

Screening of *Colpomenia Sinousa* and *Iyengaria Phaeophycota* Against Multi Drug Resistance ABCB1

Marhaba

M.phil Biochemistry, Department of Biochemistry, Abdul Wali Khan University Mardan.

Email: Marhaba.khan07@gmail.com

Asma

Ph.d Scholar, Department Biochemistry, Abdul Wali khan university Mardan

Email asmatariq.bkuc@gmail.com

Dr. Roheena

Zafar, Director Pharmacy, Peshawar General Hospital, Phase V, Hayatabad, Peshawar, Pakistan.

Email: zroheena@gmail.com

Ali Usman (Corresponding Author)

PHD Biotechnology, Department of Biotechnology University of Central Punjab, Lahore. Email: ali.usman448@yahoo.com

ABSTRACT

All living things, including bacteria and human being, have ATP-binding cassette (ABC) proteins. An extensive class of proteins known as ABC transporters is responsible for transferring a variety of substrates across cell membranes. The modular structure of ABC transporters varies, and they can have any domain arrangement, ranging from complexes of four distinct single domain polypeptides to a single four domain polypeptide. Human P-glycoprotein (ABCB1 and P-gp) is a multi-specific drug, efflux transporters which mediate the resistance of cancer cells to cytotoxic drugs. Several synthetic inhibitors ABCB1 are failed in phase III clinical trials therefore there is an urgent need of new inhibitor from natural resource. In this regard current study was conducted to evaluate solvent-solvent extraction and phytochemicals from *Colpomenia sinousa* and *Iyengaria phaeophycota* against MDR ABCB1 transports. All samples were evaluated for antioxidant (DPPH) assay and MTT assay for the inhibition of ABCB1 transporter. Ethanol, ethyl acetate and methanol of *Colpomenia* shown the lower IC₅₀ value of 14.69±0.01µg/mL, 30.01± 0.25µg/mL and 48.02±3.20µg/mL respectively for antioxidant assay, the sample of *Iyengaria* also shown high inhibition with IC₅₀ of 16.80±0.01 µg/mL, 31.73±0.23µg/mL, 35.84±0.618µg/mL and 40.80±0.241µg/mL for ethanol, n-Hexane, methanol and ethyl acetate respectively. Further active samples were assessing in ABCB1 inhibition assays. The results from

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

the above methodology shown potent inhibition for ABCB1 transporters. ABC transporter shows the expression with IC_{50} $29.57 \pm 2.128 \mu\text{g/mL}$, $77.12 \pm 3.21 \mu\text{g/mL}$ and $45.33 \pm 0.032 \mu\text{g/mL}$ for Iyengeria ethanol, ethyl acetate and methanol respectively to attenuate accumulation of cellular and tissue drugs that increase MDR across a different type of cancers. One goal is to overcome MDR in order to increase patient survival.

Keywords— ABCB1/P-glycoprotein Inhibition, Multidrug Resistance (MDR) Reversal, Marine Algae Phytochemicals, Colpomenia sinousa, Iyengaria phaeophycota, Solvent-solvent extraction, Cytotoxicity (MTT assay), Antioxidant activity (DPPH assay), Natural MDR modulators, Cancer chemotherapy resistance

Introduction

ATP-binding cassette (ABC) proteins are found in all organisms, from bacteria to humans. Moving various kinds of substrates across cell membranes is carried out by a vast family of proteins known as ATP-binding cassette (ABC) transporters. (Burke, Mutharasan et al. 2008). Its members play roles in numerous metabolic processes in by releasing energy for substrate transport across membranes through hydrolysis of ATP. According to amino acid sequence and ultimately protein domains, the 49 known ABC genes in humans are divided into seven distinct families (A-G): ABCB (11 members), ABCC (11 members), ABCA (13 members), ABCD (4 members), ABCE (1 member), ABCF (3 members), and ABCG (5 members). Among these there are three multidrug resistances that include ABCB1, ABCC1 and ABCG2. The overexpression of these transporters exports a diverse variety of drugs, which finds it challenging to treat diseases including cancer, AIDS, and diabetes (Wilkins 2015). P-glycoprotein is the first ABC transporter identified by ABCB1 gene's in 1976 by Victor Ling and known to have an impact in clinical MDR development. Numerous findings made earlier in 1968, which led to the first isolation and characterization of MDR cell lines, served as the driven for this discovery, the drug efflux function was proved via a carrier-mediated extrusion mechanism (Juliano et al., 1976) (Sharom 2014). It is thought that ABCB1 has been evolved from a half transporter through gene duplication, resulting in the domain order TMD-NBD-TMD-NBD.

The substrates which ABC transporter proteins primarily transport involve peptides, metal ions, alkaloids, vitamins, inorganic molecules, sugars, amino acids, organic molecules, glutathione, and cell metabolites. ABC transporter proteins move substrates across membranes by interacting with ATP and hydrolyzing it to release energy. One or two ATP binding cassettes or nucleotidebinding domains(NBDs), which indicate 30 to 40% homology between family members, are the basis for the naming of ABC proteins (Verrier, Bird et al. 2008). Each NBD has three different sequence motifs and 200 amino acid residues. utilized a straightforward way of identifying ABC transporter proteins, such as those that simply include transmembrane domains or nucleotidebinding domains. While TMDs are highly heterogeneous and share the structure and functionality of NBDs across families, transporters are able to recognize a variety of substrates and use the energy from ATP hydrolysis to translocate molecules across membranes irrespective of the prevailing concentration gradient. Current research indicates that while baseline ATP hydrolysis generates a constantly changing

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

conformation that may facilitate MDR1 binding and transport of a broad range of substrates, while the energy from ATP can help translocate engaged substrates. The NBD, which is also called an ABC, is the signature feature of this transporter family. The ATP-Binding Cassette transporter (ABC), subfamily B, member 1 (ABCB1/P-glycoprotein/MDR1) and subfamily G, member 2 (BCRP/MXR/ABCP) are two of the most very much described drug carrier proteins that are solidly connected to the advancement of multidrug opposition (MDR) aggregate in disease. P-glycoprotein (P-gp) is a transmembrane efflux carrier with an expansive substrate explicitness that limits intracellular medication gathering and adds to a reduction of harmfulness. It is available in common tissues connected to excretory or obstruction capabilities, as well as in cancer cells, where it is liable for protection from an enormous assortment of chemotherapeutic medications; a peculiarity known as the multidrug opposition (MDR). The construction of this carrier comprises of two balanced and homologous parts that demonstration in an organized way as a unit, each with six transmembrane spaces (TMD) and a nucleotide restricting area (NBD) situated on the cytosolic surface liable for ATP restricting and hydrolysis. ABCB1 protein intercepts drugs at the level of the plasma membrane and effluxes them before they are able to reach their intracellular target structures. Both ABCB1 and ABCG2 are known to use energy got from ATP hydrolysis to effectively ship xenobiotics, including a different scope of remedial medications, across natural membranes. Thus, the overexpression of ABCB1 or ABC malignant growth cells could diminish the intracellular gathering of cytotoxic anticancer medications, for example, anthracyclines taxanes and Vinca alkaloids , causing therapy disappointment and backslide in patients with metastatic bosom disease (MBC), lung carcinoma, esophageal squamous cell carcinoma (ESCC) various myeloma (MM) ,intense lymphocytic leukemia (ALL), intense myelogenous leukemia (AML), and constant lymphocytic leukemia (CLL) .It is in this manner critical to foster ABCB1-and ABCG2tweaking specialists for clinical application (C. P. Wu et al., 2022).

P-glycoprotein (ABCB1), which is extensively overexpressed in cells and tissues, is crucial for cell detoxification. Its physiological relevance in drug transport across various blood-organ barriers, resulting in drug release, drug-drug interactions, and drug pharmacokinetics for many therapeutically relevant drugs, is supported by its broad substrate specificity. Due to its ability to bind transporters at various states of the catalytic cycle, U1C2 (a P-gp-specific antibody) has been successfully utilized to examine the transport cycle of human ABCB1 (ABCB1H). (Frank et al., 2016).

