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Biophysical, Anticancer, Antibacterial and Antiviral Profiles of Aloe-emodin: A Mini-Review

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Abstract: Aloe-emodin belonging to anthraquinone class is an active constituent found in aloe vera (*Aloe barbadensis miller*) which is a chinese herb. Recent advances in research have explored the pharmacological and pharmacokinetic properties of aloe-emodin that are useful in ailment of different diseases. Aloe-emodin has been reported to show anti-cancer, anti-bacterial, anti-viral, anti-inflammatory, anti-neoplastic and anti-parasitic activities underlying in pharmacological properties. This review aims to provide a concise report on the biophysical and biological properties of aloe-emodin. Reports suggest that among biophysical studies, spectroscopic techniques such as UV-visible, fluorescence, Circular Dichroism, resonance light scattering are used to study the binding behaviour of aloe-emodin with DNA, proteins or enzymes. Established binding constants values with DNA is of the order of 10^3-10^5 M^{-1} and with proteins or enzymes is 10^4-10^6 M^{-1}. Its anti-cancerous activity has been demonstrated in different cancer cell-lines involved in breast cancer, cervical cancer, lung cancer, liver cancer, melanoma cancer, etc. via blocking p13/mTOR pathways, by activation of caspase-mediated pathway or by alteration in Bax/Bcl-2 ratio, etc. The anti-bacterial activity against *Staphylococcuss aureus, Aeromonas hydrophila, Bacillus subtilis, Escherichia coli, Proteus vulgaris,* etc. has been investigated and reported alongwith the minimum inhibitory concentration (µg/mL) of aloe-emodin. In some studies, aloe-emodin has been tested for its antiviral activity against poliovirus, herpes simplex virus (HSV), Pseudorabies Virus (PSV), Influenza Virus A (INF), Varicella-Zoster Virus (VZV) and the ID_{50} Values (µg/ml) has been reported by using plaque reduction assay method. This comprehensive summary of aloe-emodin might be helpful in understanding the mechanism and the various pathways involved in its action on cancerous, bacterial and viral cells and also gaining knowledge about its spectroscopic behaviour.

1. Introduction

Aloe-emodin, an anthraquinone derivative is 1, 8-dihydroxy-3-hydroxymethyl-

anthraquinone (Fig.1.) shows various advantageous biochemical properties. It poses anti-cancer, anti-bacterial, anti-viral, anti-inflammatory, hepatoprotective, anti-protozoal, immunomodulatory properties [1]. It is regarded as the major bioactive component of aloe vera (Aloe barbadensis miller) and its other derivatives like aloin and aloe-emodin-8-glucoside are also found. In cancerous cells, it inhibits their proliferation hence exhibiting anti-proliferative behaviour [2]. Anthraquinone derivatives exert cytotoxic behaviour via interaction with DNA generally at guanine and cytosine specific sites. Such interaction results in DNA damage due to conformity changes. It can also act by inhibition of topoisomerase II enzyme activity. [3]. Biological assays have displayed that aloe-emodin affects the cell growth by regulating apoptosis, cell cycle arrest, cell mobility alterations and by acting as immunomodulator. Cell-cycle arrest is generally indicated by the downregulation of cyclin and cyclin-dependent kinase activity. Regarding mechanistic approach, aloe-emodin exhibits anti-cancer activity via intrinsic (Cytochrome c/ caspase 9) and extrinsic (TNF-α and FASL) apoptosis pathways [4]. It can also induce carcinogenesis through inhibition of arylamine N-acetyl transferase (NAT) activity [5].
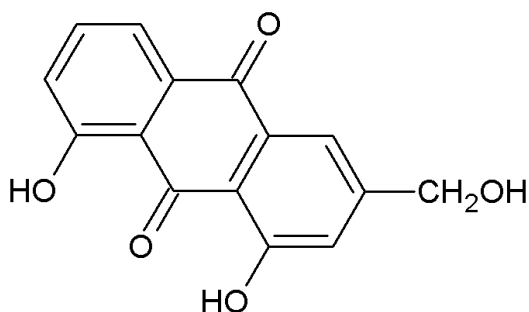

Fig 1. Chemical Structure of Aloe-emodin

Herein, this mini review summarizes last 30 years progress covering various biophysical and biological studies conducted on aloe-emodin in order to reveal its spectroscopic and biological properties. It also presents different biological assays of aloe-emodin with different cell lines in terms of anti-cancer activity. Anti-bacterial and anti-viral activity of aloe-emodin with different bacterial strains and viruses has also been illustrated respectively.

