OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2018.142.149

Research Article
Effect of Pre-treatment Methods on *in vitro* Seed Germination of Bullock's Heart (*Annona reticulata* L.)

Hemalatha Kudikala, Raghu Ellendula, Shama Nazrin, Abhiteja Sirikonda, Kasim Mood and Venkateswar Rao Allini

Department of Biotechnology, Kakatiya University, 506009, Warangal, India

Abstract

Background and Objective: *Annona reticulata* Linn. (Bullock's Heart) is traditionally an important ethnomedicinal plant of Annonaceae. It having potential role in ayurvedic for the treatments of several diseases. Different parts of this plant have various pharmacological activities. Seed germination is a major hurdle for this plant. In this sense, this study was aimed to optimize the *in vitro* germination procedures. **Materials and Methods:** *In vitro* seed germination of *A. reticulata* was done by evaluating different pretreatments such as mechanical scarification, hot water treatment with different timings and GA_3 treatment with different concentrations. **Results:** About 8.67 μM GA_3 for 24 h pre-treatment was shown better results pertaining to the maximum percentage (60%) of germination, minimum germination period (46.67 days) and maximum length (10.5 cm) of the seedling. **Conclusion:** The present investigation described an enhanced *in vitro* seed germination protocol for the first time in this woody species. It will strengthen large scale plantation and useful in the field of pharmacology.

Key words: *Annona reticulata*, seed germination, pre-treatment, tissue culture, gibberellic acid

Citation: Hemalatha Kudikala, Raghu Ellendula, Shama Nazrin, Abhiteja Sirikonda, Kasim Mood and Venkateswar Rao Allini, 2018. Effect of pre-treatment methods on *in vitro* seed germination of bullock's heart (*Annona reticulata* L.). Asian J. Plant Sci., 17: 142-149.

Corresponding Author: Venkateshwar Rao Allini, Department of Biotechnology, Kakatiya University, 506009 Warangal, India Tel: 7032717666

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The species *A. reticulata* belongs to the family, Annonaceae. It is native to tropical regions of America, particularly in West Indies and South America[1]. It is commonly called as custard apple and has many common names like Bullock's heart (English), Ramphal (India), Buah nona (Indonesia), Ramaseeta (Tamil), Rramasitapalam (Telugu), Manilanilam (Malayalam)[2]. It can grow up to a height of near about 6-7.5 m with many lateral branches and fruits are heart shaped and edible[3].

The tree has many ethnomedicinal properties such as roots are used for mental depression, spinal disorders and blood dysentery. Leaves are used for antispasmodic, anthelmintic, insecticidal and destroying lice. Fruits are used to enrich the blood, increasing muscular strength. Seeds are used in making detergents and insecticides. The bark is used as an astringent and tonic[1]. Scientifically various phytochemical and pharmacological medicinal properties have been investigated by several researchers[2]. Phytopharmacological properties such as Antipyretic[4], anthelmintic[5],antihyperglycemic[6], antiulcer[7], *in vitro* cytotoxic and recombinant caspase inhibitory[8], antinociceptive[9], analgesic and CNS depressant[10], analgesic and anti-inflammatory[11], antiproliferative[12,13], wound healing and anti-marking[14], antioxidant and antimicrobial[15], larvicidal[16] activities indicated in *A. reticulata*.

Annona reticulata plant contains a wide range of useful secondary metabolites and minerals. An efficient regeneration method from juvenile material would allow the possibility to enhance the secondary metabolites could be responsible for different therapeutic activities. Rapid propagation may be useful in the field of pharmacology to develop new drugs for human welfare. Prerequisite for this research is to establish an efficient, rapid, reliable and simple method for germination of Bullock's heart seeds *in vitro*. Exogenous dormancy of the seeds can be removed by treating the seeds with physical, mechanical and chemical methods[17]. The growth substance most commonly used for better germination for various plant species are Auxin (IAA, IBA, NAA), Gibberellic acid (GA_3), etc. Among these, GA_3 has proved to be the best for seed germination and proper seedling growth. In various species, Gibberellins have been used for dormancy breakage along with accelerating germination of non-dormant seeds[18]. The present study was carried out to improve the seed germination and to assess the efficiency of gibberellic acid (GA_3) on seed germination in *A. reticulata*.