There are several mechanisms for chemotherapy failure, the major reason is the development of drug resistance by cancer cells (Mansoori et al.,2017). The extensive range of cross resistance developed during treatment with specific cytotoxic drugs is referred to as multidrug resistance (MDR). A prominent area of study is determining the mechanism of cancer treatment resistance. Some of the key indicators of ineffective chemotherapy are increased drug efflux, decreased drug intake, cell cycle abnormalities, changes in the drug target, and drug sequestration. ATP-binding cassette (ABC) transporter overexpression is responsible for all of these mechanisms. (Jeevitha Priya et al., 2022). The MDR peculiarity is expected to the hereditary and epigenetic cosmetics of the patients that reflects in the medication adequacy and pharmacokinetics.

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

A few components have been proposed for the clinical MDR that remembers modified drug take-up and efflux for the disease cells, expanded detoxification of the drug or decreased drug enactment, changed drug targets, expanded DNA fix against chemotherapeutic mixtures, modified apoptosis, initiation of supportive of endurance flagging, and initiation of cell endurance announcing development factors. The ABCB1 limit the restorative impact of chemotherapeutic medications in malignant growth cells. Overexpression of MDR, ABC transporters including ABCB1 efflux out chemotherapeutic medications from the disease cells and adds to clinical MDR system. The ABC-carriers cause low bioavailability of chemotherapeutic medications in the disease cells through drug efflux system to the detriment of ATP hydrolysis. Thusly, the ABC transporters intervened MDR has been viewed as a significant obstruction to disease chemotherapy (Ganesan et al., 2021).

ATP-binding cassette (ABC) efflux transporters are used in breast cancer chemotherapy resistance associated with multidrug resistance events to reduce drug intracellular concentration. In order to cause cell death, photodynamic therapy (PDT) combines a photosensitizer (PS), light, and molecular oxygen. Although its therapeutic effectiveness for chemo-resistance is still unknown, this therapy approach has been thought of as a potential strategy in the fight against the phenomena of multidrug resistance. The concurrent use of chemotherapy agents, nanotechnology, stopping drug release from lysosomes by photochemical internalization and the use of structurally related compound inhibitors to block the transport function of the multidrug resistant transporters were all suggested as ways to lessen the impact of efflux transporters on drug resistance.(Aniogo et al., 2019)

The ABCB1 transporter, commonly known as P-glycoprotein (P-gp), is a transmembrane protein that is a member of the ATP binding cassette super-family of transporters. It functions as a xenobiotic efflux pump, preventing the accumulation of drugs inside of cells. P-gp has a wide range of substrate specificity and helps to reduce toxicity. Due to the process known as multidrug resistance (MDR), which causes the chemotherapeutics to be removed from the targeted cells, it contributes to the failure of many anticancer and antiviral chemotherapies. Therefore, comprehension of the specifics of the ligand-P-gp interaction is essential for the creation of medications that may counteract the MRD phenomenon and for gaining a more accurate assessment of the toxicity of particular substances.(Lagares et al., 2020).

The inhibition of P-glycoprotein (P-gp) by low molecular weight molecules has important effects on both the treatment of cancer and the development of new drugs. P-gp, commonly referred to as multidrug resistance protein 1 (MDR1), is a membrane transporter protein that is essential in pumping a variety of chemicals, including many chemotherapeutic drugs, out of cells. Resistance to drugs in the treatment of cancer can be enhanced by this pumping activity, which can result in less drug accumulation within cells. (Chiba et al., 2006) P-gp can interface with huge quantities of fundamentally different mixtures, which as indicated by their collaborations can be named substrates, inhibitors and modulators. Compounds effectively transferred by P-gp are known as substrates, while those that compromise the moving capability of P-gp are known as inhibitors. Modulators interface with P-gp diminishing substrate restricting through a negative allosteric balance. Studies have been finished attempting to figure out the idea

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

of the P-gp capacity for restricting such countless various mixtures and to explain the qualities of the medication restricting pocket. A review made by means of photoaffinity marking of P-gp with azidopine showed that there are two different restricting locales for this drug (Lagares et al., 2020).

A few dynamic mixtures from organic sources have proactively perceived for their huge demonstrative impact. The utilization of any normal items for hindrance of P-gp is not new to the examination world and that has been used in real life for thirty years. Natural products, for example, oranges, citrus foods grown from the ground, for example, glycosides, flavonoids, phenolics and alkaloids are especially among those. Natural products are famous in their exceptionally low harmfulness and more noteworthy P-glycoprotein accuracy. Investigation into finding inhibitors for P-glycoprotein have turned into a focal point of attention for improvement of science, due to their exceptionally low harmfulness profile and higher explicitness (Fletcher et al., 2010).

The use of traditional medicine gained significant momentum over the past decades. Numerous forms of traditional medicine such as traditional Chinese medicine, Ayurveda and Unani medicine have also been used worldwide. In few countries, traditional medicine serves as the main source of health care because of its accessibility, affordability, cultural acceptability and trust by people. Up to four billion individuals (80% of the world's population) in developing countries rely on traditional medicine as the primary source of health care. Natural products are naturally occurring compounds that possess biological activities derived from abundant natural resources such as plants and sea weeds. Many currently used therapeutic drugs are derived from natural resources such as alkaloids, taxanes, and flavonoids. Recently, chemotherapy using natural compounds has gained increased attention to improve the therapeutic index of specific anti-neoplastic drugs. Moreover, cancer treatment by natural compounds, including phytochemicals, minerals, and vitamins, has shown promising results against various malignant tumors. In this regard, natural compounds with potent antitumor activity and minimum toxicity in healthy tissues have been suggested to study their synergistic efficacy with traditional anticancer drugs. Natural products have displayed selective benefits against cancer cells compared to normal cells, but their chemical structures also act as models for developing novel drugs. Natural products have displayed selective benefits against cancer cells compared to normal cells, but their chemical structures also act as models for developing novel drugs. These models formulate drugs with similar or better benefits than existing natural products with lower side effects and resistance (Hashem et al., 2022).

Early on, the conception of simultaneous administration of anticancer drugs with inhibitors of P-glycoprotein has been advocated as a concept for evading resistance (Khurana 2014). To find more potent ABC transporter inhibitors, natural products often provide fascinating scaffolds and serve as lead structures, as recently reviewed by Klepsch (Mohan et al., 2016).

One of the many marine resources that can be found in the ocean is seaweed. Seaweeds have long been recognized as important, renewable, All the natural sources of bioactive substances like sterols, halogenated compounds, proteins, carbohydrates, lipids, phenolic compounds, polysaccharides, and polyketides throughout the whole oceanic biota. Seaweeds are employed in the creation of fresh pharmaceuticals (Shobier et al.,

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

2023). Novel bioactive substances—defined as drugs that, when applied to living cells, have a biological interaction or impact depending on their use. To synthesis of new functional foods or develop cosmeceutical, nutraceutical, and pharmaceutical products, these seaweed-bound substances can be isolated and introduced into the food matrix. The various bioactivities previously explored are caused by the bioactive compounds coated by seaweeds. The impact of various bioactive compounds isolated from seaweeds on antioxidant, antimicrobial, antifungal, anti-inflammatory, anti-cholesterol, anti-neurodegenerative, anti-tumor, and prebiotic properties have been addressed in several types of studies and reviews (Freitas et al., 2023). While seaweed bioactive are still largely underutilized on an industrial and commercial scale, attempts are being made to promote the use of seaweeds in applications as food ingredients. It is important to note that every sea weed contains thousands of bioactive substance or phytochemicals of which only few are therapeutically qualities. In addition, phytochemicals yield depends on extraction methods. Most medicinal plants contain micronutrients amino acids and proteins, essential oils, sterols and triterpenoids, saponins, carotenoids, alkaloids, flavonoids, phenolic acids, tannins, bitter and coumarins. The therapeutic activity of these plants depends on the interaction of several classes of phytochemicals (Katiyar et al. 2015).

Seaweeds have become goals for searching of novel compounds that exhibit prospective medical values. The produced chemical composition varies between seaweeds according to the environmental conditions, habitats, species, and maturity. The main objective of the present study was screening and comparing the ability of different solvent seaweed extracts (*Iyengeria phaeophycota* and *Colpomenia sinuosa*) gathered from the Karachi coast, Pakistan, as potential inhibitors for ABCB1.

Objectives of the Study

Keeping in view that above mentioned facts the aims and objectives of this study were as follows:

1. To extract and quantify phytochemicals from *Colpomenia sinuosa* and *Iyengeria phaeophycota*.
2. To Screen solvent-solvent extracts against MDR-ABCB1 transporter using MTT assay.
3. To evaluate inhibitory potential of phytochemicals against ABCB1.

