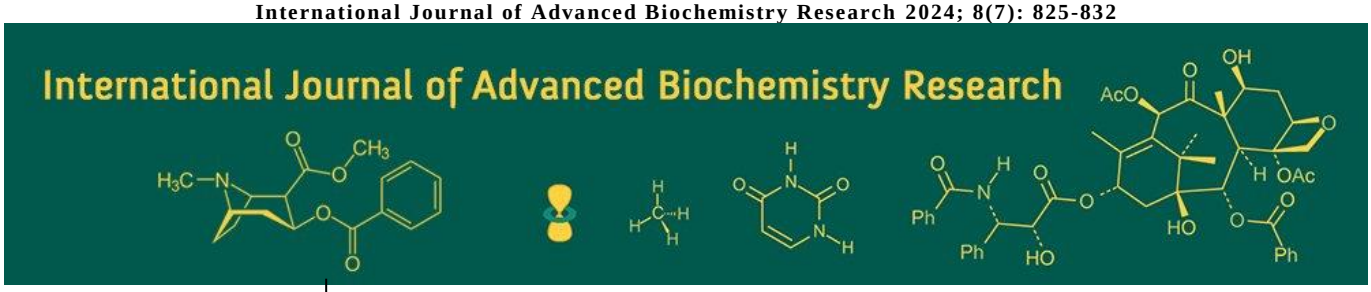

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Organoids: Revolutionising research and shaping the future of medicine

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Abstract
The aim of this study is to determine the chemo profile of the aqueous extract of Nauclea latifolia using GC-MS. The GC-MS analysis of the plant extract was performed using a GC-MS QP-2010 (Shimadzu), and the mass spectra of the compounds found in the extract were matched with the data in the National Institute of Standards and Technology (NIST) library. The results revealed the presence of eighteen compounds. The most prevailing compounds were 9-Octadecenoic acid (Z)-methyl ester, Oleic acid, and Benzamide, 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl], with percentage areas of 16.93%, 14.01%, and 9.18%, respectively. The least abundant compound was 5-methyl-2-phenylindolizine (0.66%). Some of these phytocompounds exhibit bioactivities such as anti-inflammatory, antibacterial, and antimalarial activities. Consequently, the leaf extract has the potential to be a source of novel treatments. The results of this investigation thus validate the plant's traditional usage for medicinal purposes.

Keywords: Nauclea latifolia, GC-MS analysis, phytocompounds, anti-inflammatory, antibacterial, antimalarial, traditional medicine

Introduction
Organoids are self-organizing, self-renewing three-dimensional cellular structures that resemble organs in structure and function (Eiraku *et al.*, 2008 and Sato *et al.*,2009) [8, 29]. They can be called “mini organs” as they mimic some of the key multicellular, anatomical and even functional characteristics of real organs.
The term organoid refers to a 3D culture of different cell types derived from tissue explants, tumors, stem cells or other progenitor cells, that self-organize under controlled conditions and differentiate into functional cell types to acquire the complexity, anatomy and physiology of an organ or body structure. A tumor-like organoid derived from primary tumors is referred to as a tumoroid (Lehmann *et al.*, 2019) [16].

Key events in organoid history
In 1907, it was proposed that complex organs/tissue structures could be recreated *in vitro* after Henry Van Peters Wilson showed that sponge cells in culture could self-organize and regenerate into fully-grown organisms. It was thought that this self-organizing and regenerating potential of cells in culture could probably be utilized to recapitulate parts of complete tissues or organs. Standard two dimensional (2D) cultures comprise growing and maintaining monolayers of individual cell types on the surface of flasks or Petri dishes. However, the challenge was to attain the 3D spatial conformations of cells as they are in their natural environment (Corro *et al.*, 2020) [5].
Subsequent attempts to achieve 3D culture were seen in the 1950s when Ehrmann and Gey cultivated various human cell lineages using collagen isolated from rat tails as a scaffold. Several attempts were made to model *in vivo* organ systems during the 1980s and -90s using dissociation-aggregation experiments: for example, cells isolated from chick embryos or mouse fetal lungs were dissociated and allowed to aggregate in culture. Since 1975, scientists have started to focus on developing scaffolds that facilitated cell-matrix interactions along with cell-cell interactions required for *in vitro* cell differentiation, just like in tissues and organs, and thus brought on a major leap shift from 2D cultures to 3D organoids.