MATERIALS AND METHODS

Obtaining the seeds: The seeds were collected from trees located in Suraram village, Karimnagar district of Telangana state in the month of February and they were separated from the pulp and washed with running tap water and cleaned with paper towels. Cleaned seeds were stored at room temperature (28°C) for further use.

***Ex vitro* germination:** A sample of 60 seeds was sown in trays containing cocopeat and sand. They were incubated at 25°C with 16/8 h photoperiod in greenhouse .

***In vitro* germination:** Paper bridges[19] were used as a scaffold for *in vitro* germination of seed. Medium pH was adjusted to 5.6-5.8 and 15 mL of liquid medium was dispensed into 50 mL test tubes before autoclaving at 121°C for 15 min. Culture conditions were maintained at 25±2°C under 16 h photoperiod. The cultured seeds were daily observed to check for germination.

Intact seeds were sterilized with 0.1% $HgCl_2$ for 5 min. followed by rinsing with sterile distilled water for 2-3 times. Later, they were cultured on paper bridges in DW, MS[20] liquid medium and ½ strength MS liquid medium. Observed results were used as the control.

Seed pre-treatment methods

Mechanical scarification (removal of seed coat): The seed coat was carefully removed by using the surgical blade without damaging the embryo. Later, the de-coated seeds were sterilized with 0.1% $HgCl_2$ for 5 min followed by rinsing with sterile distilled water for 2-3 times. These sterilized seeds were soaked for 24 h in sterile distilled water and cultured on paper bridges having DW, MS liquid medium and ½ strength MS liquid medium.

Hot water pre-treatment: The intact seeds were exposed to hot water treatment (100°C) for 3, 5 and 7 min. Treated seeds were immediately transferred to the normal water and sterilized with 0.1% $HgCl_2$ for 5 min. followed by rinsing with sterile distilled water for 2-3 times. Later, they were soaked in sterile distilled water for 24 h before inoculating on to paper bridges having DW, MS liquid medium and ½ strength MS liquid medium.

GA_3 hormone pre-treatment: Intact seeds were sterilized with 0.1% $HgCl_2$ for 5 min followed by rinsing with sterile distilled

Fig. 1(a-d): *In vitro* seed germination of *A. reticulata*, (a) Seeds, (b) Germinating seed on paper bridge, (c) *In vitro* raised seedling on MS basal media and (d) *In vitro* raised seedlings

water for 2-3 times and subsequently soaked in 3 different concentrations of GA_3 solutions i.e., 2.89, 5.78, 8.67 and 11.56 µM for 24 h. Later they were cultured on paper bridges having DW, MS liquid medium and ½ strength MS liquid medium supplemented with the same concentration of hormone.

All above treatments were repeated thrice with 20 seeds in each and the cultured seeds were daily observed for 9 weeks for the germination. Seeds were considered as germinated when a radicle is visible (Fig. 1).

Data analysis: Data on the effect of pretreatments on seed germination *in vitro* was subjected to IBM SPSS software statistical version 20. All the results were stated as the Mean±Standard Error (SE). Statistical analysis was done by the Analysis of Variance (one way ANOVA between the groups) and means of comparative analysis was made by (DMRT) duncan's multiple range tests ($p \leq 0.05$).

Table 1: *Ex vitro* seed germination of *A. reticulata* under greenhouse in different months (60 seeds were tested per month)

Months of sowing	Germination percentage
February	5.0
March	13.3
April	8.3
Mean Percentage	8.8

RESULTS AND DISCUSSION

The month of March seems the most favorable time for seed germination in the greenhouse. *Ex vitro* seed germination of *A. reticulata* was observed to be very poor (13.3%) (Table 1). The same was also reported by Padilla and Encina[21] in *A. cherimoya* (52.5%) and Campbell and Popenoe[22] in *A. diversifolia* (30-80%). In contrast, George and Nissen[23] opined a high germination rate (90-95%) in *Annona squamosa*.