Literature review

In Multidrug resistance (MDR) the cancer cells stop responding to one or more chemotherapeutic medicines during or after the course of treatment. Chemotherapeutic resistance severely restricts the effectiveness of treatment and continues to be a major problem for clinicians. The primary cause of MDR is the variation in intracellular drug concentration brought on by the modification of the drug's transport through the plasma membrane, which is adopted by a number of mediators, including ATP-requiring enzymes (ATPases). Among these ATPases, ABC transporters have received the most attention and have been proven to have a significant role in carcinogenesis and MDR. Therefore, to alter P-gp- and BCRP-mediated drug transport, including retinoids, new structurally diverse P-gp and BCRP substrates are being searched after. comprehensive demonstrates the precise processes regulating interactions as well as their structural

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

regions. the inhibition of the ABC transporters may impact the pharmacokinetics of other co-administered chemotherapeutic drugs, more caution should be exercised when combining retinoids with other anti-cancer drugs to prevent drug-drug interactions at the level of the membrane transporters, P-gp and BCRP. With consideration to their effectiveness against cancer, the study examined how synthetic retinoids alter the expression and operation of efflux pumps and other drug transporters. As a result, it might be possible to use synthetic retinoids as chemosensitizers that precisely target transporters that encourage MDR, assisting in the fight against chemoresistance. (Abdelaal & Haffez, 2022).

ABCB1 transporter overexpression is a significant variable in mediating multidrug resistance (MDR). In order to maintain an effective intracellular level of chemotherapeutic medicines, it is crucial to block ABCB1 function. Tepotinib is a MET tyrosine kinase inhibitor that is now undergoing clinical studies and has the potential to treat cancer. In the study, we looked into whether tepotinib may inhibit MDR caused by ABC transporters. Tepotinib significantly reduced ABCB1-mediated MDR, but not ABCG2- or ABCC1-mediated MDR, according to the results. Tepotinib dramatically reduced ABCB1-mediated MDR by attenuating the ABCB1 transporter's efflux activity, according to mechanistic investigations. Tepotinib reduced ABCB1's ATPase activity in a concentration-dependent manner, based on the results of the ATPase experiment. Tepotinib therapy did not alter the expression of ABCB1's protein or its subcellular location. Tepotinib interacted with the ABCB1 transporter's drug-binding region. Additionally, the group also perform docking using tepotinid to map drug binding interaction tepotinib interact with the ABCB1 drugbinding domain obtained a high docking score of -14.343kcal/mol. The high affinity between tepotinib and ABCB1 drug-binding pocket may be due to the hydrophobic interaction with multiple ABCB1 residues.(Z. X. Wu et al., 2019)

The overexpression of ABC transporters, which are involved in drug efflux, is linked to the MDR phenotype. The epithelial-mesenchymal transition (EMT), in which cells take on a migrating phenotype and invade other tissues, leads to metastasis. Although cells undergoing EMT show increased ABCB1 expression, the function of ABC transporters during EMT is yet unclear. After TGF-induced EMT in A549 cells, enhanced in ABCB1 expression is absorbed but no change in activity. Furthermore, ABCB1 overexpression caused by verapamil's ABCB1 inhibition increased the expression of snail and fibronectin, indicating parallel cell signaling pathways leading to the transcription of ABCB1 and EMT-related markers rather than a direct effect of transport.(da Costa et al., 2023)

An extensive analysis of the literature suggests that scaffolds have inhibitory effects, mainly on P-gp, BCRP, and MRP1, as well as on a variety of additional pathways involving the phosphorylation of transport proteins or the expression of MDR1 genes (Liew, L. et al., 2011). Given that synthetic inhibitors have a high level of toxicity. Therefore, finding more effective and benign naturally occurring P-gp inhibitors could be a desired goal for researchers who develop novel processes and methodologies for creating new inhibitors from plant sources. Natural bioactive substances have modifying effects on P-gp when compared to synthetic medicines, according to the research by Saikat Dewanjee et al. 2017. The historical backdrop shows that it is still

Online ISSN

3007-3197

Print ISSN

3007-3189

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crucial to support the development of pharmaceuticals derived from natural sources and the development of innovative P-gp inhibitors using a variety of medicinal plants that are less toxic and more effective (Dewanjee et al., 2017). MDR transporters obstruct the therapy of cancer, diabetes, and AIDS by exporting drugs from cells. Therefore, it is essential to check whether new compounds can inhibit these MDR transporters.

Acetogenins from the plant *Annona muricata* are screened by M. Jeevitha Priya et al., 2020 to identify a powerful MDR inhibitor. anonaceous acetogenins (AGEs) extensively inhibit NADH oxidase of cancer cells by performing anticancer activity. A total number of 24 AGEs have been selected and their pharmacokinetic features were studied. The human P-gp protein was modelled because an inward facing conformation of P-gp is essential to understand the interaction of AGEs at the drug binding site. The selected compounds were then docked with the modelled human P-gp's ATP binding site and drug binding site. When compared to conventional drug, Annonacin A.1, Annohexocin.1, and Annomuricin E.1 docked better with higher MM/GBSA dG binding. When compared to control inhibitor drugs, these substances had a higher docking score at the ATP binding site. Annonacin A might compete with chemotherapy medicines for binding to the Pgp drug-binding area. As a result, it has the ability to reduce the efflux of chemotherapy drugs out of cells by P-Glycoprotein/ABCB1/MDR1. according to computational study, it is concluded that this compound might potentially reverse MDR (Jeevitha Priya et al., 2022).

Yuchen Zhang et al in 2022 conducted research on the treatment of non-small cell lung cancer (NSCLC). Adagrasib (MRTX849) is currently being studied in clinical trials as a targeted inhibitor for the KRAS G12C mutation in non-small cell lung cancer (NSCLC) treatment. MRTX849 was found to both in vitro and in vivo increase the anticancer efficacy of ABCB1 substrate drugs in transporter-overexpressing cells. The MDR reversal effect was specific to ABCB1, as neither the parental sensitive cells nor the ABCG2-mediated MDR cells showed any evidence of a similar effect. In ABCB1 overexpression MDR cells, MRTX849 enhanced cellular accumulation of ABCB1 substrates such as doxorubicin (Dox) and rhodamine 123 (Rho123) by inhibiting ABCB1 efflux activity. Additionally, in a concentration-dependent manner, MRTX849 enhanced ABCB1 ATPase activity and competed with [125I]-IAAP for the photolabeling of ABCB1. However, MRTX849 did not cause any changes in the levels of ABCB1 expression or the phosphorylation of AKT/ERK at the concentrations needed to effectively reverse multi-drug resistance (MDR) (Y. Zhang et al., 2022).

Although docetaxel (DCT) is frequently used in clinical practice, but the clinical value has been restricted due to its drug resistance of breast cancer patients. Traditional Chinese medicine called chan'su is mostly used to treat breast cancer. There is limited research on reversing drug resistance in breast cancer, however bufalin (BUF), a bioactive polyhydroxy steroid isolated from chan'su, exhibits potent anticancer action. The purpose of this study is to find out if BUF can reverse DCT drug resistance and reestablish efficacy in breast cancer. Drug-resistant cell lines sensitivity to DCT was improved with the addition of BUF. BUF can decrease ATPase function, inhibit ABCB1 protein expression, and increase DCT drug accumulation in drugresistant strains (D. Zhang et al., 2023).

The development of pharmaceuticals that can reduce a specific aspect of chemo-

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

resistance may aided by an extensive knowledge of the function and kinetics of ATP-binding cassette (ABC) transporters. Qian et al., 2019 studies the function of the ABC transporter-mediated intracellular drug efflux using bioluminescence imaging. Additionally, the authors investigated the intracellular bioluminescent pharmacokinetic profiles and the anti-tumor potency of coix seed extract and gemcitabine against pancreatic cancer cells in vitro and in vivo were associated. The pharmacodynamic index (TGI) and bioluminescent pharmacokinetic parameters (IC50) were calculated. ABCB1 and ABCG2 expression levels were evaluated. The results demonstrated that coix seed extract might synergistically improve gemcitabine's anti-cancer activity (p 0.05). It was shown by Western blot and immunohistochemical assay that coix seed extract could considerably reduce the overexpression of ABCB1 and ABCG2 protein caused by gemcitabine. In conclusion, coix seed extract may improve the effectiveness of gemcitabine therapy in pancreatic cancer cells at least partly via altering ABC transporter-mediated drug efflux activity(Qian et al., 2019).