Floating gels or matrices rich in collagen and lamina were used as scaffolds for culturing fragments of tissues or cells isolated from dissociated organs in a 3D environment (Lehmann *et al.*, 2019 and Corro *et al.*, 2020) [16, 5].

Landmarks in the development of organoids came with the first successful isolation of pluripotent embryonic stem cells (ESCs) from mice in 1981 and human blastocysts in 1998. In 2006, Kazutoshi Takahashi and Shinya Yamanaka genetically modified mouse fibroblasts to develop murine pluripotent stem cells (PSCs). Induced or embryonic PSCs (iPSCs or ESCs) have effective self-renewal and self-organizing capacities that are lacking in primary cells isolated from tissues or organs. PSCs are organ progenitors that differentiate in culture to give rise to multiple cell types that self-organize to form organ-like complex structures in culture, closely resembling the process of organ formation in embryos until birth. The discovery of stem cells improved organoid technology by eliminating the need for isolation and co-culturing of several cell types and reduced the need for expensive differentiating media and complicated scaffolds. Thereafter, the establishment of human induced pluripotent stem cells (hiPSCs) in 2007 revolutionized organoid technology to facilitate the development of organoids from single individuals that could be used for diverse *in vitro* and preclinical studies (Sakalem *et al.*, 2021 and Tang *et al.*, 2022) [27, 30].

In the early 21st Century, organoids were developed that did not require the use of gels or scaffolds. Cells were cultured either in hanging droplets of medium using surface tension or in medium with nanoparticles levitated by magnetic fields. However, the scaffold-free methods did not gain popularity and did not emerge further. Extracellular matrix (ECM) plays an important role in the *in vivo* microenvironment and architecture of organs, and natural or synthetic matrix continued to impact organoid improvements significantly. From 2012 onwards, air-liquid interface (ALI) was used to initiate organoids in which cells were cultured in gels immersed in the medium such that the upper layers of the cells were exposed to air, facilitating their polarization and differentiation. ALI models were used to construct organoids from cell lines, cells differentiated from ESCs or primary cells isolated from the skin. Advanced bioengineering and bio printing techniques have also improved organoid culture techniques in the last decade by fabricating complex micro architectures of organs that act as scaffolds on which different cell lineages can be seeded and cultured in an enrichment medium to generate an "organ-on-chip" model. Multiorgan-on-a-chip models contain different types of organoids on specially designed scaffolds/compartments on a single chip to allow cross-organ studies and multi-organ modeling of diseases (Corro *et al.*, 2020 and Mittal *et al.*, 2018) [5, 20].

Use of organoids

- They offer the possibility to study human tissues at the same level of scientific scrutiny, reproducibility, and depth of analysis as has been customarily possible only with non-human model organisms (Lehmann *et al.*, 2019) [16].
- They allow investigators to recapitulate morphogenetic events in human development that lead to tissue and organ formation (Lehmann *et al.*, 2019) [16].
- Serves as a good replacement to laboratory animals.

Synthesis of organoids

ESCs, iPSCs, somatic stem cells and cancer cells can all be used to create organoids. Suspensions of cells or fragments can be obtained from tissues, organs or cryopreserved organoids using mechanical or enzymatic dissociations. These cells or tissue fragments are first seeded in differentiating medium on low attachment plates or culture vessels for 5-7 days to generate 3D cellular aggregates called spheroids. The spheroid formation can also be performed using normal suspension culture, mini bioreactors or roller bottles. These spheroids are then planted into liquid ECM, madrigals or agarose-based gels. The protocols are adapted according to the progenitor cells used and the organ/tissue structure to be recapitulated: in some cases, the cells obtained from dissociated tissues or organoids are suspended and seeded directly into liquid ECM, skipping the differentiation step. The gels are polymerized at 37 ℃ to obtain a 3D culture, which is supplemented with organoid-specific expansion and maturation medium to stimulate the growth of the desired cell types, resulting in organ-like structures. For example, a medium supplemented with the growth factor EGF stimulates the growth of epithelial and tumor organoids; whereas fibroblast growth factor 10 is needed in stomach, breast and liver cancer tumoroids (Corro *et al.*, 2020 and Xu *et al.*, 2018) [5, 35].

