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Callus Culture as an Alternative Source of Secondary Metabolites in Curtailing Malaria Epidemic

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Malaria remains a threat to public healthcare system. In 2018, more than 200 million people were exposed to this disease globally. There have been reports of drug-resistance in the recommended therapy in some endemic regions. This called for relentless efforts in the search for potential antimalarial compounds.

An *in vitro* culture technique has emerged as a promising option for sustainable and industrial propagation of plant bioactive compounds with wide range of medicinal properties. The demand for these invaluable metabolites is witnessing a continuous increase as a folk medicine, hence, endangering their existence in natural habitats. Besides its use in natural form, the nature's gift to humans seems to be restricted and limited by environmental conditions. An *in vitro* culture approach remains the most viable and sustainable alternative for the endangered plant species. Here, we

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present some plant species reported to have potential antimalarial activities and recommend further study through callus culture induction against malaria.

Keywords: Malaria; callus culture; secondary metabolites; treatment.

1. INTRODUCTION

An *in vitro* culture technique has been regarded as a powerful tool for germplasm conservation and micropropagation of plants with economic and therapeutic potentials. Recently, a deliberate attempt was made to focus on the development of renewal plant cells factories with potential medicinal values to the pharmaceutical industry. Several studies have reported the potential applications of the callus culture approach using different plant species as an anti-malarial campaign. Three steps are relevant in callus culture; aseptic preparation of explant, the ratio of suitable hormones (auxins and cytokinins) and appropriate culture medium under controlled physical environment.

Maximum callus growth with appropriate concentrations of auxins/cytokinins in the presence of appropriate precursors and eventual production of secondary metabolites are the two steps that are primarily involved in callus culture aimed at producing metabolites with anti-malarial potential [1].

Malaria, a disease transmitted by the female *Anopheles* mosquito remains a threat to overburden public healthcare delivery. It is endemic in Africa and Asia, with relative cases in the USA. In 2017, 11 nations represented around 70% of surveyed malaria cases and death across the endemic regions with 10 in sub-Saharan Africa and India. Among these nations, only India revealed progress in lessening its cases in 2017 in contrast to 2016 [2].

A WHO investigation inferred that deaths due to malaria in Sub-Saharan Africa could arrive at 769,000, double the number of deaths announced in the region in 2018. This could mean a re-visitation of malaria mortality levels witnessed 20 years prior.

There has been progressing across the endemic regions in malaria burden and death rate as a result of coverage and effective intervention measures and resources. The future control strategy towards malaria elimination is impacted by external forces such as population growth rate, migration, poverty, climate change, weak and an overburdened healthcare system, drug resistance etc. Malaria elimination would require optimization strategies and new intervention approaches. Artemisinin combination based therapy currently faces resistance at a significant rate [3].

Globally there has been an increasing demand for the use of environmentally friendly substances that discriminate against non-target organisms and are easily biodegradable with desirable products.

A number of plant species are used as a source of insecticides due to the presence of bioactive compounds they contain with little or no side effects on humans and the environment. Some of these plants have been the sources of drugs [4].

This review aims to highlight the tissue culture technique of plant species in synthesizing secondary metabolites with potential anti-malarial activity.

2. CALLUS CULTURE OF SOME PLANTS SPECIES WITH ANTIMALARIAL ACTIVITY

2.1 *Dysoxylum binectariferum*

A number of experimental investigations in the plant family of Maliaceae revealed the potential insecticidal properties of these plants. Limonoids (Fig. 1) have been isolated from the genus *Dysoxylum* of Maliaceae family. The efficacy of limonoids a bioactive compound against insects has been reported but their activity against mosquitoes is limited [5]. Hence, Uma Mansur et al. [5] investigated the callus extracts of *D. binectariferum* and they reported 98.75% mortality at 2000 ppm with LC_{50} and LC_{90} values of 907 ppm and 1961 ppm respectively. They conclude that both the leaf and callus bioactive extracts of *D. binectariferum* possessed an anti-larval activity against malaria vectors with callus extracts demonstrating greater efficacy. Hence, it is suggested that callus extracts of this plant can be used as a vector-control agent in the campaign against malaria. Aside from the genus *Dysoxylum*, a handful of other genera in the family of Meliaceae are equally reported to be potential sources of limonoids with excellent

bioactivity against insects. This plant species holds a tremendous potential in the fight against malaria as future study to determine the bioactive compounds and their mechanism of action against the malaria parasite and anti-larval remains viable, sustainable and promising.