Anna Palko-Labuz studied two cellular models to check the potential of Isobavachalcone (IBC) which is the active mass derived from medicinal plant *Psoralea corylifolia*. Two cell lines, the human colorectal cancer HT29 cell line and its resistant counterpart HT29/Dx, in which doxorubicin resistance was brought on by protracted drug use, and a kind of MDCK cells transfected with the human ABCB1 gene,were used to study the cytotoxic, MDR reversing, and ABCB1-inhibiting potency of isobavachalcone. The interaction of isobavachalcone with model phosphatidylcholine bilayers was investigated using differential scanning calorimetry because

MDR modulators are commonly membrane-active compounds. To describe how isobavachalcone penetrates membranes, molecular modelling was used. IBC, which is an ABCB1 substrate and/or competitive inhibitor, interacted with the ABCB1 transporter. Furthermore, when IBC is added to model membranes, it strongly influences how the main transition of their phospholipids takes place. isobavachalcone can impact the cell's outer layer and a transporter called ABCB1. This might make isobavachalcone useful for treating cancer cells that are challenging(Palko-Labuz et al., 2021).

A plant alkaloid known as stemofoline was isolated from the *Stemona* species, found in Northern Australasia and Eastern and Southeastern Asia. The extract from *Stemona*'s tuberous roots is used to treat various insect pests and respiratory diseases like bronchitis and pulmonary tuberculosis. In the study how stemofoline affected P-gp activity in an attempt to effectt P-gpmediated MDR was examined. The study demonstrates stemofoline's impact on P-gp activity with the goal of preventing P-gp-mediated MDR. P-gp-mediated ATP hydrolysis was accelerated by stemofoline (conc. required for 50% stimulation = 10-12 micro M). Stemofoline also failed to alter the Km for ATP, indicating that this alkaloid may interact with the P-gp drug-binding site. Stemofoline inhibited the photo-crosslinking of P-gp with [125I]-iodoarylazidoprazosin (IAAP; IC50 = 7 micro M), a transport substrate for P-gp. Stemofoline is an interesting medicine because, unlike verapamil and vinblastine, it increased ATPase activity rather than inhibited substrate-stimulated ATP hydrolysis. This suggests that stemofoline and these pharmaceuticals (verapamil and vinblastine) bind to P-gp in the drug-binding pocket at same time. Stemofoline was not cytotoxic to

Online ISSN

3007-3197

Print ISSN

3007-3189

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P-gp expressing KB-V1 or parental KB-3-1 cells up to 100 micro M concentration, but this compound at non-toxic concentration (1 – 5 micro M) sensitized KB-V1 cells to vinblastine. Stemofoline does not appear to interact with ABCG2 transporter, which is also linked to development of MDR(Ohnuma et al., 2013) .

Citrus methoxyflavone nobiletin potentially increased the resistance of ABCB1 overexpressing cells A2780/T and A549/T to chemotherapeutic drugs such doxorubicin (DOX), paclitaxel (a 433-fold reversal of MDR to PTX at 9 M), docetaxel, and dounorubicin. Nobiletin considerably enhanced the intracellular accumulation of DOX and Flutax-2 in A2780/T cells and lowered the efflux of ABCB1 substrates in Caco2 cells without changing the mRNA or protein expression of ABCB1, which indicates that it drastically inhibited ABCB1 transporter activity. Additionally, in a concentration-dependent way, nobiletin enhanced ATPase activity and reduced verapamilinduced ATPase activity, showing a direct interaction with the transporter. According to their molecular docking research, nobiletin binds well to the transmembrane region site 1 of the homology modelled human ABCB1 transporter (Ma et al., 2015).

A natural substance called tetrandrine is obtained from the stem of *Tinospora crispa*. Tetrandrine exhibited synergistic cytotoxic action in the investigation when used in mixed with chemotherapeutic drugs. In KB-C2 cells, tetrandrine boosted P-glycoprotein ATPase activity, reduced [3H]-Paclitaxel efflux, and increased the intracellular accumulation of [3H]-Paclitaxel. Additionally, P-glycoprotein expression was lowered in SW620/Ad300 and KB-C2 cells after a 72-hour pretreatment with 1 M tetrandrine without affecting its cellular position. Western blotting and immunofluorescence analyses were used to prove. Interestingly, there was no correlation between the down-regulation of P-glycoprotein expression and gene transcription in SW620/Ad300 and KB-C2 cells at the 0, 24, and 72 h treatment time points for MDR1 mRNA levels. Tetrandrine was anticipated to have inhibitory potential with the ABCB1 transporter by molecular docking study as well. which suggested that tetrandrine can inhibit MDR in cancer cells that have been drug-selected or transfected with the MDR1 gene by suppressing the expression of the ABCB1 transporter and then raising the intracellular concentration of chemotherapeutic drugs. Tetrandrine and other anticancer medications may be used in combination therapy to increase the effectiveness of ABCB1 substrate drug treatments (Liao et al., 2019).

Methodology

Sample collection

The sea weeds *Iyengeria* and *Colpomenia* were kindly received from professor Dr. Humaira Gull department of Botony Abdul Wali khan University Mardan. The samples were then identified by taxonomist.

Online ISSN

3007-3197

Print ISSN

3007-3189

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a)

b)

Figure 3.1: a) *Colpomenia sinuous* b) *Iyengeria phaeophycota*

Sample Preparation

Sea weeds were shadow dried at room temperature. The dried sample was ground to a fine powder using electric blender and stored at 4°C for further analysis. Powdered sample were extracted for 24 hours at 37°C using a mixture of organic solvents (1:10) based on polarity. Following that, the samples were filtered and again dissolved in DMSO for phytochemical analysis and ABCB1 inhibition using the MTT assay.

Solvent-Solvent Extraction

Polarity based solvent- solvent extraction was carried out using the modified method of Lapornik et al., 2005 .10g of *Colpomenia* and *Iyengaria* were weight and soaked in 100 mL of n-hexane for 24hrs in shaking incubator at 37 °C. After 48hrs the extract was centrifuged followed by filtration. The supernatant was collected in free-weight beaker and store at 4°C, pallet was redissolved in slightly more polar solvent chloroform.100ml of chloroform add to each sample, again the samples were centrifuged followed by filtration using the conditions mentioned above. The supernatant containing chloroform were collected in a free-weight beaker and store at 4°C. The pallet was again re-dissolved in 100ml of ethyl acetate the same condition was applied as mentioned above. The supernatant was separated in a free-weight beaker with the help of filter paper after centrifugation. The pallet was again dissolved in more polar solvent 100ml ethanol followed the same condition. the supernatant was filtered in free-weight beaker and the pallet again re-dissolved in 100ml methanol the same conditions were followed and the supernatant obtained after centrifugation were filtered in a weighted beaker and stored in refrigerator. The pallet was dissolved in the most polar solvent of 100ml distilled water and then placed it in a shaker incubator for 48 hours at room temperature, the supernatant was filtered off. The residues were then discarded and the supernatant were stored.

Phytochemical extraction and quantification

Quantification of Flavonoids

Determination of flavonoids was carried out using modified method of Barros, L. *et al.*, 2008. A 5 g of sample was dissolved in 50 mL of 80% aqueous ethanol to extract flavonoids. (at ratio of 1g: 10mL). The mixture was kept in a shaker incubator for 24

hours at 37°C. The extract was filtered after shaking. The pellet was removed, and the flavonoids-rich filtrate was kept at 4 °C.

The sample (500 µl) was added to 3 mL of distilled water and 75 µl of 5% NaNO₂ solution. A 150 µl of a 10% AlCl₃.H₂O was added after 5 minutes and subsequently, after 6 minutes 500 µl of 1 M NaOH and 275 µl of distilled water were mixed in the solution. The blank containing 80% aqueous ethyl alcohol was also prepared in a similar way. A spectrophotometer was used to measure the solution's absorbance at 415 nm after it had been thoroughly shaken. The absorbance values were quantified in triplicate. Quercetin was used as a standard for concentration ranging from 7.81 µg/ml to 1000 µg/ml (Barros *et al.*, 2008).