Generally, fully-grown, functional organoids are generated after 30-60 days (maybe even longer) in the maturation medium, replenished twice per week. This mature organoid can be maintained for about 100 days (sometimes up to a year) by changing the maturation medium once or twice every 10 days and passaging the organoids. For passaging, the maturation medium is removed and organoids are gently broken into smaller fragments using mild cell dissociation reagents for 15-20 minutes, followed by vigorous pipetting. After a few centrifugation and washing steps, the organoid pellets finally obtained are resuspended in liquid ECM to repeat the whole process of organoid generation. Organoids can be cryopreserved and fresh organoids can be generated by the complete dissociation of these frozen organoids.2, 6 The protocol is highly adaptable to the target organ or body structure to be recreated and a broad range of specialized media and media supplements, as well as other materials such as trans wells, well-plates or matrices, are available commercially to uplift the various systems of organoid technology.

Organoid culture

Organoid formation and maturation are preceded by a single cell or small cell-cluster expansion and reorganization (Sasai *et al.*, 2012) [28]. There are two main types of organoids based upon the choice of stem cells. The first is derived from PSCs that include both embryonic stem cells (ESCs) and iPSCs and the second type is derived from organ-specific adult stem cells (ASCs) (Vaart and Clevers., 2020). A variety of workflows have been developed to generate organoids; however, specialized organoid types require unique culture methods, and not all general workflows are appropriate. The choices of cell culture conditions and the 3D matrix are critical for this complex organization.

Organoids can be generated by both unguided and guided methods. The unguided approach depends on PSC aggregates to undergo spontaneous morphogenesis and their capacity for intrinsic differentiation, whereas the guided approach induces PSCs to differentiate towards desired lineages using the supplementation of external patterning factors. (Qian *et al.*, 2019) [24].