2. Biophysical Studies/Spectroscopic Studies

The biophysical assays for binding studies are carried out by different techniques such as UV spectroscopy, Fluorescence spectroscopy, isothermal titration calorimetry [6], circular Dichroism (CD) spectroscopy, differential scanning calorimetry (DSC) [2]. For small molecules, DNA is the target molecule for their interaction. The interaction occurs either non-covalently or co-valently [3]. A report suggested that polycyclic planar aromatic compounds are generally the DNA intercalators. Other interaction modes are groove binding, hydrogen bond, pi-pi interaction, van der Waals forces, molecular orbital interactions. Interestingly, aloe-emodin is a planar and polycyclic aromatic molecule falls under anthraquinone class [7]. Aloe-emodin and its derivatives exhibit DNA binding which has been established through various spectroscopic or biophysical methods (Fig.2.b). Apart from DNA binding, recent research also shows its binding behaviour with proteins and enzymes (Fig.3.a). Among spectral characteristics, it has shown the maximum absorption band at 430 nm. When aloe emodin excited at 430 nm, it exhibited peaks at 565, 530 and 495 nm in fluorescence emission spectrum (Fig.2.a) The phototoxicity and photocarcinogenity study of aloe-emodin suggests different mechanisms for inhibition of cell activity. Amongst them the popular mechanism involves the singlet oxygen formation upon irradiation with UV-light [8]. Aloe-emodin in transient state shows absorption band at 410 nm, 480 nm and 670 nm due to triplet state formation. Upon photoexcitation with UV or visible light, aloe-emodin has caused cytotoxic effect and oxidative damage to cellular

RNA and DNA exhibiting photosensitizing effect [9].

The biophysical Studies conducted on aloe emodin have been listed in Table 1. Giving an insight into the binding mode of aloe emodin with DNAs and other materials, techniques used to study the interaction alongwith their important results. Molecular modeling studies on aloe-emodin with DNA, proteins or enzymes have also been conducted in order to reveal its binding characteristics. These studies are performed via molecular docking with the help of computer programme software such as Autodock 4.0, cDocker, (Fig.3.c) etc. giving the value of binding energy, information about interaction mode and site of action [10, 12]. For example, Zeng HJ and co-workers have furnished the molecular docking studies of aloe-emodin with tyrosinase using Autodock 4.0 [18]. It was revealed that aloe-emodin induced conformational changes in tyrosinase enzyme due to its binding in tyrosinase cavity. Binding free energy for this complex was estimated to be -22.61 KJ mol^{-1}. Similarly, with trypsin and pepsin the binding free energy was found to be -23.032 KJ mol^{-1} and -19.532 KJ mol^{-1}(Fig.3.b). Aloe-emodin was found to be located in the binding cavity of trypsin and pepsin. These results are also consistent with thermodynamic parameters obtained from experimental studies [16, 17]. Molecular Docking of Aloe emodin with duplex DNA (Fig.2.c) has revealed the binding forces such as electrostatic forces, hydrogen bonding, minor groove binding, etc. [10].

3. Anticancer Activity

3.1. Breast Cancer Cells

Researchers have examined the effect of aloe-emodin on estrogen-positive (ER+) breast cancer cell line (MCF-7) using WST-1 assay. Aloe-emodin induced dose and time-dependent
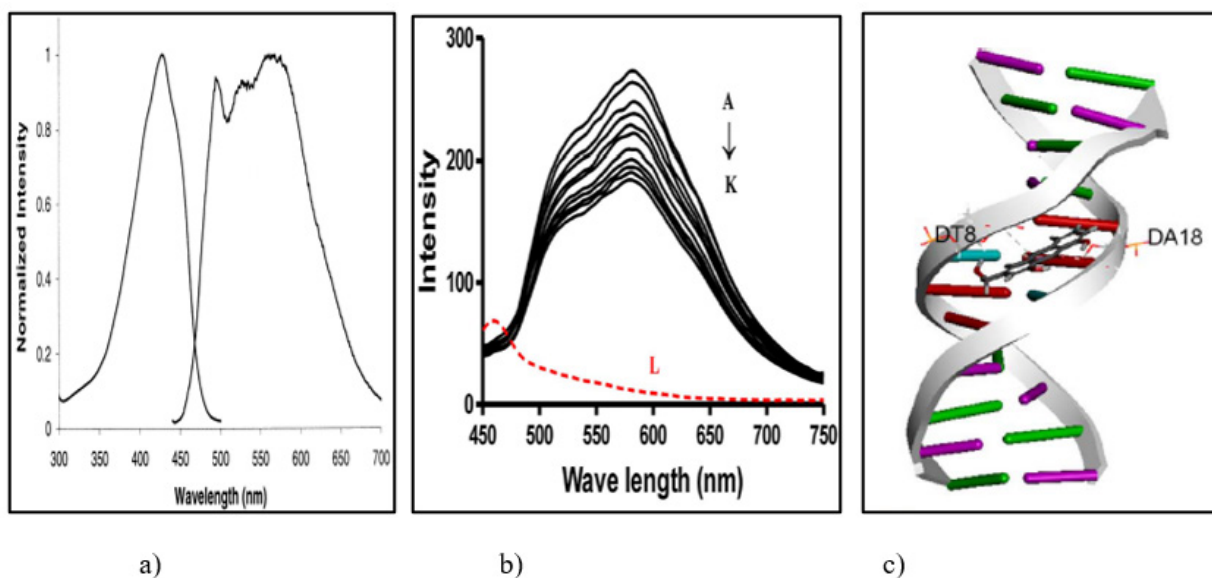