Quantification of Phenols

Total phenol was determined through soxhlet extraction method of Leonardo Satrio Putra *et al.* 2022. Then, de-fatted phenols were quantified using the calorimetric assay procedure published by Barros, L. *et al.*, 2008. A 1g of the tasted sample dissolved in 25mL of diethyl ether for 15 minutes in a water bath to extract the total phenol. A 500 µL of sample mixed with 1 µL of FolinCiocalteu reagent after 15 minutes. of saturated 20% sodium carbonate Na₂CO₃ saturated solution 20% in amount of 500 µL was introduced after 3 minutes, the total volume was adjusted to 3.5mL by adding distilled water. For 90 minutes the solution was then kept in dark. The solution's absorbance was analyzed using a spectrophotometer at a wavelength of 725 nm. Different concentrations of Gallic acid (7.81 µg/ml - 1000 µg/ml) were employed to produce phenols standard curves. The absorbance values were quantified in triplicate (Barros *et al.*, 2008).

Quantification of Tannins

Tannins were extracted using the standard procedure of Akindahunsi, A. A. and Oyetayo, F. L., 2006 by dissolving 1g of sample in 200ml of 70% acetone at a ratio of 1g in 200mL. For 24hrs the mixture was left in shaking Incubator, at 4°C the extract was then stored. The initial stock solution prepared for tannic acid was 50mg /100ml of 70% acetone. the standard stock solution of tannic acid was prepared by serial dilution ranging from 0.39mg to 50mg of different concentrations from stock solution, then 250 µl of folin and 1.25mL of 20% Na₂CO₃ were added. The blank was prepared containing 1mL of DMSO and the reagents as mentioned above. The absorbance were quantified at 725 nm on spectrophotometer (Akindahunsi and Oyetayo, 2006).

Quantification of Alkaloid

Using acid base methodology alkaloids were isolated and quantified (Edeoga *et al.* 2007). 10ml ethanol were added to 1g of sample and the mixture was shaken in incubator at 37°C for 24 hrs. After the incubation, the solution underwent filtration, and the obtained extract was preserved for further use. Next, the extract was redissolved by introducing the solution of 1% HCl in ethanol. the mixture was then refrigerated for 3 days. 1M NaOH was used to maintain the Ph from 8 to 10 of the filtered solution. Chloroform was added to the PH maintained ethanol in separate funnel. Chloroform layer contains alkaloid because chloroform is immiscible with water. chloroform layer containing alkaloid was preserved in pre-weighed beaker and the ethanol layer was discarded. the alkaloid content was determined using initial and final weights.

ABCB1 inhibition Using Thiazolyl Blue Tetrazolium Bromide (MTT) Assay Cell Culture

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Prof. Dr. Volker Gekeler provided the P-glycoprotein over-expressing cell line CCRF-CEM VCR1000 (Gekeler et al., 1992). The Cell line was maintained active using 10% FBS (Cat. No. 10082139) and Penstrep (Cat. No. 15070063) in RPMI1640 medium (Gibco, Cat. No. 11875093). After that, the cells were put in a CO₂ incubator set to 37°C. Cells were counted using a hemocytometer cell counter. The entire procedure was carried out in a laminar flow hood under sterile conditions.

Cytotoxicity Assay (MTT Assay)

A Cytotoxicity assay was performed for solvent-solvent extracts and phytochemicals. for MTT assay 96-well plates containing 5000–10,000 cells each were used to culture the cells. A range of sample concentrations of 0.7µg/ml to 50µg/ml were added, then the mixture was incubated overnight at 37 °C with 5% CO₂. On next day, MTT of 20µl at the concentration of 5mg /ml was added to each well and left for 24hrs in incubator. In order to dissolve the blue color, 100 µL of DMSO was poured in to each well and let to stand for 20 minutes. On the ELISA, the absorbance was measured at 450 nm and 520 nm.

Radical Scavenging DPPH Assay

The antioxidant activity of solvent-solvent extracts were assists against 2,2-diphenyl-1picrylhydrazyl hydrate following the method derived by Brand *et al.*, (Brand-Williams et al., 1995). The solution contains 100 µL of the extract of sea weed with concentration of 30 to 1000 µg/mL in range and 200µL of 0.1 mM DPPH solution. L-Ascorbic acid as standard solution range from 1 to 100 µg/mL was also prepared. The blank was prepared by mixing 1mL methanol with 2 mL DPPH solution. the scavenging activity of each sea weed extract is shown by incubating the reaction mixture for 5 min at room temperature and place in the dark. Absorbance measured at 517 nm using UV-Vis spectrophotometer. To calculate IC₅₀ values the doseresponse curve was generated. The procedure was conducted in duplicate.

Statistical Analysis

Statistically analysis was performed using Graph pad Prism9. Standard curve was plotted for the quantification of phytochemicals dose-response curve were generated using Non-linear regression analysis to calculate IC₅₀ values, which was the mean of two separate experiments. The Graph were plot with absorbance on the Y- axis and concentration on the X- axis using the log inhibitor. IC₅₀ values of the data was defined as mean ± Standard error.

Results

Result of solvent-solvent extraction

Polarity based solvent-solvent extraction solutions were prepared from low polarity to highly polar solvent of Colpomenia and Iyengeria as shown in Table 4.1 and 4.2. **Table 4.1:** Polarity based Solvent-solvent extraction of Colpomenia

S.No	Solvent	Colpomenia initial weight in g	Colpomenia final weight in g	Net weight in g	Stock solution 15mg/ml in DMSO
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1	N-Hexane	56.8343	56.8479	0.0139	0.92ml
2	Chloroform	55.762	55.7822	0.0202	1.34ml
3	Ethyl-acetate	62.7982	62.7996	0.0014	0.093ml
4	Ethanol	70.7881	71.1521	0.322	22.13ml
5	Methanol	152.899	153.133	0.234	15.6ml
6	Distilled Water	55.7894	56.7048	0.1964	13.0ml

Table 4.2: Polarity based Solvent-solvent extraction of *Iyengeria*

S.No	Solvent	<i>Iyengeria</i> Initial weight in g	<i>Iyengeria</i> Final weight in g	Net weight in g	Stock solution 15mg/ml in DMSO
1	N-Hexane	51.8931	51.892	0.0011	0.073ml
2	Chloroform	27.748	27.532	0.0212	1.413ml
3	Ethyl-acetate	98.686	98.706	0.0208	1.386ml
4	Ethanol	55.7968	55.9247	0.1279	8.52ml
5	Methanol	56.7968	57.4836	0.686	45.7ml
6	Distilled Water	55.803	56.482	0.6791	45.72ml

Bioactive Compounds Determination

Phytochemicals from *Colpomenia sinousa* and *Iyengeria phaeophycota* were extracted and measured in the first phase of examinations. The data showed that phytochemicals like flavonoids, phenols, tannins, and alkaloids were present. Sections 4 have discussed the results of the phytochemical analysis.

Result of Flavonoids Determination

The spectrophotometric approach was used to identify flavonoids. Quercetin concentrations ranging from 7.81 g/ml to 1000 g/ml have been used to generate a standard curve. The results are displayed in Figure 4.1.1. On the Y-axis, absorption was plotted, and the X-axis represented concentration. The figures were calculated in triplicate.

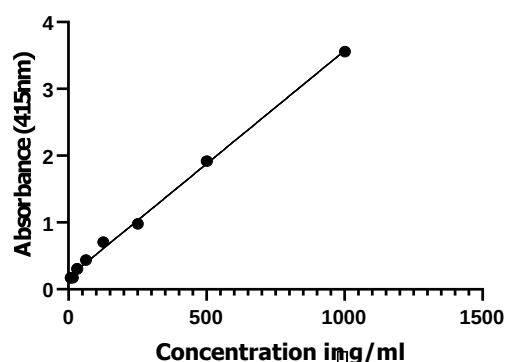


Figure 4.1: Standard curve for quantifying flavonoids. To generate a standard curve for the measurement of flavonoids, quercetin values ranging from 7.81 to 1000 µg/ml

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were employed. The x-axis represents concentration, while the y-axis represents absorbance, which was determined at 415 nm. We measured each value in triplicate.

Result for Phenols Determination

Phenols were measured at various gallic acid concentrations (7.81 µg/mL to 1000 µg/mL). The results are shown in Figure 4.2. At 725 nm, the absorbance and concentration were plotted along opposing axes. These values were examined in triplicate.

