Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 3517-3523
Received: 22-03-2019
Accepted: 24-04-2019

Jayanthi D
Research Scholar, R&D Centre, Bharathiar University, Coimbatore, Tamil Nadu, India

Martin Paul A
Research Scholar, R&D Centre, Bharathiar University, Coimbatore, Tamil Nadu, India

Thamizhseran N
Associate Professor, Department of Botany, St. Joseph's College (Autonomous), Bengaluru, Karnataka, India

Correspondence
Jayanthi D
Research Scholar, R&D Centre, Bharathiar University, Coimbatore, Tamil Nadu, India

Dual source phytohormones involved in flowering of *Spathodea campanulata* P. Beauv.

Jayanthi D, Martin Paul A and Thamizhseran N

Abstract
The process of flowering involves various hormones produced and translocated endogenously by plants. Exogenous applications of synthetic hormones have revolutionized the horticulture industry. In *Spathodea campanulata* floral part development occurs within water calyces, under watery fluid which is unique among flowering plants. The present study was carried out to identify plant hormones and quantify them from water calyx fluid, corolla and calyx tissues of *S. campanulata* in order to establish the role of water calyx and the bathing fluid as the exogenous source of hormones for floral development. Phytohormones *viz.*, IAA, Kinetin and GA_3 were identified and quantified by using RP-HPLC and were found to be present and occur in varying concentrations in the bathing fluid and in the floral tissues, exhibiting quantitative correlation to the process of flowering. It can be concluded that the flower development and flower opening in *S. campanulata* is also due the influence of phytohormones present in the calyx fluid besides the endogenous concentrations of tissues as an exogenous source of hormones. The present work provides the first preliminary evidence for the water calyx fluid as nature's exogenous source of hormones for development of floral parts.

Keywords: Water calyces, phytohomones, RP-HPLC

Introduction
The vegetative-to-reproductive transition is a milestone in entire plant life cycle and the phenomenon of flowering is the exciting cum integral aspect of plant biology [1]. Flower opening is a process involving sequences of elongation and expansion varying from plant to plant [2]. The whole phenomenon of flowering includes initiation of flower primordia and development of primordia into mature flowers till anthesis controlled by various factors like photoperiod, temperature, plant age and growth enhancing hormones. Plant-environment and gradation in endogenous biomolecules of plants govern flowering [3]. Organic substances like phytohormones influence various physiological process at low concentrations mainly on overall growth, differentiation and development [4]. As plant metabolism provides power and necessary building blocks, it is the plant hormones which govern and accelerate the process of growth thereby integrating the plant organs. Phytohormones are usually classified as auxins, cytokinins, gibberellins, abscisic acid, ethylene and epibrassinolide [5]. Of which auxins, cytokinins, gibberellic acids and ethylene have been well correlated with flowering [6, 7]. Plant hormones operate flowering temporally and spatially by varying their concentrations [8]. These small molecules produced through secondary processes by plants regulate many cellular processes either singly or influences single process as a whole [9]. The concentration of the compounds does not merely control the regulatory developmental processes but it is the sensitivity of the tissues [4]. These facts clearly state that the flower structure and flowering process is the most complex part of any given plant [10]. In case of S*pathodea campanulata* it is much more complex, peculiar and unique where the floral whorls differentiates and develops completely immersed in watery fluid secreted by the glands of calyx. The bud liquid is proven to be beneficial and obligatory for floral development. The fluid is found to be in alkaline containing various organic compounds, salts and essential osmotyles thus serving as optimum metabolomics by nourishing the developing floral whorls [11, 12]. Thus, identification and quantification of plant hormones during flowering in *S. campanulata* in the water calyx fluid and tissues will be providing information on the role of water calyx, the fluid and its contents.

Materials and Methods
Flower collection and sample preparation
Flower buds of *S. campanulata* were collected from in and around the city of Bengaluru. The healthy buds were selected and grouped into small, medium and large sizes based on the size of intact flower buds.

Hundred ml of the water calyx fluid from each different stage of buds was collected by using sterile syringe. The collected fluid was evaporated to dryness by incubating at 60^0 C in a hot air oven for 5 days. The evaporated residue was used for hormonal studies. The calyx and corolla were also collected separately at different stages of bud development and shade dried. The dried, powdered material was used for hormonal studies.

Sample preparation for HPLC analysis

1 gram of each sample was homogenized with 10mL of 0.1M Phosphate buffer (pH 7). The homogenate was centrifuged at 6000 rpm for 10 minutes at 4°C. The supernatant was transferred to a clean bottle and evaporated until dry. 10mg of the sample was used for the further analysis.