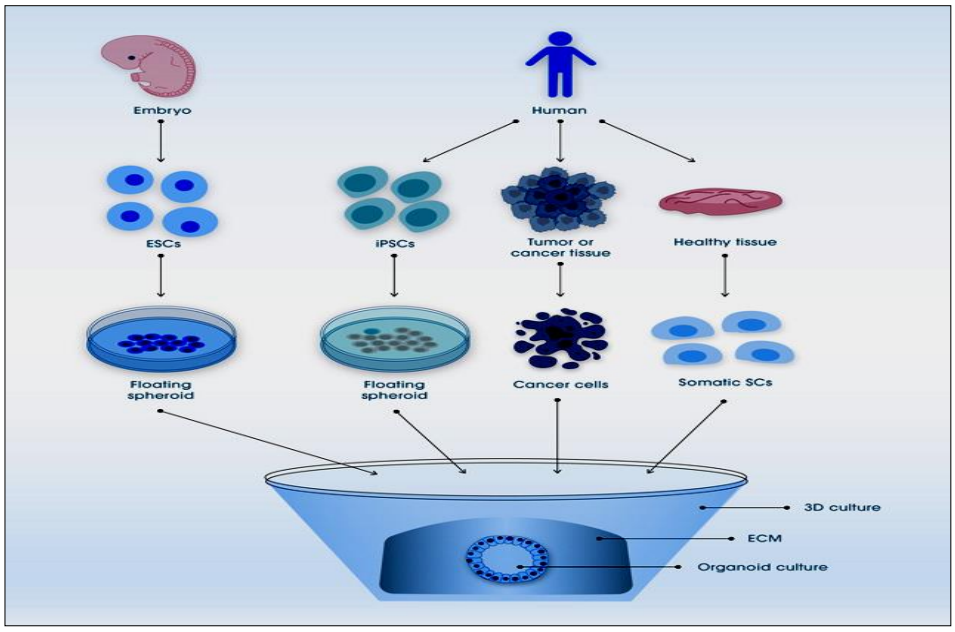

Fig 1: Schematic representation of the generation of organoids from human embryos, organs or tumors. Abbreviations: ECM, extracellular matrix; ESC, embryonic stem cell; iPSCs, induced pluripotent stem cells; SC, stem cell; 3D, three-dimensional.

Charecterization of orgnoids

Several conventional strategies can be applied to characterize organoids. These techniques include high-resolution microscopy, histology, immunofluorescence, and bulk gene expression assays (Wu and Humphreys, 2020) [34]. Cell counting is a necessary step for *in vitro* work involving cell culture.Using the correct organoid concentration will ensure that downstream experiments are reproducible and accurate. Organoid counts are important for monitoring overall health and proliferation rates, seeding for experiments, and preparing for assays (Ongena *et al.*, 2010) [22]. High-resolution microscopy can further be used to assess organoid quantity and morphology. Simple visual observation can determine organoid counts to determine organoid multiplicity before conducting experiments and for experiments that require normalization to controls (Urbischek *et al.*, 2019) [31]. Organoids quantity and size can also be determined using the analysis of 8-bit binary images of whole organoid drops (Boonekamp *et al.*, 2019) [3]. Alternatively, whole-well z-stack images, using a transmitted light inverted microscope with a motorized X/Y scanning stage, can be used to capture all organoids in a well. Organoid culture can then be evaluated based on morphology by taking measurements to quantify variables such as shape and area (McGray *et al.*, 2019). Morphological analysis is an important tool, especially when optimizing growth factor concentrations for organoid formation (Urbischek *et al.*, 2019) [31]. A variety of techniques exist to assess morphology and differentiation such as bright field image analysis, and FFPE, and whole-mount staining techniques (McGray *et al.*, 2019).

Properties of organoids

They have multiple organ specific cell types (Fatehullah *et al.*, 2016 and *Lancaster et al.*, 2014) [9, 15]. They are capable of recapitulating some specific function of the organ (eg. excretion, filtration, neural activity, contraction) origin and capable of recapitulating the pathology of the disease when cultured with tissues derived from clinical patients. (Bigorgne *et al.*, 2014) [2]. They are grouped together and spatially organised similar to an organ. Organoid cultures can typically be maintained for very long times, months or even longer than a year. (Akbari *et al.*, 2019 and Matsui *et al.*, 2018) [1, 18]. Organoids can be easily cryopreserved (Lu *et al.*, 2017) [17] and cultures can be restored from cryopreserved stocks, retaining functionality similar to that of the tissue of origin. ECM provides the main structural organization and allows cellular functions and communications for the proper functioning of biological tissues and organs (Lehmann *et al.*, 2019 and Corro *et al.*, 2020) [16, 5].

Examples of organoids

Pioneering works in the last decade have led to the establishment of human derived organoids that model tumors, embryos and a broad range of tissues and organs, some of which are highlighted below.

Brain organoids

Organoids derived from human PSCs comprising several cell lineages can recapitulate cellular and regional interactions as well as the formation of blood vessels in the brain. Brain organoids resembling specific regions of the brain or combinations of multiple brain regions and/or cell lineages, serve as representative models to study the development, function and dysfunction of the human brain. Human PSC-derived brain organoid models have been used to study gene mutations in microcephaly, the abnormal inhibitory neurons in autism spectrum disease and epilepsy, the pathogenesis of Parkinson's disease and mechanisms of Zika virus infections in the brain (Lehmann *et al.*, 2019) [16].

Lung organoids

Human airway organoids have been employed successfully to study the infectious mechanisms of the respiratory syncytial virus and influenza virus. Additionally, they served as a representative experimental model for studying severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infection with subsequent drug screening. Several other alveolar organoids derived from different cell types that mimic specific areas or a combination of multiple areas in the lungs have been developed to study SARS-CoV-2

pathogenesis and facilitate the development of new therapies.