Experiments were carried out with untreated and decoated seeds for *in vitro* germination and the results were

Table 2: Effect of different pre-treatment methods on measured characteristics in *A. reticulata* seed germination

Pre-treatment techniques	Type of media	Mean (%) of germination (±SE)[a]	Mean number of days for germination (±SE)[a]	Average length of seedling (cms) (±SE)[a]
Control	DW	0.000±0.000[f]	0.000±0.000[g]	0.000±0.000[f]
	MS	5.000±0.000[f]	61.00±0.577[a]	7.167±0.166[c]
	½ MS	5.000±0.000[f]	60.67±0.333[a]	7.100±0.378[bc]
Mechanical scarification	DW	0.000±0.000[f]	0.000±0.000[g]	0.000±0.000[f]
	MS	5.000±0.000[f]	58.00±0.577[b]	9.167±0.266[b]
	½ MS	5.000±0.000[f]	57.33±0.333[c]	7.333±0.166[b]
Hot water treatment (min)				
3	DW	8.333±1.666[de]	51.67±0.333[b]	8.267±0.371[cd]
	MS	8.333±1.666[f]	50.33±0.333[c]	8.500±0.288[b]
	½ MS	6.666±1.666[f]	50.33±0.333[d]	7.433±0.233[b]
5	DW	10.000±0.000[d]	49.67±0.333[c]	8.000±0.288[cd]
	MS	15.000±0.000[e]	48.67±0.333[d]	8.833±0.166[b]
	½ MS	11.666±1.666[e]	48.33±0.333[e]	8.500±0.288[a]
7	DW	5.000±0.000[e]	58.33±0.333[a]	6.733±0.266[e]
	MS	5.000±0.000[f]	58.00±0.333[b]	6.600±0.305[c]
	½ MS	5.000±0.000[f]	58.67±0.333[b]	6.333±0.333[cd]
GA_3 treatment (µM)				
2.89	DW	20.000±0.000[c]	48.67±0.333[d]	9.067±0.066[b]
	MS	25.000±0.000[d]	47.67±0.333[d]	8.833±0.166[b]
	½ MS	20.000±0.000[d]	48.67±0.333[e]	9.167±0.166[a]
5.77	DW	35.000±0.000[b]	47.67±0.333[e]	8.667±0.333[bc]
	MS	40.000±0.000[b]	47.33±0.333[d]	8.667±0.333[b]
	½ MS	31.666±1.666[b]	47.67±0.333[e]	7.500±0.288[b]
8.67	DW	53. 333±1.666[a]	45.67±0.333[f]	10.000±0.288[a]
	MS	60.000±0.000[a]	41.67±0.333[e]	10.500±0.288[a]
	½ MS	50.000±0.000[a]	45.33±0.333[f]	8.833±0.441[a]
11.56	DW	35.000±2.886[b]	58.67±0.333[a]	7.500±0.288[d]
	MS	30.000±2.886[c]	59.00±0.577[b]	6.833±0.166[c]
	½ MS	25.000±2.886[c]	58.00±0.577[bc]	6.000±0.000[d]

Mean±SD. Error, DW: Distilled water, MS: Murashige and Skoog's medium

presented in Table 2. Results shown that the seeds were grown on three different medium i.e., distilled water, MS medium and ½ strength MS medium shown similar germination (%). It was also recorded that the variation was observed in the number of days to germination (Fig. 4, 5) and an average length of seedlings. Decoated seeds were germinated 3-4 days earlier than untreated seeds and their average length of the seedling is also considerably increased. Decoated seeds of *A. muricata* were germinated on half strength MS medium[24]. The seed coat removal followed by 24 h pre-soaking in DW enhanced the early germination than intact seed germination. This may be due to seed coat involvement in prevention of water uptake exerting a mechanical restraint on the growth of the embryo. Similarly, removal of seed coat is necessary to *A. squamosa* seeds to germinate them *in vitro*[25]. In *A. diversifolia*[26], *A. squamosa* and *Atemoya*[27] the germination process was not regulated by the permeability of seed coat.