Estimation of Auxin

Auxin analysis was done using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), C-18 column (Make: Waters, Model: 550; Waters Corp., Milford, MA, USA, column symmetry with 4·6mm*250mm) at a flow rate of 1 ml/minute in isocratic mode. Methanol and water in ratio of 6:4 were used as mobile phase. The standard IAA (Indole-3-Acetic Acid) sigma grade with a concentration of 0.4mg/mL was dissolved in mobile phase and a concentration of 10 mg/ml of the extracted sample was dissolved in mobile phase. The sample and standard of 20µL was injected and the elution was monitored at 254nm. The amount of IAA present in the sample was estimated using the formula,

Sample area x standard amount x dilution x mean weight/standard area x dilution standard x sample amount

Estimation of Gibberellic acid

Gibberellic acid analysis was done using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), C-18 column (Make: Waters, Model: 550; Waters Corp., Milford, MA, USA, column symmetry with 4·6mm*250mm) at a flow rate of 1 ml/minute in isocratic mode. Acetonitrile and 0.01% ortho-phoshphoric acid in the ratio of 6:4 were used as mobile phase. The standard GA_3 sigma grade with a concentration of 0.4mg/mL was dissolved in mobile phase and a concentration of 10 mg/ml of the extracted sample was dissolved in mobile phase. The sample and standard of 20µL was injected and the elution was monitored at 206nm. The amount of GA_3 present in the sample was estimated using the formula,

Sample area x standard amount x dilution x mean weight/standard area x dilution standard x sample amount

Estimation of Cytokinin

Cytokinin analysis was done using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), C-18 column (Make: Waters, Model: 550; Waters Corp., Milford, MA, USA, column symmetry with 4·6mm*250mm) at a flow rate of 1 ml/minute in isocratic mode. Methanol and water in ratio of 4:6 were used as mobile phase. The standard Kinetin of sigma grade with a concentration of 0.04mg/mL was dissolved in mobile phase and a concentration of 10 mg/ml of the extracted sample was dissolved in mobile phase. The sample and standard of 20µL was injected and the elution was monitored at 270nm. The amount of cytokinin present in the sample was estimated using the formula,

Sample area x standard amount x dilution x mean weight/standard area x dilution standard x sample amount

Results and Discussion

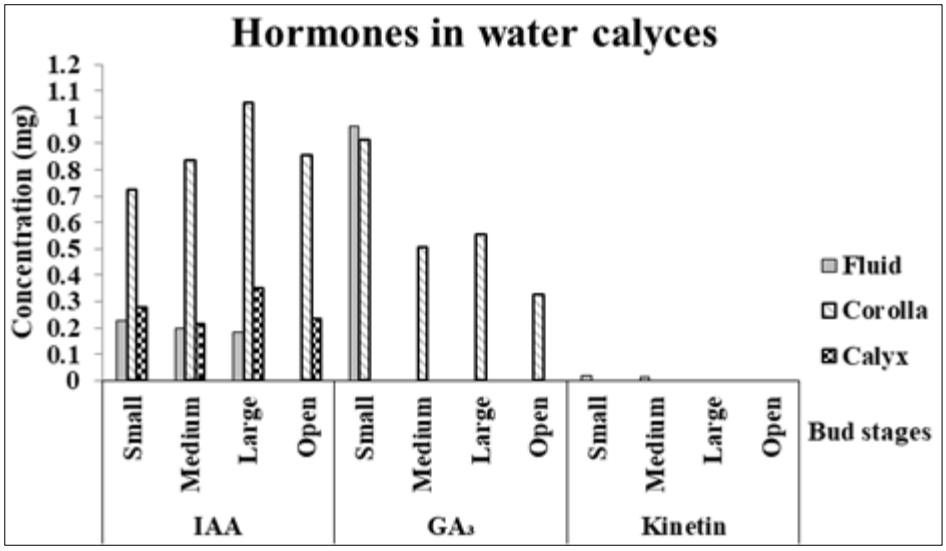

Fig 1: Graph showing different concentrations of phytohormones in different stages of water calyx fluid, corolla and calyx

The amount of IAA in the calyx fluid decreased linearly across different stages of bud maturity with 0.225 mg in small sized bud, 0.1954 in medium sized buds and 0.1839 mg in large sized buds (fig 1, 2). The higher amount of IAA in small bud signifies that it might play an active role in bud initiation and formation [13]. The IAA content increased linearly in corolla tissue with 0.726 mg in small sized corolla, 0.8366 mg in medium sized corolla, and 1.056 mg in large sized corolla and decreased in open corolla with 0.8563 mg (fig 3). Whether IAA is found endogenously or exogenously, they are known to induce flowering [14, 15]. Since, the fluid is also reported to contain appreciable amount of amino acids particularly tryptophan, the precursor for IAA formation could also be a possible reasons for IAA content [11, 12]. In calyx, maximum amount of auxin was found in large sized calyx (0.3518mg), followed by small sized calyx with 0.2772 mg, 0.2334mg in open calyx and 0.2139mg in medium sized calyx (fig 4). The overall increase in amount of IAA

estimated during the development of water calyces could be the cause for rapid elongation of floral buds, expansion of corolla and other inner whorls as IAA is considered to be the main regulator in flower timing [16].