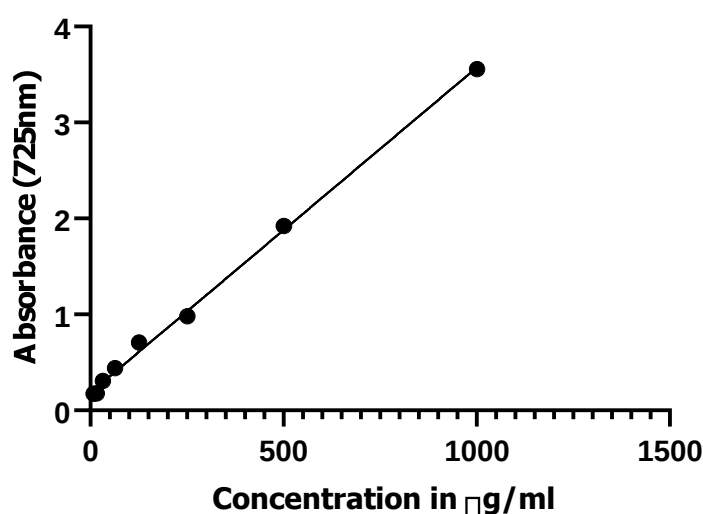


Figure. 4.2 Standard curve for quantification of phenols. Gallic acid was used in a range of concentrations 7.81 µg/ml to 1000 µg/ml to generate a standard curve for the determination of phenol. The x-axis represents formulation concentration, and the y-axis represents absorbance. At 725nm, absorbance was measured. We measured each value triplicate.

Result for Tannins Determination

Figure 4.3 displays the conventional tannin curve at various tannic acid concentrations 7.81 g/mL to 1000 g/mL. The X-axis was used to represent concentration, while the Y-axis was used to quantify absorbance at 725 nm. Each value were measured in triplicate.

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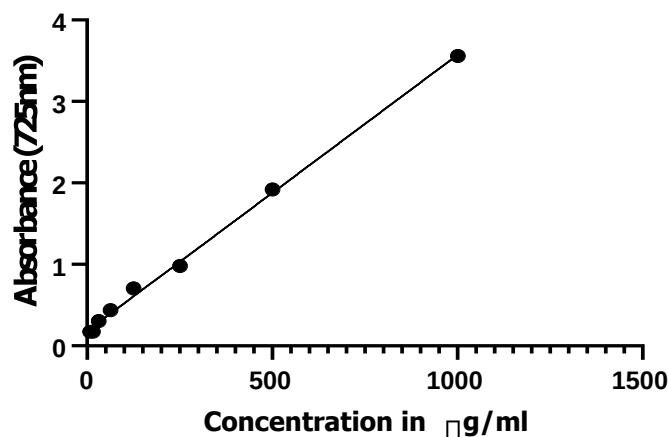
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Figure 4.3: Standard curve for Tannins Quantification. Tannic acid was used in various concentration range from 7.81 µg/ml to 1000 µg/ml to generate a standard curve for determining tannins. The absorbance is plotted on the y-axis, and the formulation concentration is plotted on the x-axis. At 725nm, absorbance was measured. We measured each value at least three times.

Table 4.3: Concentration of flavonoids, phenols and tannins in *Iyengeria* and *Colpomenia*.

S. No	Samples formulation	Concentration (µg/mL) Mean±SD of <i>Iyengeria</i>	Concentration (µg/mL) Mean±SD of <i>Colpomenia</i>
1	Flavonoids	701.64±146.11 µg/ ml	694.164±126.79 µg/ ml
2	Phenols	286.97±1.92µg/ ml	390.26±18.8µg/ ml
3	Tannins	513.79±110.45µg/mL	421.29±104.19µg/ ml
4	Alkaloid	0.0218µg/ml	0.0113 µg/ml

Results for Antioxidant activity

Free radical scavenging activity for Solvent-Solvent extraction

The Antioxidant activities for solvent-solvent extraction were assessed by DPPH scavenging assay. For generating a dose response curve, a variety of concentrations have been assessed. Figure 4.4 shows that activity was found to rise as concentration did. On the basis of IC_{50} values, a comparison was done. the results showed that the IC_{50} values of several sea weeds varied widely and ranged from 16.80 ± 0.01 µg/mL to 545.1 ± 42.062 µg/mL. the highest antioxidant activity is shown by *Iyengeria* and *Colpomenia* ethanol with IC_{50} values of 16.80 ± 0.01 µg/mL and 14.69 ± 0.01 µg/mL, respectively in the assay. *Colpomenia* distilled water shows the least antioxidant activity with IC_{50} value 545.1 ± 42.062 µg/mL gave high IC_{50} values indicating less antioxidant potential. the formulations were then compared, both were found to be effective in scavenging free radicals as compared to their respect to each sample. thus, showing again the

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synergistic effect of each sample. ascorbic acid was used as standard showed IC_{50} value $20.8 \pm 2.0 \mu\text{g/mL}$.

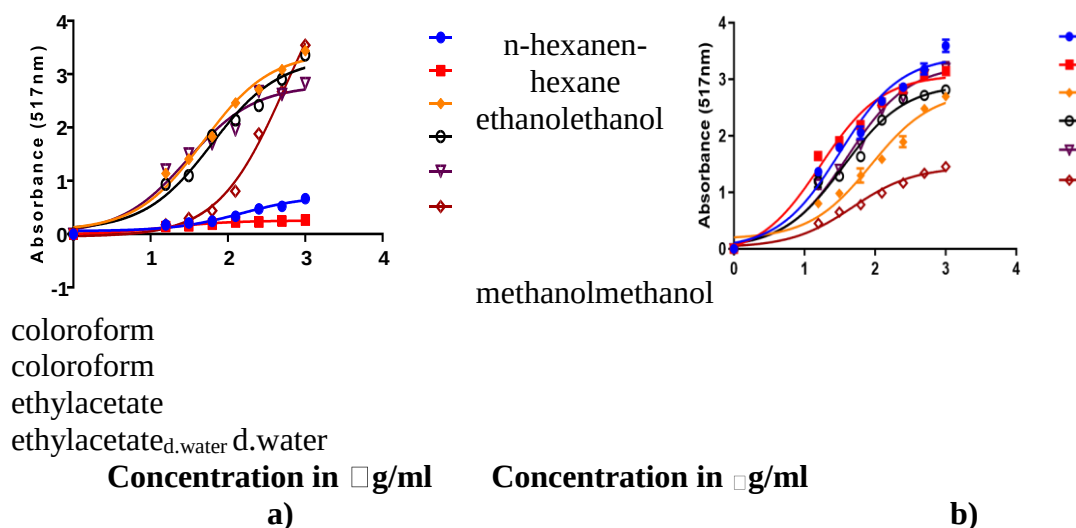


Figure 4.4: Result of Antioxidant activity. The antioxidant activity of samples at various concentrations between $15.75 \mu\text{g/mL}$ to $1000 \mu\text{g/mL}$ was assessed. Spots stand for data points, and the curve depicted the activity trend. Log-inhibitor concentration and absorbance were plotted on a graph using a graphpad prism. a) *Colpomenia* solvent-solvent extraction activity graphs for antioxidants. b) *Iyengeria* solvent-solvent extraction activity graphs for antioxidant assay.