Later, *A. reticulata* seeds were treated with hot water at different time periods i.e., 3, 5 and 7 min and allowed them to germinate on different media. Results indicated that low germination (%) was recorded in all the hot water treatments (DW 8.3, 10 and 5%, in MS 8.3, 15 and 5% and in ½ MS 6.6,11.6 and 5%, respectively) (Fig. 2). Immersing in hot water for 5 min significantly resulted in earlier germination (Fig. 4, 5) compared to control and mechanical scarification. Whereas as in 7 min treatment, it was recorded that germination (%) was decreased and days of germination was considerably increased. The reason may be a longer period contact of seed with hot water effects the damage of embryo. Similarly, Amusa[28] reported in *Afzelia africana* a 12 h treatment with (100°C) hot water. Hence hot water pre-treatment was not appropriate pre-treatment for the seeds of *A. reticulata*. It perhaps leads to the seed embryo being killed because of prolonged contact with boiled water.

In GA_3 treatment, 8.67 µM GA_3 concentration produced a high percentage (in DW 53%, in MS 60% and in ½ strength 50%, respectively, Fig. 2) of germination in all three media tested and substantially improved plantlet development (highest length of seedling i.e., 10.5 cm. Fig. 6). GA_3 effects in stimulating cell division, cell elongation, auxin metabolism, cell wall plasticity and permeability of cell membrane leading to enhanced growth[29]. About 8.67 µM GA_3 treatment also has considerably decreased the number of days to germination (Fig. 3-5) when compared with other treatments earlier tested. For these reasons, it was selected as standard. An improved

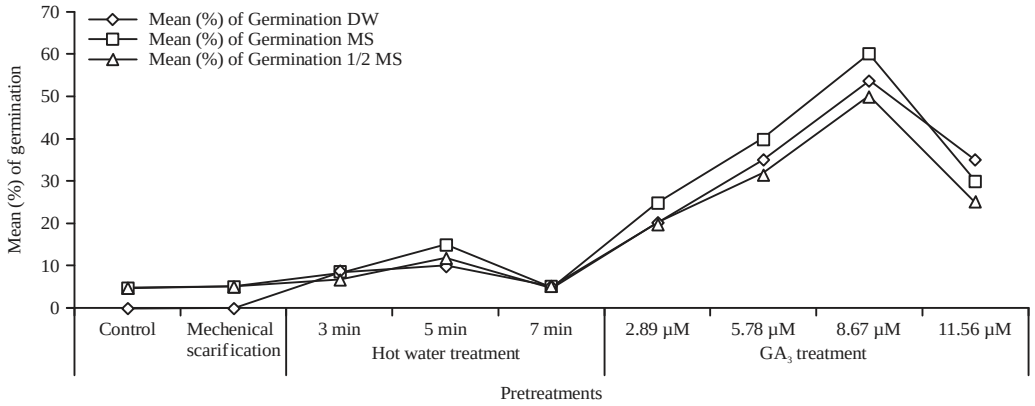

Fig. 2: Effect of pre-treatment methods of seed germination (%) in *A. reticulata*
DW: Distilled water, MS: Murashige and Skoog's medium

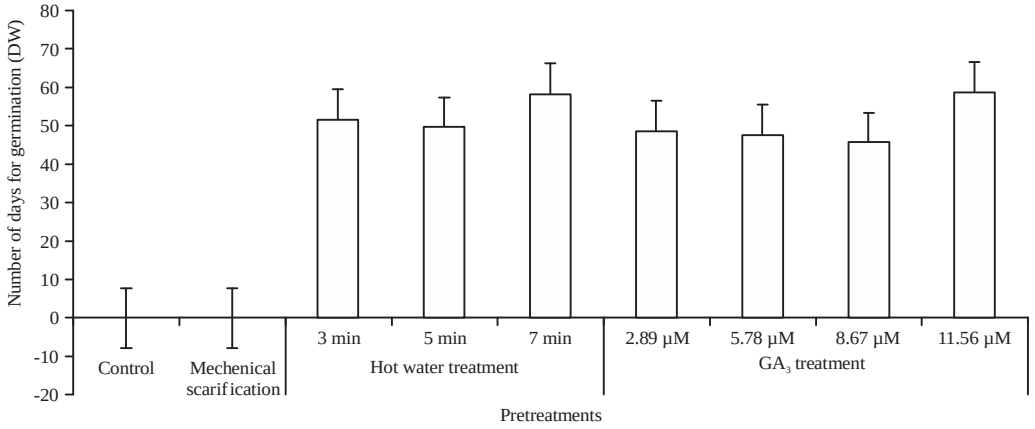

Fig. 3: Effect of pre-treatment methods on No. of days for germination in *A. reticulata* using distilled water