Kinetin usually promotes cell division and cell enlargement [17]. Kinetin has the ability to promote cell division in the presence of IAA. Endogenous kinetin also contributes to cell division. Though, major cell division activity is controlled by IAA, cytokinesis is governed by kinetin [18]. Kinetin in fluid was found only in small and medium sized buds with 0.018mg and 0.0142 mg respectively (fig 1, 5), which is similar to the findings of Chen [19, 20]. Kinetin is known to increase the number of inflorescences and in promotion of essential whorls in *Jatropha* when supplemented [21]. Application of kinetin exogenously increases fresh weight, dry weight, nitrogen, potassium, phosphorous, total carbohydrates in tissues [22]. Similar role may be played by the kinetin present in the fluid by nourishing the floral whorls during its growth and development. The presence of kinetin could be one of the reasons for the floral whorls to develop in completely alkaline medium as they can mitigate and rescue plant tissues under saline conditions [23]. Abscissic acid and kinetin plays a significant role in plant-water relation in regulation of stomata. Flower development is itself energy driven process and as floral development in *S. campanulata* requires watery fluid throughout its development the kinetin identified might play a role in formation of water calyces [24].

Endogenous gibberellins regulate and initiates flower growth [25-28]. GA_3 content was found to be maximum in small sized corolla with 0.9131 followed by medium sized corolla with 0.5554 mg, 0.5038 mg in large sized corolla and 0.3238 in open corolla (fig 1, 7) which is similar to studies conducted on safflower [13, 29]. Gibberellins control the growth and opening of corolla as well as for normal growth of anthers [30]. Gibberellin concentration increase as corolla development takes place similar to the study [31]. Corolla elongation and expansion is also accompanied by production of anthocyanin pigments. In order for the corolla to produce coloring pigments gibberellins are also essential [32, 33]. GA_3 content promotes flower development by increasing number of essential whorls and inflorescences [21]. The inflorescence of *S. campanulata* produces 80-120 floral buds under natural conditions. All the examined floral buds exhibits good growth of anther filament and brightly colored reddish-orange corolla which could be because of the higher gibberellin content reported in the present study. Water calyx fluid also showed presence of GA_3 only in small sized bud of about 0.9663 mg (fig 1, 6) which could be the reason for the anther-filament development and anthocyanin production in corolla as these traits were prominently found all examined smaller sized buds [26]. In terms of cellular requirement macronutrients and carbohydrates could also be promoted by GA_3 present in the fluid [22, 12].