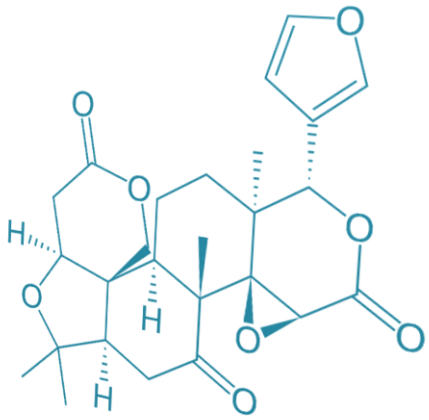

Fig. 1. Chemical Structure of Limonoid

2.2 *Artemisia annua* L.

Artemisia annua L. also known as Sweet Wormwood, Sweet Annie, Sweet Sagewort or Annual Wormwood, a native Chinese plant was identified to have an anti-malarial potential. Artemisinin (Fig. 2) a derivative of the plant has now been the standard line of treatment against malaria recommended by WHO [2]. Antioxidant phenolic phytoconstituents of *A. annua* were reported to be implicated in resistance to *Plasmodium falciparum* parasites and other diseases [6]. *In vitro* culture in *Artemisia* sp. for the production of potent medicinal products has been reported. Protocols for callus culture in this plant species has been documented [7]. Zoyava et al. [8] reported that both, leaf- and stem-derived callus tissues contained a high quantity of phenolics when Naphthalene acetic acid and 6-Benzyl Amino Purine (NAA/BAP) are appropriately combined and significantly less when 2,4-dichlorophenoxyacetic acid (2,4-D) was used. The flavonoids content according to their report in all experimental variants remained insignificant and it seemed independent from the type of auxin and more abundant in stem explants [8].

Callus cultures developed in the presence of plant regulators are found to produce bioactive compounds in significant concentration in contrast to natural plants [9].

The conventional cultivation of this plant is inadequate to meet the increasing demand for secondary metabolites; hence in vitro techniques are regarded as the excellent approach for mass propagation ensuring sustained and contaminant-free production under suitable and controlled physical conditions devoid of climatic changes.

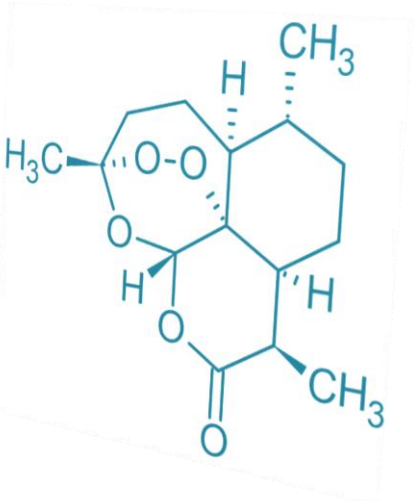

Fig. 2. Chemical Structure of Artemisinin

2.3 *Sonchus arvensis* L.

Sonchus arvensis L (Tempuyung) is an Indonesian native plant with therapeutic potentials that contains different bioactive compounds like flavonoids, saponins and polyphenols. The plant is broadly utilized as antioxidants, antihepatotoxicity, diuretic (water pills) and antimalarial [10]. They can be found with ease but the bioactive constituents fluctuate due to plantation and direct utilization from the field can result in genetic drift [11]. Tissue culture obviously remains the best option for the cultivation of such plants, which may suggest that the levels of active metabolites in them are the same per unit weight. In vitro cultivation is one of the techniques used to improve the quality of crops, increase the biomass and yield of plants. Tissue culture can produce secondary metabolites of high economic value in a relatively short, continuous-time with constant and controlled quality at higher concentrations than directly harvested [12,13].

Conservation and large scale production of these bioactive secondary metabolites are additional benefits of *in vitro* techniques. It's on the strength of this technique that Wahyuni et al. [14] reported a protocol for callus induction explants (leaf) from *S. arvensis* and determined its antimalarial activity by *in vitro* assay. According to their

report, the combination of 1mg/L 2,4-D and 0.5 mg/L BAP yield maximum callus within a short time and the *in vitro* assay has a significant antimalarial activity [14]. The report suggests that *S. arvensis* callus extract is an excellent potential candidate for malaria treatment. Further characterization of bioactive compounds from this plant with antimalarial potential is significant for specific applications.