Table 4.4: IC_{50} value for antioxidant activity of solvent-solvent extraction

S. No	Samples formulation	Concentration($\mu\text{g/mL}$) $IC_{50} \pm SD$ of <i>Colpomenia</i>	Concentration ($\mu\text{g/mL}$) $IC_{50} \pm SD$ of <i>Iyengeria</i>
1	N-Hexane	$148.6 \pm 1.27 \mu\text{g/mL}$	$31.73 \pm 0.23 \mu\text{g/mL}$
2	Ethanol	$14.69 \pm 0.01 \mu\text{g/mL}$	$16.80 \pm 0.01 \mu\text{g/mL}$
3	Methanol	$48.02 \pm 3.20 \mu\text{g/mL}$	$87.25 \pm 0.713 \mu\text{g/mL}$
4	Chloroform	$61.83 \pm 1.02 \mu\text{g/mL}$	$35.84 \pm 0.618 \mu\text{g/mL}$
5	Ethyl acetate	$30.01 \pm 0.25 \mu\text{g/mL}$	$40.80 \pm 0.241 \mu\text{g/mL}$
6	Distilled water	$545.1 \pm 42.062 \mu\text{g/mL}$	$47.97 \pm 0.312 \mu\text{g/mL}$

Free radical scavenging activity for Phytochemicals

Antioxidant activity of flavonoids, phenols, tannins, and alkaloids for poly-herbal formulation was examined in this set of experiment. A 517 nm UV spectrophotometer was used to measure the absorbance, and the IC_{50} values were then calculated for each sample concentration. Figures 4.5 show the results for this series of the formulation sample. These graphs showed that the level of activity varies. The greatest comparison, nevertheless, was done using the IC_{50} values. according to the experiment the

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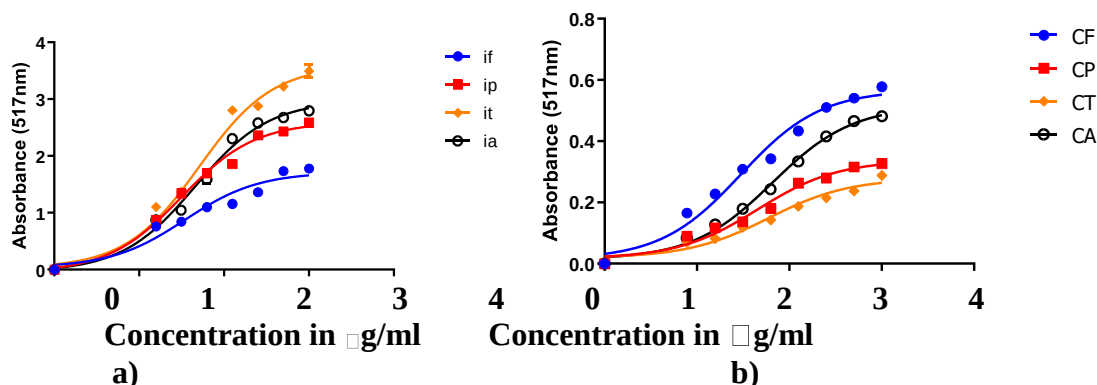
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Figure 4.5: Result for antioxidant activity of phytochemicals. The antioxidant activity of samples at various concentration 7.81 g/ml to 1000 g/ml was assessed. Spots stand for data points, and the curve depicted the activity trend. Log-inhibitor concentration and absorbance were plotted on a graph using a graph pad prism. a) Graph showing the antioxidant activity of *Iyengeria* flavonoids, phenol, tannin, and alkaloid extract. b) Graph showing the antioxidant activity of *Colpomenia* flavonoids, phenol, tannin, and alkaloid extract.

Table 4.5: IC₅₀ value for DPPH activity of phytochemicals.

S.No	Phytochemical	Concentration (µg/mL) IC ₅₀ ±SD of <i>Iyengeria</i>	Concentration (µg/mL) IC ₅₀ ±SD of <i>Colpomenia</i>
1	Flavonoids	34.56 ±1.543µg/ mL	29.73±0.53µg/mL
2	Phenols	31.47±2.487µg/mL	45.28±0.31µg/mL
3	Tannins	50.9±3.35µg/mL	59.42±1.52µg/mL
4	Alkaloid	45.92±2.83µg/mL	65.51±0.498µg/mL

Result of ABCB1 inhibition Assay (MTT Assay)

MTT assay has been used to analyze the cytotoxic effects in ABCB1 overexpressing cell line in order to determine the inhibitory capacity of polyherbal formulations against ABCB1. Figure 3.8 displays the results of the cytotoxicity assay for solvent-solvent extraction and phytochemicals. The log inhibitor concentration on the X-axis and the absorbance on the Y-axis were used to create the dose response curve. nonlinear regression analysis was used to determine IC₅₀ values. Data shows potential inhibition of CCRF CEM VCR1000 overexpressing cells expression showing IC₅₀ values of 29.57 ±2.128 µg/mL, 77.12±11.128 µg/mL, and 104.1±8.642 µg/mL for *Iyengeria* methanol, ethyl acetate and d.water respectively. Moreover, *Colpomenia* shows the highest IC₅₀ value 13165±68.762 µg/mL.

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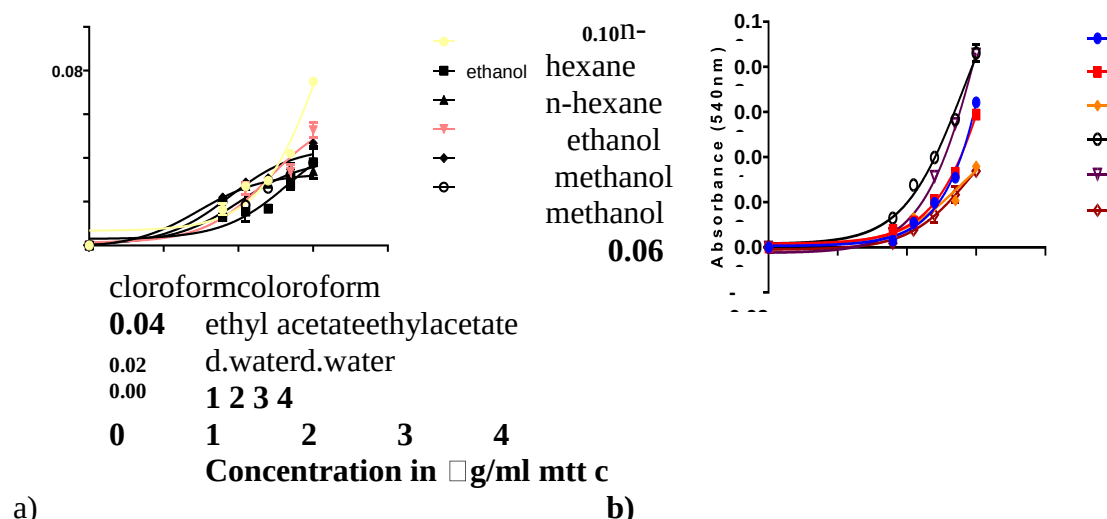
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Figure 4.6: Cytotoxicity of solvent-solvent extracts of sea weeds against ABCB1 overexpressing cell line using MTT assay. the concentration 61.5-1000 µg/mL in range was used. For cell line inhibition Dose response curve was generated to calculate IC₅₀ values in graph pad prism. a) Graphs for MTT assay of *Iyengeria* solvent-solvent extraction. b) Graphs for MTT assay of *Colpomenia* solvent-solvent extraction. **Table 4.6:** IC₅₀ value for MTT of solvent-solvent extraction

S. No	Samples formulations	Concentration (µg/mL) IC ₅₀ ±SD of <i>Colpomenia</i>	Concentration (µg/mL) IC ₅₀ ±SD of <i>Iyengeria</i>
1	N-Hexane	13156±68.762 µg/mL	1539±31.8 µg/mL
2	Ethanol	2424 ±38.31 µg/mL	460.8±21.79 µg/mL
3	Methanol	562.3±28.8 µg/mL	29.57 ±2.128 µg/mL
4	Chloroform	653.2±11.614 µg/mL	257.0±42.601 µg/mL
5	Ethyl acetate	1414± 7.34 µg/mL	77.12±3.21 µg/mL
6	Distilled water	798.5±14.63 µg/mL	104.1±8.642 µg/mL

ABCB1 inhibition of Phytochemicals

The cytotoxicity assay was carried out for flavonoids, phenols, tannins, and alkaloids. the IC₅₀ values were then calculated for each sample concentration in the GraphPad prism. Figures 4.9 show the results where dose-response curve was generated by plotting concentration of log inhibitor in X-axis and on wavelength of 540nm absorption was plotted on Y-axis. Different concentration of flavonoid, phenol, tannin and alkaloid were used for the inhibition against CCRF-CEM VCR₁₀₀₀ cell line. These graphs showed that the level of activity varies. According to the experiment the *Iyengeria* have high flavonoid content than *Colpomenia* with IC₅₀ value of 45.33±0.032 µg/mL and 845.7±42.7 µg/mL. The data reveal *Iyengeria* have the highest phenolic formulation. The value was found to be 51.64±3.814 µg/ml. the tannin content of *Iyengeria* was 366.0±9.307 µg/mL and *Colpomenia* is 105.9±5.288 µg/mL. IC₅₀ of alkaloid for *Iyengeria* was 663.3±11.624 µg/mL and for *Colpomenia* are 748.4±28.22 µg/ml.