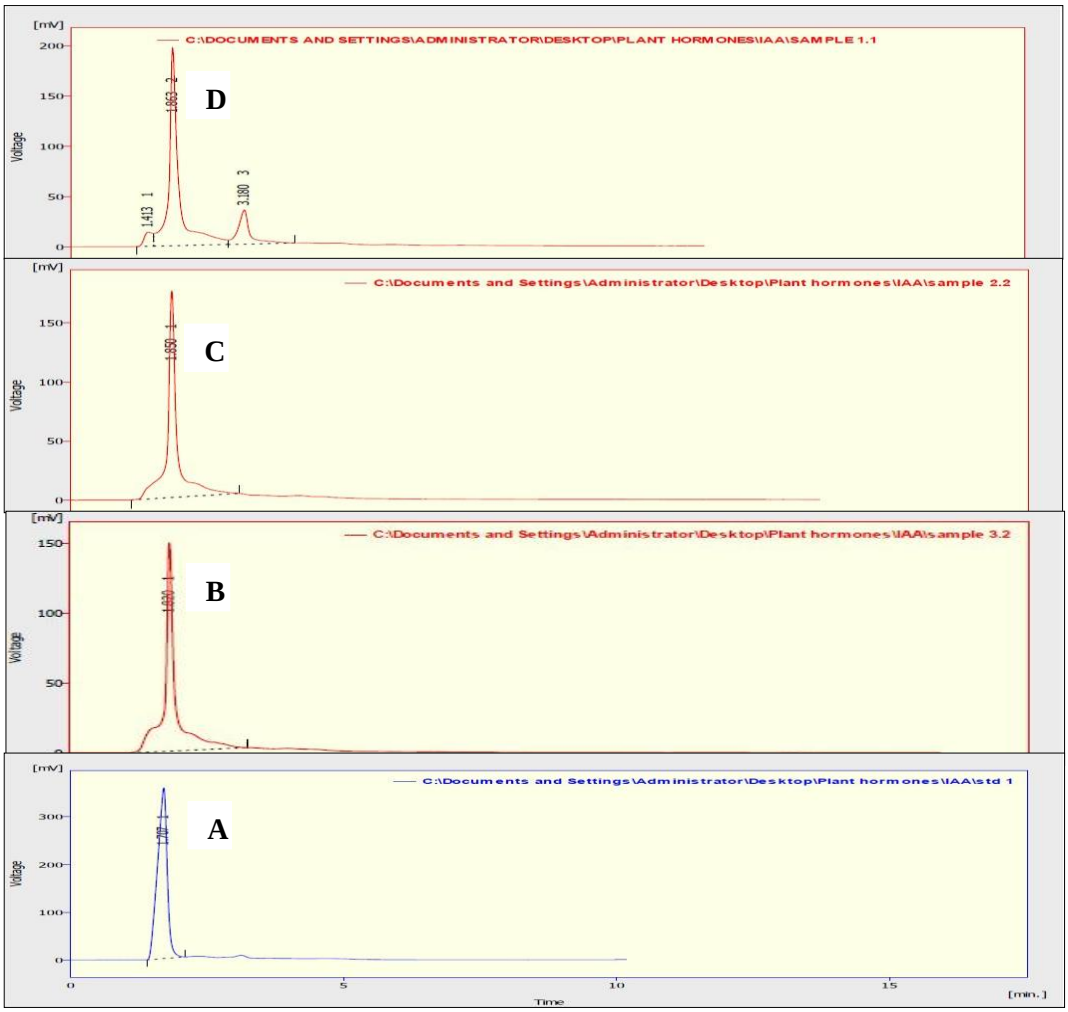

Fig 2: Chromatograph of IAA in water calyx fluid; A: Standard; B: large bud; C: medium bud; D: small bud