Intestinal organoids

Complex asymmetric structures of intestines, including the crypts and villi, are recreated in organoids from single intestinal stem cells. The highly specialized epithelial barrier plays a major role in the functioning and homeostasis of the intestines. Organoids have allowed the successful identification of drug gable biological targets as well as small molecule drugs as regulators in the composition and operation of the barrier epithelia. The intestinal epithelia for both the small intestine and colon recreated using organoids serve as promising models for studying the infectivity of the Omicron variant of SARS-CoV-2 (Jang *et al.*, 2022) [11].

Liver organoids

Multicellular liver organoids from human primary hepatic stellate and iPSCs have been applied effectively to model liver fibrosis, lipid metabolism and genetic stability in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Liver organoids have been employed to differentiate progenitor cells or PSCs into hepatocytes and liver buds. These could be transplanted with high engraftment efficiency in mice for the generation of mature hepatocytes or even vascularized into fully functional livers *in vivo*. These findings imply a potential role of organoids in liver transplantations in cases of organ damage or organ failure (Corro *et al*, 2020) [5].

Kidney organoids

Improved bioengineering techniques have achieved highly complex kidney organoids in recent years that have allowed several diseases to be modeled including polycystic kidney disease, cystic fibrosis, renal cell carcinoma and viral infections. In clinical trials, 19% of drugs fail because of nephrotoxicity. Consequently, several common medications such as cisplatin, gentamicin, aspirin and penicillin G, have recently been tested in kidney organoids for preselection (Khosdel *et al.*, 2022) [14].

Tumor organoids

Recent improvements in organoid technology have recapitulated the tumor microenvironment utilizing primary cells or cancer cell lines for the lung, liver, kidney and heart in culture. Organoids from numerous cancer types can be frozen or cultured for a long time, maintaining their morphologies, histopathologies, epigenetic mechanisms and genetic profile to study cancer progression and clinical features of the tumors (Gunti *et al.*, 2021) [10].

Retinal organoids

Retinal organoids have been developed successfully from retinal stem cells and independently from ESCs that are capable of largely mimicking the visual cycle and responses to light in healthy eyes. Gene mutations involved in the pathogenesis of the retinal disorders retinitis pigmentosa, Leber congenital amaurosis, retinoblastoma and glaucoma have been modeled effectively using retinal organoids.

Applications of organoids

Considering the advantages mentioned above, organoids offer potential applications and advancements in the fields of basic research, developmental biology, tissue engineering, drug testing and screening (Lehmann *et al.*, 2018) [16].

Organoids serve as fast and reliable methods for drug toxicity testing, drug discovery, screening and validation. Toxicity of 238 marketed drugs in liver organoids, 25 cardio active drugs in cardiac organoids, cisplatin and gentamicin in kidney organoids, 39 marketed diarrhea genic drugs in intestinal organoids and vincristine and rotenone in brain organoids have been tested and reported so far (Matsui *et al.*, 2021) [18].

Human iPSC-based organoids are susceptible to pathogens and provide a potential means of modeling host-pathogen interactions during bacterial or viral infections. Cerebral and neural organoids have been employed to model the zika virus infection. Intestinal organoids have successfully modeled rotavirus pathobiology and gastric organoids have been used to model bacterial infection by *Helicobacter pylori*. Human PSC-derived lung organoids have shed light on the various aspects of SARS-CoV-2 infection including cell tropism, how genetic profile influences susceptibility to virus infection, mechanisms of viral entry and replication in the host, host cell responses and cellular and metabolic changes after infection (Rowe *et al.*, 2019) [26]. Organoids can be used to mimic morphogenetic events to provide detailed insights into the development and functioning of tissues and organs. Organoids made up of ESCs from mice and humans self-organize and gastrulate to form embryos in culture, providing novel insights into early mammalian development. Also, organoids offer model systems to study the mechanisms of self-organization under different conditions, increasing our understanding of self-organization and controlling of complex multicellular behaviors *in vitro* (Rossi *et al.*, 2018) [25].

