely, the effect of chemical agents such as colchicine [25], methyl methanesulfonate and ethyl methanesulfonate [25], and sodium azide [26]. Evidence obtained also revealed the occurrence of cytomixis due to the use of herbicides [27] and during pathological invasion [21]. It may also arise due to the inhibition of cytokinesis during microsporogenesis. Cytomixis might result in aneuploid, polyploid cells, and binucleate condition. Although these are considered aberrations, now they have acquired importance in plant breeding and improvement efforts. Aneuploid and polyploid crops have been of great interest nowadays for its increased vigor, stress tolerance, buffering of deleterious mutations, and their "gigas effect." The secondary metabolites in the plant extract might be the reason to trigger the phenomenon of cytomixis and this, in turn, was found to be directly proportional to the increasing concentration of the leaf extract of *A. edulis*. The aberration in the mitotic cells was also remarkably hiked according to the increasing concentrations of the extract. The secondary metabolites in the plant extract might hinder the cell cycle by acting against spindle formation, structural and functional proteins involved in the cell cycle, chromatin, or membranes. Although cytological aberrations including both clastogenic and non-clastogenic ones were observed, the most prominent among them was found to be cytomixis. The potential phytoconstituents detected in the leaf extract might be the responsible factors for triggering cytomictic migration in *Allium* root meristem. This is a novel report that exposes the cytomixis inducing ability of the plant *A. edulis.* 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.

## ACKNOWLEDGMENT

The authors are grateful to the Director, CSIF (Central Sophisticated Instrumentation Facility), University of Calicut, for providing the system microscope facility.

## 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.

## FINANCIAL SUPPORT

The authors also acknowledge the Council for Science and Industrial Research for providing financial support in the form of Junior Research Fellowship (Grant no: 09/043(0181)/2017-EMR-I).

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