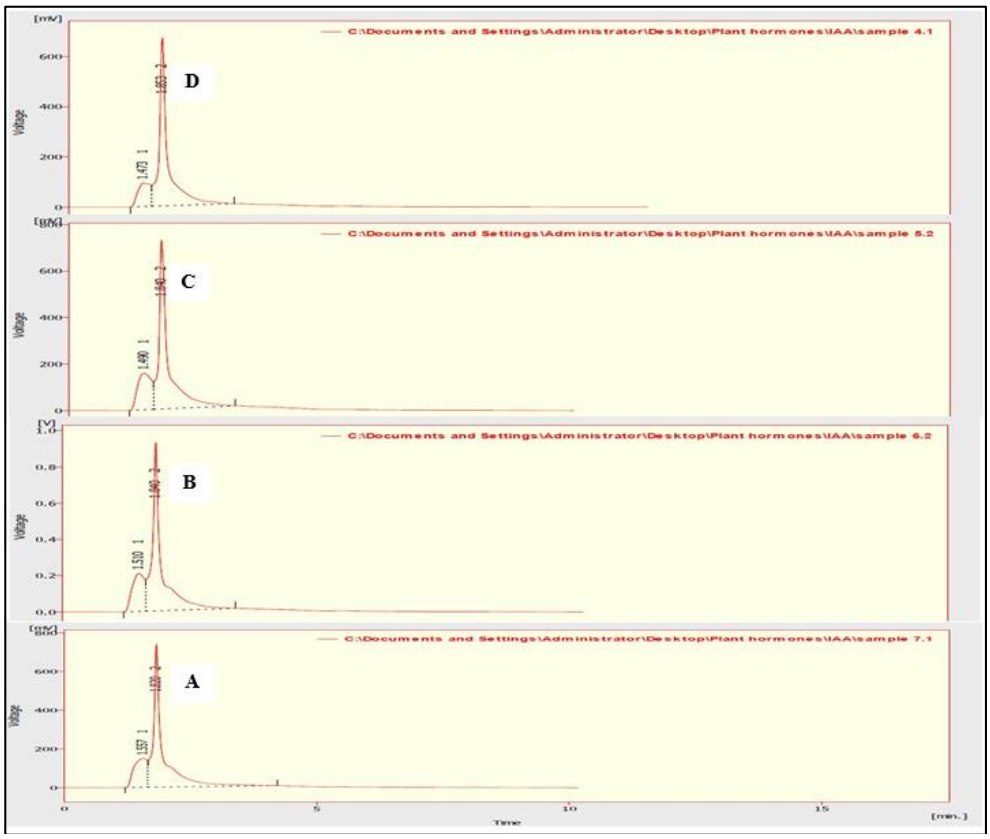

Fig 3: Chromatograph of IAA in corolla; A: Open; B: large; C: medium; D: small

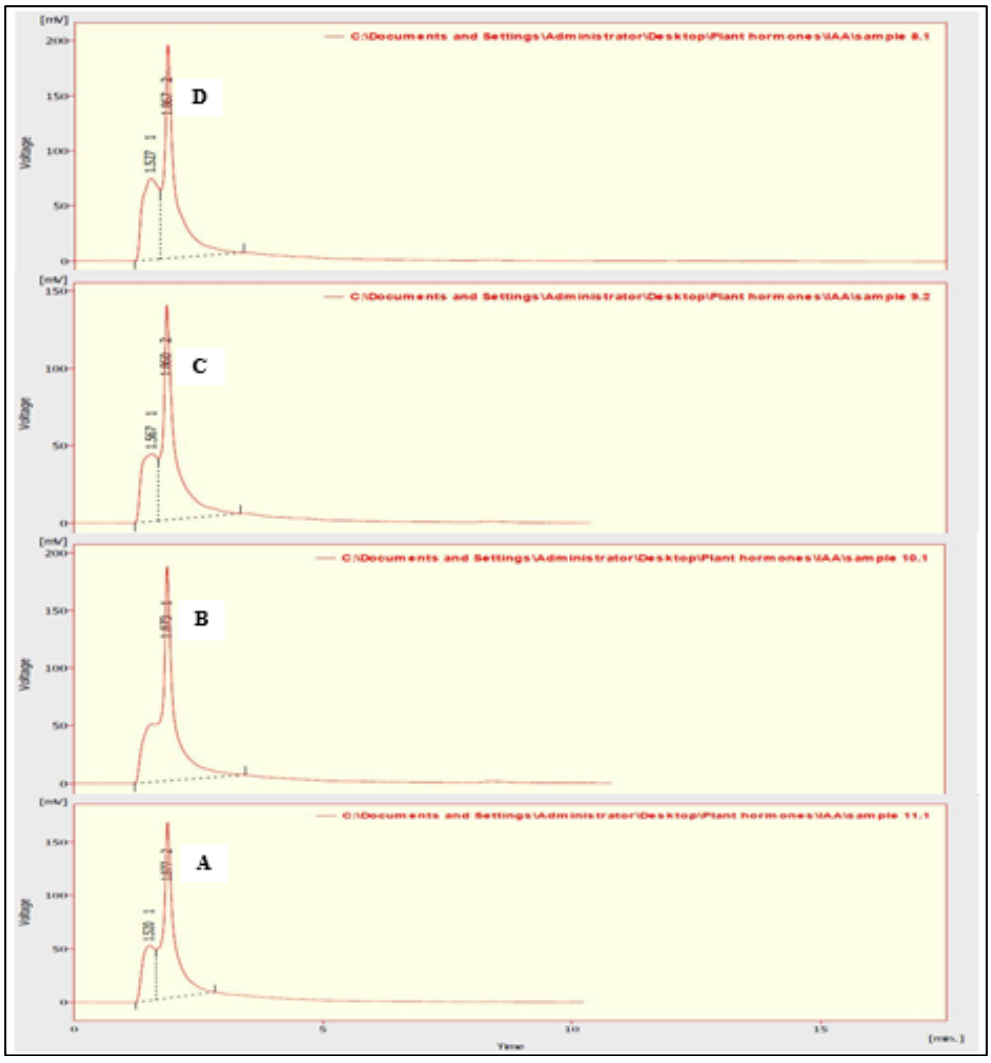