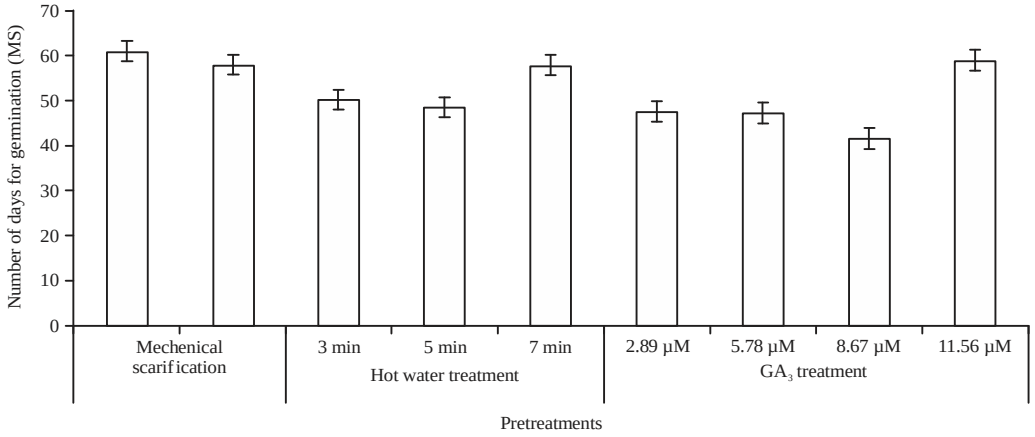

Fig. 4: Effect of pre-treatment methods on No. of days for germination in *A. reticulata* using MS medium

germination in cherimoya seeds also has been shown by GA_3 treatment[30]. The increasing seed germination parameters might be due to the involvement of GA_3 in the activation of cytological enzymes along with increase in cell wall plasticity and better water absorption. GA_3 acts as a directly on embryo relieving them from dormancy through promoting protein

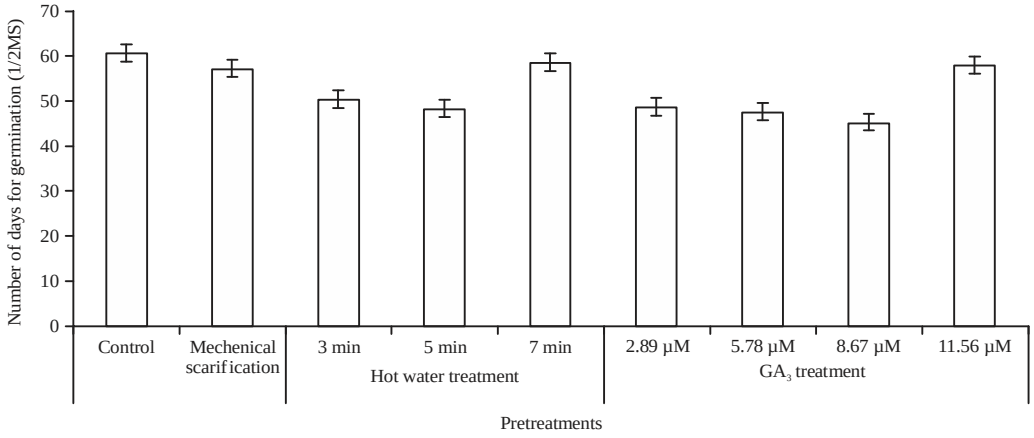

Fig. 5: Effect of pre-treatment methods on No. of days for germination in *A. reticulata* using ½ MS medium