Organoids serve as the best models to study person-to-person variability in the pathogenesis of diseases, individual responses to therapies and contribute to treatments tailored to individual needs (Lehmann *et al.*, 2019) [16] Organoids offer the potential to study various shared or unique mechanisms in the development and functioning of organs, disease progression or infections/allergies that might differ from the mechanisms involved in immortalized cell lines or animal models (Rossi *et al.*, 2018) [25] Screening of the organoid biobank showed correlations between genetic profiles and drug responses for colorectal, breast, prostate and liver cancers.

Organoids hold great promise in regenerative medicine and tissue engineering. By coaxing stem cells to differentiate into specific cell types and self-organize into complex structures, researchers can create organoids that resemble functional human tissues. These organoids can potentially be transplanted into patients to replace damaged or diseased tissues, offering a personalized and regenerative treatment option. Additionally, organoids can be used to study tissue regeneration processes and identify strategies to enhance tissue repair.

Organoids enable detailed investigations into the molecular and cellular mechanisms underlying normal organ function and disease progression. By combining techniques such as gene editing, live-cell imaging, and single-cell sequencing, researchers can unravel the complex interactions between different cell types, identify key molecular players, and study dynamic processes in real-time. These mechanistic insights contribute to our understanding of organ physiology and pathogenesis.

Three-dimensional cell culture systems for translational research

Drug development

Historically, 2D *in vitro* cultures have been used as a tool to evaluate the performance and toxicity of new bioactive molecules under development for therapeutic use. The availability of a wide range of cell types, together with ease of handling and their cost effectiveness has made 2D cultures a critical evaluation tool for screening molecules with potential as therapeutic drugs (Jensen *et al.*, 2020) [12]. While this initial pre-screening proceeds animal studies it often provides a crucial 'stop/go' checkpoint that can halt the progression of development of a drug. In recent years there has been a move away from 2D *in vitro* cultures towards 3D cultures that more accurately reflect the micro architecture and cell-to-cell interactions found in the target tissue and therefore provide a more relevant platform for drug discovery (Jensen *et al.*, 2020) [12] (Breslin *et al.*, 2013) [4]. The use of 3D cultures allows greater predictability of efficacy and toxicity before moving into clinical trials. Currently, the use of 3D organoids for drug discovery is being pioneered in human medicine, but has profound implications for drug discovery for veterinary medicine (Driehuis *et al.*, 2020) [7]. Recently, it has been shown that the *in vitro* response of patient derived organoids is predictive of patient's response to therapy (Ooft *et al.*, 2019; Vlachogiannis *et al.*, 2018) [23, 32]. Thus, 3D organoids have the potential to accurately predict response to drug treatment and therefore have great promise for future drug discovery platforms for both human and veterinary medicine.

Vaccine development

The tonsils are a lymphoid organ in which B-cell and T-cell stimulation takes place as part of an immune response. They also represent a readily available source of lymphoid tissue from human patients, through routine tonsillectomies. As a result, human tonsil organoids have recently been reported to be an effective model of adaptive immunity to investigate the development of primary and secondary antibody responses to live and killed viral vaccines (Wagar *et al.*, 2021) [33]. This organoid system was also used to determine the effects of different adjuvants on the antibody response. Similar methods for the cultivation of human tonsil organoids could be applied in the development of lymphatic organoids from animals of veterinary importance, for example to generate organoids from peripheral and GI lymph nodes. The development of a host-relevant model that allows researchers to accessibly study immune cell maturation *in vitro* would be a major research development. Such models could be applied to translational research studies to determine which antigens induce the strongest antibody responses, the particular epitopes of the antibodies generated, and the influence of adjuvants on the immune response. To date, research in this area has largely depended on *in vivo* animal models and therefore lymphatic organoids would also contribute to the reducing dependency on animals for research. Another potential usage of organoids for vaccine research is to use them as a model for antibody neutralization in a physiologically relevant context. For example, in an intestinal organoid model challenged with a GI pathogen, antibodies could be applied to assess their ability to limit pathogen invasion, proliferation and survival. Such an experiment could be setup in a relatively high-throughput manner, allowing different antibody subsets to be tested for their efficacy and to help determine which antigens represent the most suitable vaccine candidates for restricting pathogens.