Fig 4: Chromatograph of IAA in calyx; A: Open; B: large; C: medium; D: small

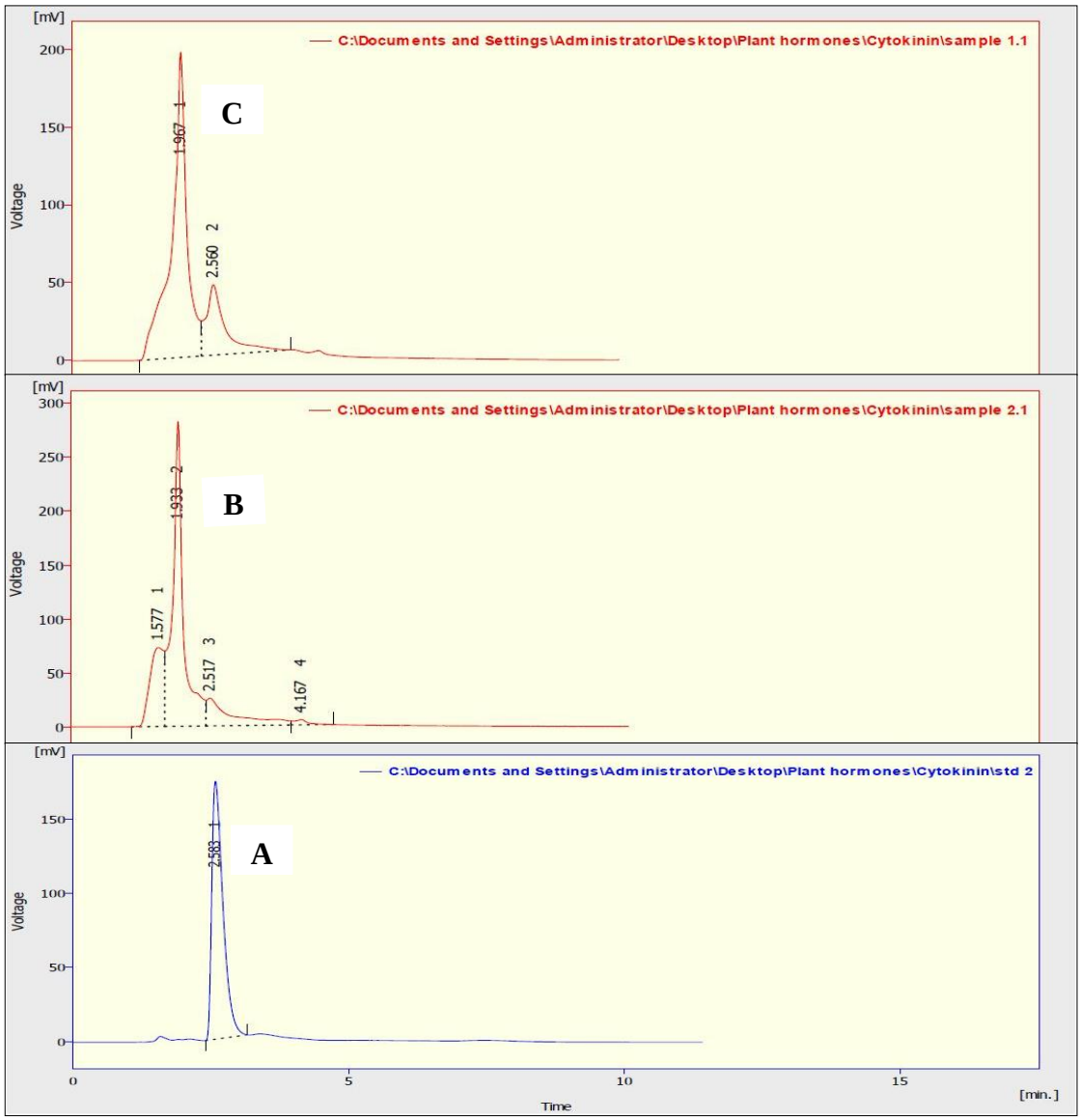

Fig 5: Chromatograph of Kinetin in water calyx fluid; A: Standard; B: medium bud; C small bud