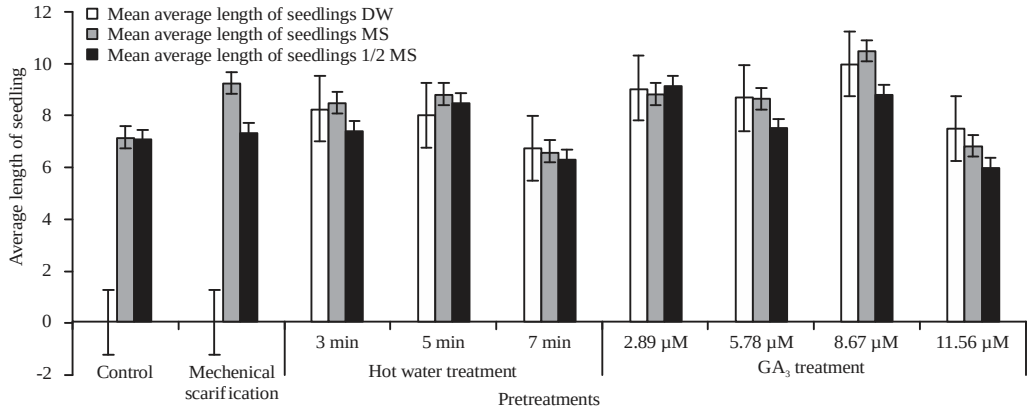

Fig. 6: Effect of pre-treatment methods on length of *A. reticulata* seedlings

DW: Distilled water, MS: Murashige and Skoog's medium

Table 3: *Ex vitro* and *in vitro* seed germination of *A. reticulata*

Germination/ treatment	Number of seeds sown	Number of seeds germinated	Germination (%)
Ex vitro	180	16	8.80
In vitro			
with GA_3	720	255	35.00
without GA_3	900	57	6.30

synthesis and elongation of coleoptiles and leaves and also helps in the production of ethylene. This ethylene invokes the synthesis of hydrolases, especially amylase, which favours the seed germination[31]. GA_3 also stimulates seed germination by formation of a-amylase enzymes which converts insoluble starch into soluble sugars and it also initiates the radical growth by removing some metabolic blocks[32]. In the case of *A. reticulata* GA_3 could increase the germination rate considerably.

Pre-soaking period of 24 h improved *in vitro* germination of *A. reticulata* seeds. Ferreira *et al.*[27] reported pre-soaking period for 4-5 h was beneficial to *A. squamosa* seeds and for Atemoya (*A. squamosa*× *A. cherimola*) seeds more than 12 h allowed the best results. Seeds treated with GA_3 for 24 h pre-soaking and standard method of sterilization with 0.1% $HgCl_2$ solution has given no contamination. Elongated pre-soaking (more than 24 h) produced more contamination. Lemos *et al.*[33] stated that a longer pre-soaking period required more than 24 h for Annonaceae seeds.

Finally, the results revealed that the *ex vitro* seed germination percentage was very low when compared with GA_3 treatment of *in vitro* seeds germination (Table 3). Treatment of the seeds with GA_3 resulted in earlier germination, well-developed seedling and high germination (%) compared with seeds without GA_3 treatment. Therefore the GA_3 treatment of *A. reticulata* seeds was only effective for increasing the speed and high percentage of seed germination.

CONCLUSION

An efficient protocol has been optimized for *in vitro* seed germination of *A. reticulata* using different seed pre-treatment methods. According to our observations, pre-soaking of seeds in 8.67 μM GA_3 for 24 h has shown maximum germination percentage and highest length of seedlings in short time duration. Therefore in this study, it concluded that GA_3 treatment can only be used to accelerate the *in vitro* seed germination of *A. reticulata*.

SIGNIFICANCE STATEMENT

For the First time, procedures were established by applying several pre-treatments. GA_3 observed to have an important role in improving germination percentage. This will be useful for exploration and development of the plant for pharmaceutical use.

ACKNOWLEDGMENT

The authors are grateful to the Head, Department of Biotechnology, KU for providing necessary facilities, working environment and Prof. A. Sadanandam, Department of Biotechnology, KU for his valuable suggestions and encouragement.