2.4 *Holarrhena antidysenterica* L. (Wall)

Holarrhena antidysenterica L. belongs to Apocynaceae family with a global distribution. It has wide application due to its medicinal value for time immemorial in the treatments of diarrhoea, haemorrhoids, amoebic dysentery, chest infection, dyspepsia, diuresis, dropsy, diabetes etc. with few reports on its larvicidal potential against mosquito vectors [15,16]. It has been reported that conessine (Fig. 3) derived from this invaluable plant has a disruptive action against insect mosquitoes and demonstrate a potential inhibitory activity on larval growth [17].

Kumar et al. [17] reported the concentration of conessine from dry barks, green barks, nodes, and leaves from *H. antidysenterica* and revealed that the concentration varied across the components; conessine was maximum in green bark (1735.56 ± 0.28 µg/g dry wt.)

In another report, Ramirez-Estrada et al. [18] examined different parts of *H. antidysenterica* and its green bark showed the highest yield of bioactive molecule conessine, however, the source, chemical synthesis and isolation of bioactive molecule from this plant is not cost-effective. It was against this background that Kumar et al. [17] opted for *in vitro* induction of callus and elicitation. The MS medium enhanced with 5 µM BA and 5 µM 2,4-D yielded the most excellent callus as far as conessine content was concerned. Be that as it may, no callus was seen when the explants were refined on MS medium with no development regulators.

Improvement in the conessine content through different elicitors like sucrose, NaCl, substantial metals ($Pb(NO_3)_2$, As_2O_3, $CuSO_4$, and $CdCl_2$), adjuvants (casein hydrolysate, chitosan, yeast extricate), amino acids, or antecedent of the biosynthetic pathway (proline, phenylalanine, tyrosine, and tryptophan) has been accomplished at fluctuated levels.

Conessine content supposedly was upgraded with expanding groupings of sucrose from 1 to 7.5% and arrived at greatest at this level. Sucrose is a significant energy source and carbon skeleton and plays a significant part in osmoregulation, digestion related organogenesis, breath, signal transduction, regulation of gene expression, and general turn of events. Their report showed potent larvicidal activity against *A. stephensi* hatchlings and could be considered as a successful and normal larvicide that is eco-friendly. In view of this investigation, it has been recommended that the green bark-inferred callus can be utilized as an excellent source of conessine on an industrial scale utilizing phenylalanine [17].

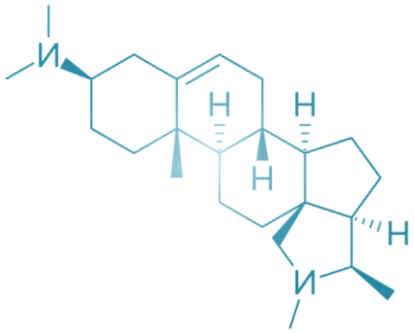

Fig. 3. Chemical Structure of Conessine

2.5 *Ajuga bracteosa* Wall ex. Benth

Ajuga bracteosa Wall ex. Benth is commonly known as 'bungle' that belongs to the family Lamiaceae. It is a perennial herb with limited distribution in the sub-tropics. It is reported to be effective against jaundice, hypertension, sore throat and as a blood cleanser. Other reports suggested its anti-inflammatory and anti-cancerous potentials. The antimalarial potential was also reported and suggested to be an alternative to artemisia. The restricted dissemination of *A. bracteosa* combined with its unlimited usage due to its therapeutic properties has made it an imperilled plant species [19].

It was in light of this that Jan et al. [20] reported a callus induction of *A. bracteosa* from leaf explants although the study was limited to callus culture and shoot differentiation of this plant without further examination to its potential antimalarial activity. They assessed plant development regulators auxins and cytokinins utilized either exclusively or in various blends cultivated callus from various explants of *A. bracteosa*. Most extreme callus cultivation was acquired from leaf explants vaccinated on MS

medium containing BAP (5 mg/l). According to their findings, juvenile leaves reacted better when contrasted with more established leaves. The callus cultivated was small and green. Callus from leaves after sub refined cultivated numerous shoots. The highest shoot development was accomplished on MS medium enhanced with BAP 5 mg/l. This report, however, is in clear contrast to Srivastav et al. [21] who reported higher callus production from leaf explants of *A. bracteosa* on MS medium enhanced with BAP (5 mg/l) and IAA (2 mg/l) following 10 days of inoculation [19]. We, therefore, recommend further investigation for callus induction from *A. bracteosa*, determine its potential antimalarial activity and to characterize the bioactive compounds.