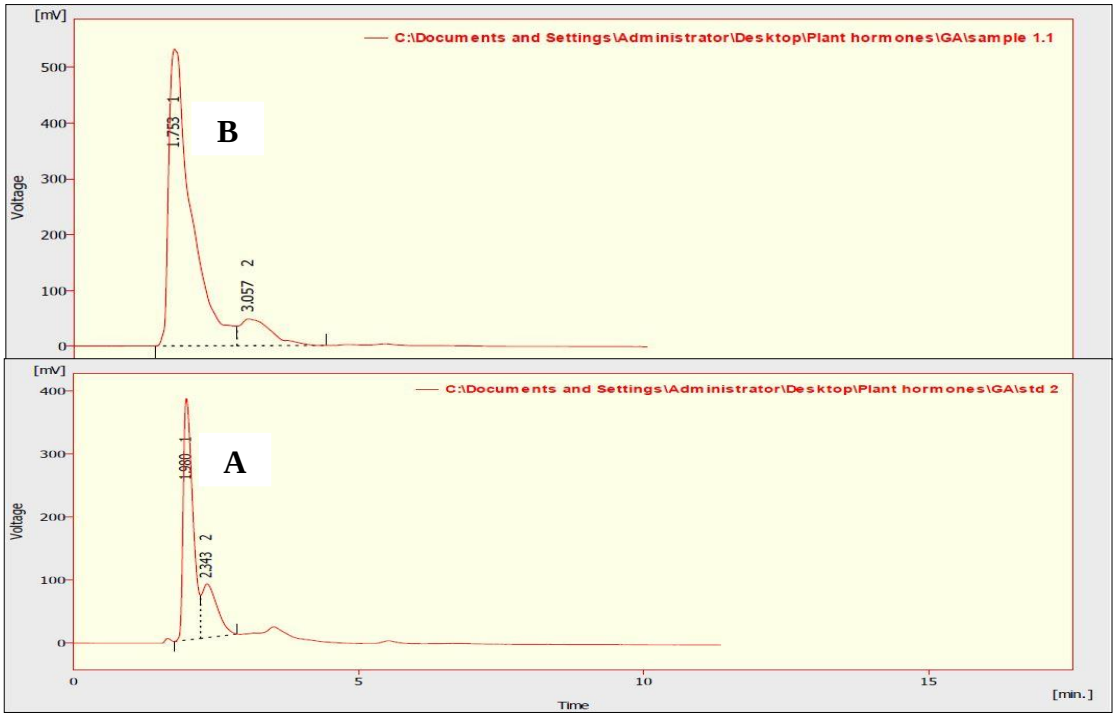

Fig 6: Chromatograph of GA_3 in water calyx fluid; A: Standard; B: small bud

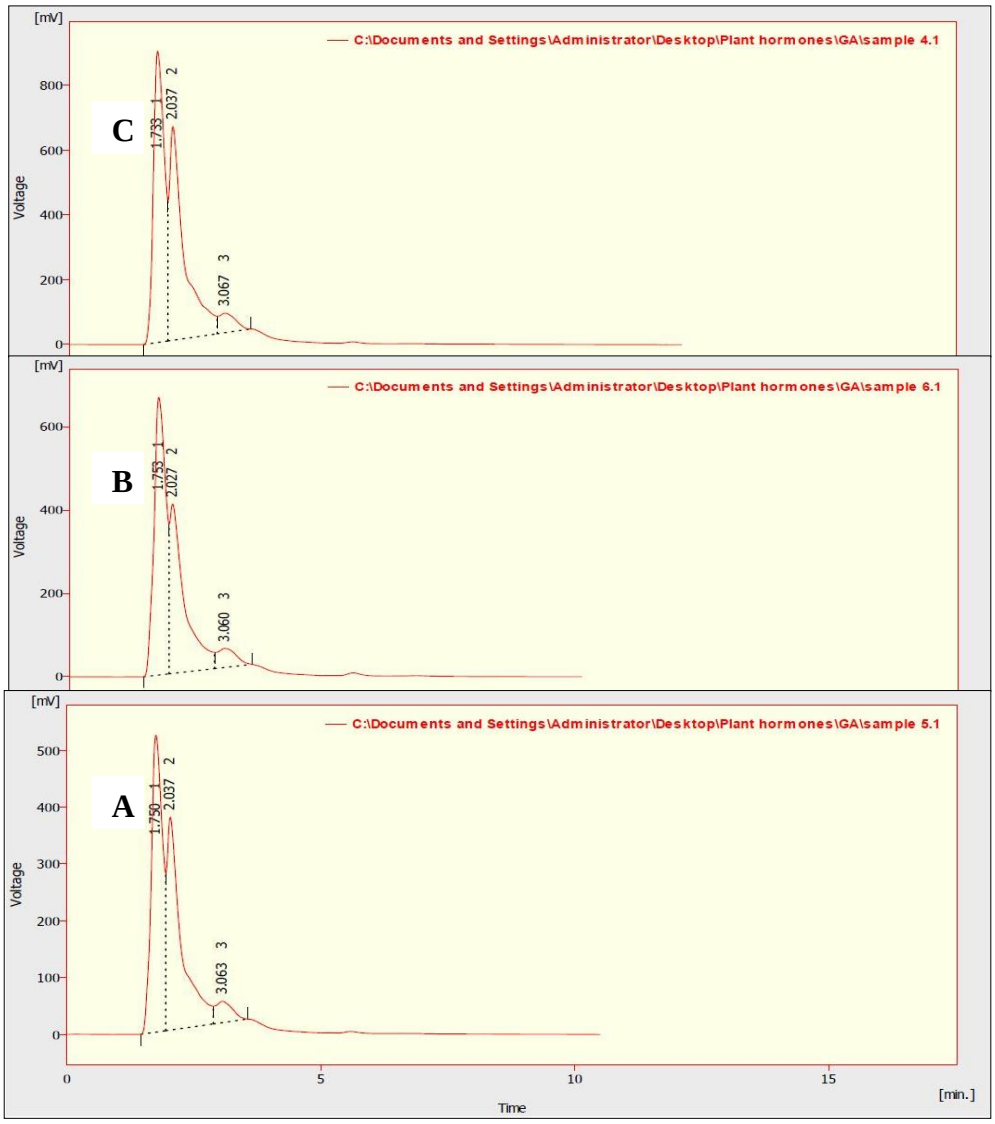

Fig 7: Chromatograph of GA_3 in corolla; A: large; B: medium; C: large

Acknowledgements
Authors are thankful to St. Joseph's College (Autonomous), Bengaluru for providing lab facilities to carry out research work. We also extend our sincere thanks to Dr. Jayashankar. M, Assistant Professor, St. Joseph's College (Autonomous), Bengaluru for his critical inputs.

Conclusion
The *S. campanulata* has a regular flowering rhythm with different concentrations of examined plant hormones. Decrease in IAA content in calyx fluid and its corresponding linear increase in corolla correlates with its size increase and expansion. It is clearly evident that the phytohormones studied play a major role in bud formation and floral whorl development and appreciable quantity of the hormones are contributed externally from the water calyx fluid beside the endogenous quantity. Hence, the water calyces with bathing fluid and its constituents, especially the hormones, provides preliminary evidence, similar to external application of hormones, a role in floral development of *S. campanulata.*

References
1. Frank BS. The flowering process. Pergamon press. 1963, 233.
2. Georges B, Jean-Marie K, Roy MS. The Physiology of flowering. Transition to Reproductive Growth. CRC Press, 2018, 2.
3. Noushina I, Nafees AK, Antonio F, Alice T, Alessandra F, Khan MIR. Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. Front. Plant Sci. 2017; 8:475. https://doi:10.3389/fpls.2017.00475
4. Peter Davies J. Plant Hormones and their Role in Plant Growth and Development. Martinus Nijhoff Publishers, 1987, 1-3.
5. Pan XQ, Welti R, Wang XM. Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by liquid chromatography-electrospray tandem mass spectrometry. Phytochemistry. 2008; 69:1773-81.
6. Ross EK. The roles of plant hormones in the growth of the corolla of *Gaillardia grandiflora* (Asteraceae) ray flowers. Amer. J Bot. 1984; 71(1):1-8.
7. Baktir I, Ulger S, Kaynak L, David GH. Relationship of seasonal changes in endogenous plant hormones and alternate bearing of Olive trees. Hort Science. 2004; 39(5):987-90.
8. Mariko O, Kensuke M, Kiyotaka O, Junichi U. Auxin Polar Transport and Flower Formation in *Arabidopsis*