REFERENCES

1. Pathak, K. and K. Zaman, 2013. An overview on medicinally important plant-*Annona reticulata* Linn. Int. J. Pharmacogn. Phytochem. Res., 5: 299-301.
2. Jamkhande, P.G. and A.S. Wattamwar, 2015. *Annona reticulata* Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties. J. Traditional Complement. Med., 5: 144-152.
3. Pinto, A.C., M.C.R. Cordeiro, S.R.M. Andrade, F.R. Ferreira, H.A. Filgueiras, R.E. Alves and D.I. Kinpara, 2005. *Annona* species. International Centre for Underutilized Crops Southampton UK, University of Southampton, England, pp: 4-12.
4. Patil, S.B., G.M. Chavan, D.S. Ghodke, N.S. Naikwade and C.S. Magdum, 2009. Screening of some indigenous plants for their antipyretic activity. Res. J. Pharmacol. Pharmacodyn., 1: 143-144.
5. Nirmal, S.A., S.B. Gaikwad, V.V. Dhasade, R.S. Dhikale, P.V. Kotkar and S.S. Dighe, 2010. Anthelmintic activity of *Annona reticulata* leaves. Res. J. Pharm. Biol. Chem. Sci., 1: 115-118.
6. Rahman, S.M., M.I. Rashedul, S. Rahman, T. Mosaiab, R. Ahmed and F. Khatun, 2011. Antihyperglycemic studies with methanol extract of *Annona reticulate* L. (Annonaceae) and *Carissa carandas* L. (Apocynaceae) leaves in Swiss albino mice. Adv. Nat. Applied Sci., 5: 218-222.
7. Singh, J., S.V. Kumar and V. Kadam, 2012. Antiulcer activity of *Annona reticulate* leaves extract in rats. Int. J. Pharm. Pharm. Sci., 4: 412-414.
8. Mondal, S.K., N.B. Mondal and U.K. Mazumder, 2007. *In vitro* cytotoxic and human recombinant caspase inhibitory effect of *Annona reticulata* leaves. Indian J. Pharmacol., 39: 253-254.
9. Islam, M.R., S.M. Rahman, M. Ahmed, P.R. Das and M. Tabibul *et al*., 2012. Antinociceptive activity studies with methanol extract of *Annona reticulata* L. (Annonaceae) and *Carissa carandas* L. (Apocynaceae) leaves in Swiss albino mice. Adv. Nat. Applied Sci., 6: 1313-1318.
10. Bhalke, R.D. and M.J. Chavan, 2011. Analgesic and CNS depressant activities of extracts of *Annona reticulata* Linn. bark. Phytopharmacology, 1: 160-165.
11. Chavan, M.J., D.R. Kolhe, P.S. Wakte and D.B. Shinde, 2012. Analgesic and antiinflammatory activity of kaur 16 en 19 oic acid from *Annona reticulata* L. Bark. Phytother. Res., 26: 273-276.
12. Suresh, H.M., B. Shivakumar, K. Hemalatha, S.S. Heroor, D.S. Hugar and K.R.S. Sambasiva Rao, 2011. *In vitro* antiproliferative activity of *Annona reticulata* roots on human cancer cell lines. Pharm. Res., 3: 9-12.
13. Duval, R.A., P. Duret, G. Lewin, E. Peris and R. Hocquemiller, 2005. Semisynthesis and biological activity of aminoacyl triesters of squamocin, an annonaceous acetogenin. Bioorg. Med. Chem., 13: 3773-3781.
14. Royal, G., 2012. Formulation and evaluation of herbal ointment for wound healing and anti marking activity by using Vitis venifera and *Annona reticulata* seeds ex-tracts. PharmaTutor.
15. Jamkhande, P.G., A.S. Wattamwar, S.S. Pekamwar and P.G. Chandak, 2014. Antioxidant, antimicrobial activity and *in silico* PASS prediction of *Annona reticulata* Linn. Root extract. Beni-Suef Univ. J. Basic Applied Sci., 3: 140-148.
16. Mallick, S., R. Banerjee and G. Chandra, 2015. Mosquito larvicidal potential of ethanol leaf extract of the plant, *Annona reticulata* L. against *Aedes aegypti* L. and Culex quinquefasciatus Say (Diptera: Culicidae). J. Mosquito Res., 5: 1-7.
17. Willan, R.L., 1987. Seed Pretreatment. In: A Guide to Forest Seed Handling, Willan, R.L. (Ed.). FAO, Rome.
18. Patel, M.S., K.H. Nurbhanej, M.J. Rathod, S.V. Vyas and N.H. Bhutiya, 2017. Effect of media and GA_3 on seed germination of custard apple (*Annona squamosa* L.) cv. Sindhan. Int. J. Pure Applied Biosci., 5: 969-975.