2.6 *Piper longum* L.

Piper longum L. belonging to the family Piperaceae is known to contain piperine (Fig. 4) as its major components as well as alkaloids, amides, lignans, esters, volatile oils and organic acids. The plant is reported to possess pharmacological activities as an anti-oxidant, anti-inflammatory, antimicrobial and potential candidate for an antimalarial drug [22].

Putri and Noli, [22] reported the potential therapeutic values of *Piper* genus but their continued utilization in its natural state may further fuel the extinction of this limited plant. With this in mind, they reported a comprehensive summary of an *in vitro* approach using *Piper* genus to encourage maximum and sustainable utilization for its wide range of benefits. According to their report, the potential antimalarial candidates extracted from a few *Piper* families include 20,60-Dihydroxy-40-methoxydihydro-chalcone, 3-Farnesyl-p-hydroxybenzoic corrosive, piperine, chabamide, benzoic corrosive subsidiaries, guineensine, pellitorine, brachystamide B, sarmentine, and sermentosine, 5,8-Hydroxy-7-methoxyflavone, prenylated hydroxybenzoic corrosive, 4-Nerolidylcatechol, piperitone, champor, and viridiflorol. Supplemented with different plant growth regulators, callus induction for antimalarial potential in *P. betle, P. colubrinumm, P. crocatum, P. longum, P. nigrum, P. permucronatum* and *P. solmsianum* has been reported. Efforts were made to induced callus culture from *P. longum* and *P.nigrum* for bioactive metabolite (piperine) as antimalaria pharmaceuticals with remarkable success, however, efforts to replicate same in *P. hostmannianum, P. tricuspe, P. chaba, P. sarmentosum, P. piedecuestanum, P. heterophyllum, P. peltatum,* and *P. aduncum* is limited. The induction of callus culture for antimalarial metabolites seems to be very promising; we, therefore, encourage further investigation especially with species that have a paucity of data to ensure a sustainable supply of antimalarial if we must curtail the scourge of malaria.

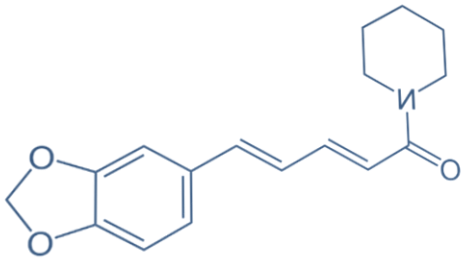

Fig. 4. Chemical Structure of Piperine

2.7 *Phyllanthus niruri* (amarus).

Phyllanthus niruri (amarus) a yearly plant broadly distributed in the coastal areas has generally been recognized as a significant enemy of plasmodial which can be utilized to treat the sickness

Subeki et al. [23] have shown that 1-O-galloyl-6-O-luteoyl-alpha-d-glucose (Fig. 5) gotten from bubbled concentrates of *P. niruri* had antiplasmodial potential. In addition to its antimalarial potential, extracts from this plant was reported to have a potent effect against hepatitis B and C viruses, diabetes, jaundice, and as a treatment for liver disorders [24,25,26].

The nonstop usage of *P. niruri* for medicinal purposes in its natural form endangers its existence, hence, Liang and Keng, [27] suggested that an *in vitro* culture technique remains the viable alternative for commercial and sustainable propagation of this amazing plant. Calli induction in several species of *Phyllanthus* has been documented.

Adusei-Fosu et al. [28] reported that *in vitro* callus induction is important for commercial propagation of the whole plant and as a source of secondary metabolites with pharmacological and medicinal values. Their report recorded a level of success in nodal and calli cultures of *P. niruri* regeneration. The study, however, failed to examined callus culture efficacy

against *Plasmodium* parasite; hence, we recommend further study to determine the level of activity of its extract from callus culture as a sustainable effort against the malaria epidemic.