A final promising use for organoids in vaccine research is to evaluate mucosal vaccine delivery systems. Such delivery systems have been historically difficult to evaluate in veterinary species *in vivo* due to the difficulty in accessing 'immune inductive sites' in the mucosal epithelium. Such sites include Peyer's patches in the intestine and nasal associated lymphoid tissue, which are covered by follicle associated epithelium (FAE) containing micro fold or membranous cells (M cells), specialized cells involved in translocation of particulate antigens across the epithelium to underlying antigen-presenting cells to initiate mucosal immune responses (Neutra *et al.*, 2001) [21]. Recently, culture conditions have been defined in mice (Kanaya *et al.*, 2018) [13] and humans (Ding *et al.*, 2020) [6] which promote differentiation of M cells within intestinal organoids. These M-cell-enriched organoids are now being used to evaluate particulate systems for efficient delivery of antigens across the epithelial barrier (Tong *et al.*, 2020). If M-cell inducing culture conditions could be defined for intestinal and/or respiratory organoids from veterinary species, this would allow evaluation of different particulate delivery systems for mucosal vaccines targeting the intestinal or respiratory tracts.

Advantages of organoid technology

Organoid technology offers several advantages in the field of biomedical research and drug discovery. Here are some key advantages of organoid technology: Recapitulation of tissue architecture and function Organoids are three-dimensional structures derived from stem cells that closely resemble the structure and function of specific organs or tissues in the human body. They can replicate the complexity of various cell types, tissue organization, and organ-specific functions, allowing researchers to study disease processes and drug responses more accurately. Disease modeling Organoids can be generated from patient-derived induced pluripotent stem cells (iPSCs), which carry the genetic background and disease mutations of the individual. This enables the creation of disease-specific organoids, allowing researchers to study and understand the underlying mechanisms of diseases in a personalized manner. Organoids have been successfully used to model a wide range of diseases, including cancer, neurodegenerative disorders, and genetic diseases.

High-throughput drug screening

Organoids provide a scalable platform for drug screening and testing. Multiple organoids can be generated simultaneously, allowing for high-throughput screening of potential drug candidates or therapeutic interventions. This helps in identifying drugs that are effective and safe, thereby reducing the time and cost associated with traditional drug development processes.

Personalized medicine

The use of patient-specific iPSC-derived organoids enables personalized medicine approaches. By studying an individual's own organoids, researchers can predict how a patient will respond to specific drugs, helping in the development of personalized treatment strategies. This has

the potential to revolutionize drug development and improve patient outcomes.

Reduced reliance on animal models

Organoid technology provides an alternative to animal models for studying human biology and diseases. While animal models have their limitations, organoids offer a more human-relevant system for research, reducing the need for animal testing and potentially leading to more reliable results. Studying early human development Organoids derived from embryonic stem cells or iPSCs can be used to model early human development and study the formation of various organ systems. This provides insights into the processes and defects underlying developmental disorders and offers opportunities for regenerative medicine.

Investigation of rare diseases

Organoid technology allows researchers to investigate rare diseases that have limited patient samples or are difficult to model in traditional systems. By generating organoids from patient-specific cells, researchers can study the disease mechanisms and test potential therapies in a controlled and relevant system.

Overall, organoid technology offers a powerful and versatile tool for studying human biology, disease modeling, drug discovery, and personalized medicine, thereby advancing our understanding of diseases and improving patient care.