19. Heller, R., 1965. Some aspects of the inorganic nutrition of plant tissue cultures. Proceedings of the International Conference on Plant Tissue Culture, (ICPTC'65), Berkeley, pp: 1-17.
20. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plantarum, 15: 473-497.
21. Padilla, I.M.G. and C.L. Encina, 2003. *In vitro* germination of cherimoya (*Annona cherimola* Mill.) seeds. Scient. Hortic., 97: 219-227.
22. Campbell, C.W. and J. Popenoe, 1968. Effect of gibberellic acid on seed dormancy of *Annona diversifolia* Saff. Proc. Trop. Region Am. Soc. Hortic. Sci., 11: 33-36.
23. George, A.P. and R.J. Nissen, 1987. Propagation of *Annona* species: A review. Scient. Hortic., 33: 75-85.
24. Bejoy, M. and M. Hariharan, 1992. *In vitro* plantlet differentiation in *Annona muricata*. Plant Cell. Tissue Organ Cult., 31: 245-247.
25. Nair, S., M.V. Shirgurkar and A.F. Mascarenhas, 1986. Studies on endosperm culture of *Annona squamosa* Linn. Plant Cell Rep., 5: 132-135.
26. Gonzalez-Esquinca, A.R., L. Luna-Cazares, J.G. Alvarez-Moctezuma and Y. de Paz-Velazco, 1997. Estudios sobre el letargo de *Annona diversifolia* saff. Proceedings of the Congreso Internacional de Anonaceas, (CIA'97), Chapingo, Mejico, pp: 229-239.
27. Ferreira, G., E. Cereda, C.P. de Silva, R.J.P. Cunha and A. Castaneo, 1997. Imbibition study of sugar apple (*A. squamosa* L.) and atemoya (*A. cherimola* Mill. x *A. squamosa* L.) seeds. Proceedings of the Congreso Internacional de Anonaceas, CIA'97), Chapingo, Mejico, pp: 210-224.
28. Amusa, T.O., 2011. Effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex pers). J. Hortic. Forest., 3: 96-103.
29. Rahemi, M. and A. Baninasab, 2000. Effect of gibberellic acid on seedling growth in two wild species of pistachio. J. Hortic. Sci. Biotechnol., 75: 336-339.
30. Vargas, M.E., 1986. [Effect of gibberellic acid, 6-benzyl-amino-purine and stratification on cherimola (*Annona cherimola* Mill.) cv. Bronceada seed germination]. M.Sc. Thesis, University of Valparaiso, Chile.
31. Stewart, E.R. and H.T. Freebairn, 1969. Ethylene, seed germination and epinasty. Plant Physiol., 44: 955-958.
32. Anjanawe, S.R., R.N. Kanpure, B.K. Kachouli and D.S. Mandloi, 2013. Effect of plant growth regulators and growth media on seed germination and growth vigour of papaya. Ann. Plant Soil Res., 15: 31-34.
33. Lemos, E.E.P., R.L.R.R. Cavalcanti, A.A. Carrazoni and T.M.L. Lobato, 1988. Germinacao de sementes de Pinha submetidas a tratamrntos para quebra de dormencia. Proceedings of the Anais do Congresso Brasileiro de Fruticultura, Volume 2, (ACBF'88), Fortaleza, Brazil, pp: 675-678.