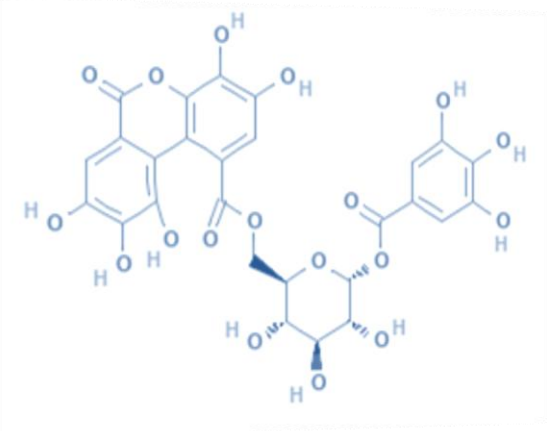

Fig. 5. Chemical Structure of 1-O-galloyl-6-O-luteoyl-alpha-d-glucose

2.8 *Phyla nodiflora* L.

Phyla nodiflora L. belongs to Verbenaceae family known for its medicinal benefits. The plant extracts cure adenopathy, chronic indolent ulcers, diuretic and aphrodisiac and is also used for the treatment of heart diseases, bronchitis, fevers, and cold. It was also reported to have anti-cancer, anti-inflammatory and anti-pyretic, anti-tumour, anti-malarial, antifungal, cytotoxic activities and a cure for multiple skin diseases [29].

Ahmed et al. [29] reported the callus induction of this plant. According to their study, Callus induction and propagation was better in 2,4-D and NAA than IAA, IBA in all media. Auxin, 2,4-D (0.6 mg/L) with ascorbic acid (10 mg/L) induced higher embryogenic callus in leaf explants (94.5 %) than stem explants (76.8 %) after 25 days. The study was limited to the somatic embryogenesis of *P. nodiflora* by picloram in suspension culture. We also suggest further study to assay for the potential antimalarial activities of *P. nodiflora* callus and to characterize the bioactive compounds.

2.9 *Allamanda cathartica*

Allamanda cathartica is also known as called golden trumpet has emerged among other species of *Allamanda* as a potential medicine for human health especially in the treatment of jaundice, malaria and cancer [30]. They study the effects of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP) on callus induction from the leaf and stem explants of *A. cathartica.* According to their study, callus induction from leaf and stem explants cultured on 1.0 mg/L 2,4-D and 1.0 mg/L BAP yielded excellent callus (100%) with yellow-white, greenish friable callus (0.0707 ± 0.0549) g following initiation after 6 days and brown-white, greenish friable callus (0.0207 ± 0.0009) g with callus induction after 5 days, in that order. Having established, the protocol for maximum callus induction of this plant that possesses a potential for antimalarial activity, we recommend further assay of *A. cathartica* callus to establish its antimalarial effect and toxicity level as an alternative candidate in the campaign against malaria and to determine the available bioactive compounds.

3. DISCUSSION

Plant species with antimalarial potential have been identified and the reports are readily available. Extensive utilization of these plants materials that are limited in supply for folk medication and other purposes gradually endangers the existence of these plants. To conserve and produce these plants species in commercial quantity sustainably and without harm to the environment, tissue culture techniques remains at the forefront. An *in vitro* culture research is well established in the literature Induction of callus culture for their therapeutic potentials has also been reported in some plant species. Callus induction for antimalarial bioactive compounds has been equally reported even though there is limited available data in contrast to plant species that are identified to have possessed antimalarial activity. Callus culture for antimalarial is a viable approach to develop alternative treatment options in the fight against malaria. Putri and Noli, reports that callus culture could be the most promising approach to obtain high yield of secondary bioactive compounds with antimalarial activities [22]. Uma Masur, et al. [5] equally reported the use of extracts from callus and leaf of *D. binectariferum* with high activity against *An. stephensi* larvae. Higher mortality rate was observed in the presence of low concentration of callus and this could be due to higher synthesis of bioactive metabolites in callus tissues in contrast to the leaf extract. Other reports suggest similar observation of callus extract showing higher antimicrobial activities when compared to extracts from other parts of the same plant [31].The extraction of bioactive compounds that

is effective against the plasmodium parasite is cost-effective in callus suspension when compared to extraction from the whole plant in its native form. We, therefore, recommend further investigation for antimalarial candidates using callus culture approach with different plant species and subsequent characterization to consolidate the current efforts against malaria. It is important to mention that the plant species mentioned in this brief report is limited. The induction of transgenic callus culture targeted against malaria is a future possibility.