Challenges in organoid technology

While organoid technology holds tremendous potential, there are several challenges that researchers face in its implementation and application. Here are some of the key challenges in organoid technology:

Complexity and variability

Creating organoids that faithfully recapitulate the complexity of human organs is a significant challenge. Human organs comprise multiple cell types arranged in intricate architectures, and reproducing this complexity *in vitro* is difficult. Additionally, organoids derived from different individuals or even different regions of the same organ can exhibit variability, making it challenging to establish standardized protocols and compare results across studies.

Maturation and Functionality

Organoids often lack full maturation and functionality compared to their *in vivo* counterparts. While they can mimic certain aspects of organ development, they may not fully recapitulate the intricate cellular interactions, tissue organization, and functional properties of mature organs. Achieving complete maturation and functionality remains a challenge, limiting their applications in certain research areas and clinical translation.

Reproducibility and scalability

The production of organoids can be technically demanding and resource-intensive, limiting reproducibility and scalability. Variations in culture conditions, differentiation protocols, and quality control measures can lead to inconsistent results between different laboratories or batches. Developing standardized and scalable methods for organoid production is essential for widespread adoption and clinical translation.

Vascularization and innervation

The development of a functional vascular network and innervation within organoids is crucial for their long-term survival, nutrient supply, and accurate modeling of organ physiology. However, replicating the complex vasculature and neural networks found in human organs remains a significant challenge. Without proper vascularization and innervation, organoids may have limited size, functionality, and lifespan.

Ethical and Legal Considerations

Organoid research raises ethical and legal concerns, particularly when it involves the use of human embryonic stem cells or genetic manipulation. The generation and use of organoids from human tissue samples may also raise issues related to patient consent, privacy, and ownership of biological materials. Ethical frameworks and guidelines need to be developed and implemented to address these concerns and ensure responsible use of organoid technology.

Cost and Accessibility

Organoid research can be costly due to the specialized equipment, reagents, and expertise required. The expense associated with organoid culture and analysis may limit access to this technology for many research institutions, especially in resource-constrained settings. Improving cost-effectiveness, sharing protocols, and promoting collaboration can help increase accessibility and accelerate progress in the field.

Despite these challenges, ongoing research efforts continue to address these limitations and advance the field of organoid technology. Overcoming these hurdles will enhance the fidelity, functionality, and clinical relevance of organoids, enabling their broader application in biomedical research and clinical practice.

Future directions

In the field of organoid technology, several promising future directions can be pursued:

- **Organoids maturation and functionalization**: Efforts can be directed towards improving the maturation and functional capabilities of organoids to better resemble adult organs. This includes enhancing vascularization, promoting organoid growth and differentiation, and achieving functional integration of various cell types within the organoid.
- **Multi-Organoid Systems and Body-on-a-chip:** Developing advanced platforms that integrate multiple organoids or create "body-on-a-chip" systems can enable the study of complex organ interactions, systemic responses, and drug metabolism. These models could provide a more comprehensive understanding of infectious diseases and the effects of therapeutics on the whole body.
- **Organoid Biobanks and Personalized Medicine:** Establishing organoid biobanks, where organoids from diverse patient populations are preserved, can enable personalized medicine approaches. These biobanks can facilitate the screening of patient-specific drug responses, identification of biomarkers, and the development of tailored treatment strategies for infectious diseases.
- **Organo-Pathogen Interactions:** Investigating the interactions between organoids and pathogens in greater

detail can provide insights into the mechanisms of infection, host responses, and pathogen variability. High-resolution imaging and single-cell analysis.

- **Regulatory and Ethical Considerations**: As organoid technology progresses, addressing regulatory and ethical challenges surrounding its use becomes crucial.

Conclusion

This study successfully determined the chemo-profile of the aqueous extract of Nauclea latifolia using GC-MS analysis, identifying eighteen compounds with significant bioactivities such as anti-inflammatory, antibacterial, and antimalarial properties. The presence of compounds like 9-Octadecenoic acid, Oleic acid, and Benzamide highlights the potential of the leaf extract as a source of novel treatments. These findings support the traditional medicinal use of Nauclea latifolia and underscore its potential for future drug development. Further research is recommended to explore and validate the therapeutic applications of these bioactive compounds.

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