thaliana Transformed with Indoleacetamide Hydrolase (iaaH) Gene. Plant Cell Physiol. 1999; 40(2):231-37.
9. Dilworth LL, Riley CK, Stennett DK. Plant Constituents: Carbohydrates, Oils, Resins, Balsams, and Plant Hormones. Pharmacognosy. Elseiver. 2017; 101:61-80. http://dx.doi.org/10.1016/B978-0-12-802104-0.00005-6
10. Elena RA, Mariana B, Adriana C, Álvaro CC, Stefan de F, Alicia G de B *et al*. The Arabidopsis Book. Flower Development. American Society of Plant Biologists, 2010, 57.
11. Charyulu NVN, Rao KVN. Changes in the nitrogen metabolism during growth and development of the flower in S*pathodea companulata* Beauv. Indian J Plant Physio. 1990; 13(1):7-15.
12. Martin PA, Jayanthi D, Thamizhseran N. Bio-Physicochemical Studies on Water Calyx Fluid in the African Tulip Tree, *Spathodea campanulata* P. Beauv. Pharmacog J. 2019; 11(3):594-99.
13. Hasan B, Salih Ü. Correlations between Changes in the Amount of Endogenous Phytohormones and Flowering in the Safflower (*Carthamus tinctorius* L.) Tr. J of Biology. 1998; 22:421-5.
14. Goodwin PB. Phytohormones and growth and development of organs of the vegetative plant. In Phytohormones and Related Compounds: a Comprehensive Treatise, Edited by Letham, DS, Goodwin PB, Higgins TJV. Elsevier/North Holland Biomedical Press, Amsterdam 1987; 2:31-173.
15. Liverman JL, Lang A. Induction of flowering in long day plants by applied indole acetic acid. Plant Physiol. 1956; 31:147-50.
16. Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW. Auxin response factor1 and auxin response factor2 regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development 1. 2005; 32:4563-74.
17. Sohair K, El-Saeid HM, Magda S. The Role of Kinetin in Flower Abscission and Yield of Lentil Plant. J Appl Sci Res. 2006; 2(9):587-91.
18. Pooja. Physiology of Angiosperms. Discovery Publishing House, 2004, 292-96.
19. Chen WS. Endogenous growth substances in relation to shoot growth and flower bud development of mango. J Amer. Soc. Hort. Sci. 1987; 112(2):360-3.
20. Chen WS. Endogenous growth substances in xylem and shoot tip diffusate of lychee in relation to flowering. Hort.-Science. 1990; 25(3):314-5.
21. Dasumiati, Miftahudin, Triadiati, Alexhartana, Dibyopronowo. Increasing Hermaphrodite Flowers using Plant Growth Regulators in Andromonoecious *Jatropha curcas*. HAYATI Journal of Biosciences. 2014; 21(3):111-20.
22. Abou-El-Ghait, Eman M, Gomaa AO, Youssef ASM, EL-Nemr AM. Effect of kinetin and GA3 treatments on growth and flowering of *Dendranthema grandiflorium* cv. Art Queen plants. Middle East J Agric. Res. 2018; 7(3):801-15.
23. Muhammad H, Anwar H, Sumera AK, Muhammad I, Abdul LK, Muhammad W *et al*. Kinetin modulates physio-hormonal attributes and isoflavone contents of Soybean grown under salinity stress. Front. Plant Sci. 2015; 6:377. https://doi:10.3389/fpls.2015.00377
24. Poljakoff-Mayber Gale AJ. Plants in Saline Environments. (Ecological studies V 15). Springer-Verlag Berlin Heidelberg, 1975, 169-72.
25. Ramanuja RIV, Mohan RHY. Specificity of Gibberellin and Sucrose-promoted Flower Bud Growth in *Gladiolus*. Ann. Bot. 1982; 50:473-9.
26. Murakami Y. The role of gibberellins in the growth of floral organs of *Pharbitis nil*. Ibid. 1973; 14:91-102.
27. Murakami Y. The role of gibberellins in the growth of floral organs of *Mirabilis jalapa*. Ibid. 1975; 16:337-45.
28. Sircar PK, Dey B, Sanyal T, Ganguly SN, Sircar SM. Gibberellic acid in the floral parts of *Cassia fistula*. Phytochemistry. 1970; 9:735-36.
29. Yazici K, Ulger S, Kaynak L. The relationship between flower development and endogenous ga3 in pomegranate (*Punica granatum* "hicaznar"). Acta Horticulturae. 2011; 890:341-6. https://doi:10.17660/actahortic.2011.890.47.
30. David W, Arnold van der L, Erik K, Evert V, Joseph NMM, Jan MK. Identification of Endogenous Gibberellins in Petunia Flowers. lnduction of Anthocyanin Biosynthetic Gene Expression and the Antagonistic Effect of Abscisic Acid. Plant Physiol. 1995; 107:695-702.
31. Pharis RP, King RW. Gibberellins and reproductive development in seed plants. Annu Rev Plant Physiol. 1985; 36:517-68.
32. Weiss D, Halevy AH. Stamens and gibberellin in the regulation of corolla pigmentation and growth in *Petunia hybrida*. Planta. 1989; 179:89-96.
33. Weiss D, Van Blokland R, Kooter JM, Mol JNM, Vim Tunen AJ. Gibberellic acid regulates chalcone synthacie gene transcription in the corolla of *Petunia hybrida*. Plant Physiol. 1992; 98:191-7.