4. CONCLUSION

The role of plants in human healthcare cannot be overemphasized. In the resolved to curtail the scourge of malaria-related cases on the healthcare delivery system, there's a need to explore and focus on potential candidates plant species that demonstrates antimalarial activity instead of channelling all efforts and resources to only a few that are established. The reported cases of drug resistance in some parts of malaria endemic regions with the current recommended choice of treatment calls for urgent research for alternative treatment. The use of callus culture for the production of valuables products has been well documented. We therefore, recommend the optimization of callus culture protocols for the production of bioactive compounds as the technique remains viable and sustainable in contrast to folk medicinal usage of plant material in the treatment of malaria and other related diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Efferth T. Biotechnology Applications of Plant Callus Cultures. Engineering 2019; 5(1) 50–59
2. WHO Report: The World malaria report 2018.
3. Global Technical Strategy for Malaria 2016-2030. (Accessed 18th August 2021; https://www.theglobalfund.org/media/9683/strategydevelopment_2020malaria_landscapeanalysis_en.pdf)
4. Bhutani K. and Gohil, V. Natural products drug discovery in India: status and appraisal. Indian J. Exp. Biology, 2010;48: 199-207.
5. Uma M, Hemanth K, Ashwani K. Anti-larval effects of leaf and callus extract of Dysoxylum binectariferum against urban malaria vector, Anopheles stephensi; Journal of Natural Products, 2014;7:147-154
6. Suberu J, Gorka AP, Jacobs L, Roepe PD, Sullivan N, Barker GC, and Lapkin AA. Antiplasmodial polyvalent interactions in Artemisia annua L. aqueous extract – possible synergistic and resistance mechanisms, PLoS One 2013;8:| e80790.
7. Ali M, Abbasi BH, Ahmad N, Khan H, and Ali GS. Strategies to enhance biologically active secondary metabolites in cell cultures of Artemisia - current trends. Crit. Rev. Biotechnol. 2017; DOI:10.1080/07388551.2016.1261082
8. Zayova E, Nedev T, Petrova D, Zhiponova M, Kapchina V. and Chaneva G. Tissue Culture Applications of Artemisia annua L. Callus for Indirect Organogenesis and Production Phytochemical. Plant Tissue Cult. & Biotech. 2020;**30**(1): 97-106. DOI:https://doi.org/10.3329/ptcb.v30i1.47795
9. Benjamin ED, Ishaku GA, Peingurta FA, and Afolabi AS. Callus Culture for the Production of Therapeutic Compounds. American Journal of Plant Biology; 2019; 4(4): 76-84. doi: 10.11648/j.ajpb.20190404.14
10. Sukandar EY, Safitri, D. Evaluation of teratogenic effect of tempuyung (Sonchus arvensis) extract on Wistar Rats. International journal of farmakognosy and phytochemical research. 2016;8(5):761-766.
11. Thomas TD. and Yoichiro H. In vitro propagation for the conservation of a rare medicinal plant Justicia gendarussa Burm.f. by nodal explants and shoot regeneration from callus. Acta Physiologiae Plantarum, 2010;32(5):943-950
12. Sitorus EN, Hastuti ED, Setiari N. Induksi kalus binahong (Basella rubra L.) secara in vitro pada media Murashige & Skoog dengan konsentrasi sukrosa yang berbeda. Bioma, 2011;13(1):1-7.
13. Ariati SN, Waeniati, Muslimin, Suwastika, IN. Induksi kalus tanaman kakao (Theobroma cacao L.) pada media MS

dengan penambahan 2,4-D, BAP dan air kelapa. Natural Science: Journal of Science and Technology, 2012;1(1): 74-84.