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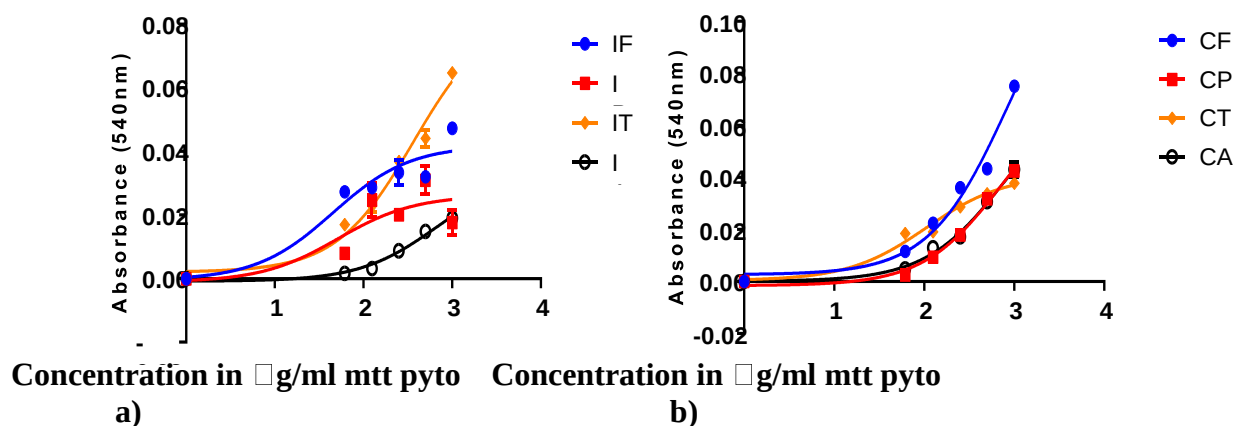
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Figure 4.7: Result for cytotoxicity assay of phytochemicals. For the MTT experiment, sample concentrations ranging from 61.5 g/ml to 1000 g/ml were examined. Spots stand for data points, and the curve indicated the activity trend. Graphs were generated by log-inhibitor concentration on x-axis and absorbance on y-axis plottation in graphpad prism. a) Graph for MTT assay for flavonoids, phenol, tannin and alkaloid extract of *Iyengeria*. b) Graphs for MTT assay for flavonoids, phenol, tannin and alkaloid extract of *Colpomenia*.

Table 4.7: IC₅₀ value of MTT assay of the phytochemicals.

S.N	Phytochemical	Concentration (µg/mL) IC ₅₀ ±SD of <i>Colpomenia</i>	Concentration (µg/mL) IC ₅₀ ±SD of <i>Iyengeria</i>
1	Flavonoids	845.7±42.7µg/ ml	45.33±0.032µg/mL
2	Phenols	725.0±25.689µg/mL	51.64±3.814µg/mL
3	Tannins	105.9±5.288µg/mL	366.0±9.307µg/mL
4	Alkaloid	748.4±28.22µg/mL	663.3±11.624µg/mL

Discussion

A comprehensive overview of the significance of ABCB1 in cancer cells and its broader impact on cellular environments, drug interactions, and pharmacokinetics. ABCB1, also called by Pglycoprotein, is a well-studied transporter protein that plays a critical role in pumping various substances out of cells. The involvement of multidrug resistance with in cancer cells is especially remarkable, where it can actively eject drugs used for chemotherapy from cancer cells by reducing the treatment effectiveness. Recently, there has been a lot of interest in investigating the medicinally beneficial substrate from sea weeds, especially those that may have anticancer properties. In the present study, the study highlights the important role of drugs that can combat multidrug resistance (MDR) by focusing after efflux pumps that can lessen the efficacy of chemotherapy. This emphasis shows that the study is interested in finding drugs that might improve the effectiveness of already-available anticancer medicines. Here we present a total number of 20 samples of two different sea weeds were used against the overexpressed ABCB1 cell line. From cytotoxicity assays it is revealed that these

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3007-3189

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samples actively regulate the cell proliferation as indicating the IC₅₀ values in low micro-molar range, the most active sample IC₅₀ value range are 29.57, 45.33, 77.12, 104.1 and 105.9 µg/ml.

In the present study several solvent extracts of *Iyengeria*, *colpomenia* and their phytochemicals have been screened for Antioxidant and cytotoxicity (MTT assay). Initially total contents of phytochemicals like flavonoid, phenol and tannin have been quantified spectrophotometrically and the concentrations were found 701.64±146.11 µg/ ml, 513.79±110.45 µg/mL and 286.97±1.92 µg/ ml to be for *Iyengeria* flavonoid, tannin and phenol respectively. *Colpomenia* also shows tannin, flavonoid and phenol with concentration of 421.29±104.19 µg/ ml, 694.164±126.79 µg/ ml and 390.26±18.8 µg/ ml respectively.

Grina.F et al., 2019 conducted an antioxidant activity of five different sea weed *Bifurcaria bifurcata*, *Cystoseira humilis*, *Cystoseira stricta*, *Fucus spiralis* and *Gelidium sequipedale* on DPPH method. The IC₅₀ value of their samples was *Cystoseira stricta* 78.73 ± 1.1 µg/ml, *Cystoseira humilis* 121.1 ± 1.3 µg/ml, *Fucus spiralis* 47.23 ± 3.8 µg/ml, *Bifurcaria bifurcata* 208.5 ± 1.4 µg/ml and *Gelidium sequipedale* 84.61 ± 3.9 µg/ml. which show weak inhibition than our study extracts of *colpomenia* ethanol and ethyl acetate 14.69 ± 0.01 µg/ml and 30.01 ± 0.205 µg/mL respectively was found good antioxidant activity, *Iyengeria* extracts also lower IC₅₀ value than the above sea weeds *Iyengeria*, n-hexane, ethanol, chloroform and ethyl acetate have 31.73 ± 0.23 µg/ml, 16.80 ± 0.02 µg/mL, 35.84 ± 0.61 µg/mL and 40.80 ± 0.241 µg/ml respectively (Grina et al., 2020).

Yin Chia in 2015 investigate Three species of seaweeds *Caulerpa racemosa*, *Padina tetrastrum* and *Turbinaria ornata*. The Study indicate cytotoxicity of the above seaweeds, A Five different solvents were used for the isolation of sea weeds, i.e., hexane, dichloromethane, ethyl acetate (EA), methanol and acetone. *P. tetrastrum* hexane extract showed an IC₅₀ value 130.0 ± 1.72 µg/ml, *C. racemosa* acetone showed an IC₅₀ value 100.0 ± 2.41 µg/ml and *T. ornata* hexane and acetone showed IC₅₀ value 240.0 ± 1.89 µg/ml and 480.5 ± 2.27 µg/ml. (Chia et al., 2015). The results from our cytotoxicity assays demonstrated the high effectivity of our samples in inhibiting cell proliferation. This was evident through the low micro-molar range IC₅₀ values. the most active compounds were *Iyengeria* methanol and ethyl acetate, showed the IC₅₀ values of 29.57 ± 2.12 µg/ml and 77.12 ± 3.21 µg/ml. *Iyengeria* Flavonoid also show good IC₅₀ value 45.33 ± 0.032 µg/ml.

Conclusion

conclusions of the above study given in the following. Firstly, by displaying IC₅₀ values in the low micromolar range, several solvent extracts have been found to be the most effective inhibitors of P-gp. The samples exhibit strong inhibition in the DPPH assay for antioxidants. secondly, the invitro cytotoxicity assay was performed for the highly active seaweed extracts. The cancer cell line CCRF-CEM VCR₁₀₀₀ to the methanol and ethyl acetate extract of seaweeds and their viability was reduced in a concentration-dependent manner after 48 hrs. The current study conclude that the sea weed *Iyengeria* have an excellent pharmacological activities especially anticancer activity. Additionally, this study emphasizes the importance of bioprospecting research, highlighting the value of marine biodiversity as a rich source of potential natural

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compounds possessing pharmacological or biotechnological properties. The interesting activity observed in all active extracts require specific attention in further examinations, encompassing detailed chemical characterization of the bioactive compounds, as well as more advanced biological assays.

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