14. Wahyuni DK, Purnobasuki H, Kuncoro EP, and Ekasari W. Callus Induction of Sonchus arvensis L. and its Antiplasmodial Activity. Afr., J. Infect. Dis. 2020;14 (1): 1-7 https://doi.org/10.21010/ajid.v14i1.1
15. Verma G, Dua VK, Agarwal DD, Atul PK. Anti-malarial activity of Holarrhena antidysenterica and Viola canescens, plants traditionally used against malaria in the Garhwal region of north-west Himalaya. Malar J 2011; 10(1):20. https://doi.org/10.1186/1475-2875-10-20
16. Siriyong T, Chusri S, Srimanote P, Tipmanee V, Voravuthikunchai SP. Holarrhena antidysenterica extract and its steroidal alkaloid, conessine, as resistance-modifying agents against extensively drug resistant Acinetobacter baumannii. Microb Drug Resist 2016; 22(4):273–282. https://doi.org/10.1089/mdr.2015.0194
17. Kumar D, Kumar G, Das R, Kumar R, and Agrawal V. In vitro elicitation, isolation, and characterization of conessine biomolecule from Holarrhena antidysenterica (L.) Wall. callus and its larvicidal activity against malaria vector, Anopheles stephensi Liston. Environmental Science and Pollution Research. 2017; https://doi.org/10.1007/s11356-017-1038-3
18. Ramirez-Estrada K, Vidal-Limon H, Hidalgo D,Moyano E, Golenioswki M, Cusidó RM, Palazon J. Elicitation, an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. Molecules 2016;21(2):182. https://doi.org/10.3390/molecules21020182
19. Kaul S, Das S, and Srivastava PS. Micropropagation of Ajuga bracteosa, a medicinal herb. Physiology and Molecular Biology of Plants. 2013;19(2); 289296 DOI:10.1007/s12298-012-0161-3
20. Jan M, Singh S, Kaloo ZA, and Maqbool F. Callus induction and multiple shoot regeneration in Ajuga bracteosa Wall ex. Benth.-An important medicinal plant growing in Kashmir Himalaya. Journal of Scientific and Innovative Research; 2014; 3(3): 319-324
21. Srivastav A. and Joshi AG. Control of shoot tip necrosis in shoot cultures of Portulaca grandiflora Hook; Not. Sci. Biol. 2013;5(1):45-49
22. Putri NS. and Noli ZA. Callus culture as the method in providing antimalarial compounds of Piper genus. Journal of Environmental Science for Sustainable Society. 2021;10, PP03.
23. Subeki M, Takahashi K, Yamasaki M, Yamato O, Maede Y. et al. Anti-babesial and anti plasmodial compounds from Phyllanthus niruri. J. Nat. Prod. 2005; 68(4):537-9.
24. Shim SB, Kim NJ, Kim DH. b-Glucuronidase inhibitory activity and hepatoprotective effect of 18 b glycyrrhetinic acid from the rhizomes of Glycyrrhiza uralensis. Planta Med. 2000; 66:40–43.
25. Bhattacharyya R. and Bhattacharya S. Establishment of Phyllanthus amarus root clone with significant activity against bovine viral diarrhoea virus ând surrogate model of hepatitis C virus: Cur. Sci. 2003; 84:529-533
26. Banerjee A.and Chathopadhay S. Effect of overexpression of Limum usitatissimum pinorresinol lariciresinol reductase (LuPLR) gene in transgenic Phyllanthus amarus. Plant Cell Tiss. Org. Cult. 2010;103(3): 315-329.
27. Liang OP. and Keng CL. In vitro plant regeneration, flowering and fruiting of Phyllanthus niruri L (Euphorbiaceae). Int J. Bot. 2006;2(4):409-414.
28. Adusei-Fosu K, Elegba W, Annor C, Klu GYP, and Danso KE. In vitro regeneration and morphogenesis in Phyllanthus niruri L. An anti-plasmodial herb. African Journal of Biotechnology 2012;11(80):14542-14552 DOI: 10.5897/AJB12.1591
29. Ahmed ABA, Rao AS, Rao MV, and Taha RM. Effect of Picloram, Additives and Plant Growth Regulators on Somatic Embryogenesis of Phyla nodiflora (L.) Greene. Braz. Arch. Biol. Technol. 2011;54 (1): 7-13
30. Wong KF. and Taha RM. The effect of 2,4-dichlorophenoxyacetic Acid and 6-benzylaminopurine on Callus Induction and Plant Regeneration of Allamanda

cathartica; A Valuable Medicinal Plant. Res. J. Biotech. 2012;7(3).

31. Radfar M, Sudarshana MS, Kavitha HU, Satish S, Niranjan MH. Evaluation of antibacterial and antifungal activity of root and root callus extracts of Trianthema decandra L. Afr. J. Biotechnol., 2012;11: 510-515.

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