a)　　　　　　　b)　　　　　　　c)

Fig.2. a) Adapted from [8] showing reported Absorption and Emission spectra of Aloe-emodin in Methyl Cyanide Solvent. b) Adapted from [10] showing reported fluorescence emission spectra of Aloe-emodin (λ_{exc} = 400 nm) with varied DNA concentrations (A-K) and bottom red line 'L' showing 100 μM DNA alone emisssion spectrum (λ_{exc} = 400 nm) c) Adapted from [12] showing reported Molecular docked pose of Aloe-emodin with CT DNA representing minor groove binding (Cytosine-Purple, Thymine-Cyan, Guanine-Green, Adenine-Red).
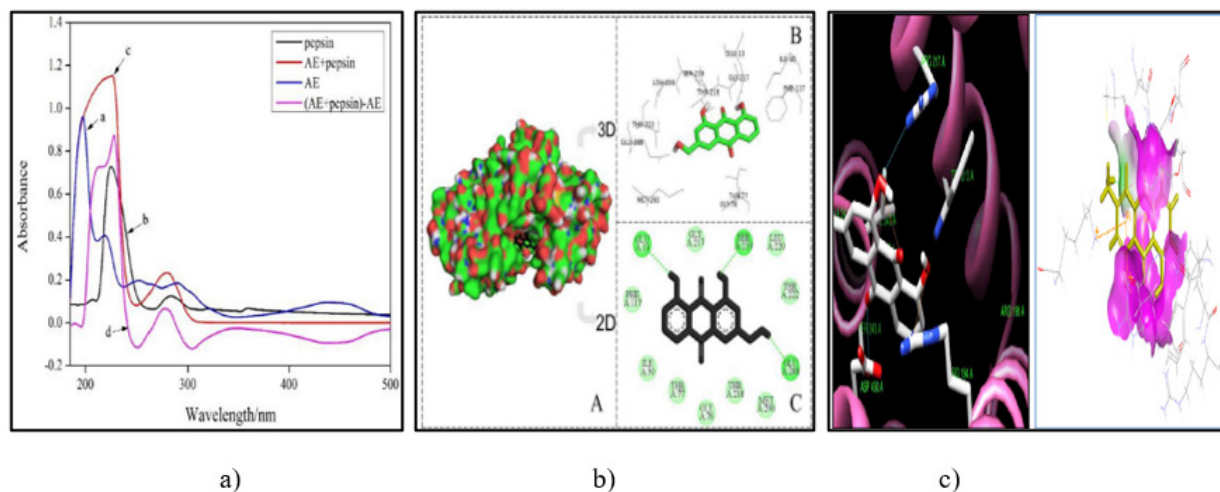
Fig. 3. a) Adapted from [17] showing reported UV absorption spectrum of Aloe –emodin with pepsin. b) Adapted from [17] showing reported docked conformations (A) of Aloe-emodin with pepsin in 2D (B) and 3D (C) view. c) Adapted from [12] showing reported molecular interactions of aloe-emodin with Bovine serum albumin using AUTODOCK (black background) and CDOCKER (white background) programmes.

inhibition effect. The IC_{50} value was observed to be 104 µM, 125 µM and 80 µM for 24 hrs, 48 hrs and 72 hrs respectively for aloe-emodin treatment and the cytotoxicity has also been compared with tamoxifen (Table 2) [19]. In another study, it was found that aloe-emodin affected ER+ breast cancer cell (MCF-7) but did not affect control breast cells (MCF-10A). Tamoxifen was non-selective as it inhibited the viability of both cells with IC_{50} of 27 µM and 38 µM. It downregulated expression of insulin-like growth factor-1 receptor (IGF-1R), insulin-like growth factor binding protein (IGFBP)-2 and B-raf gene. Annexin V-FITC/PI staining, QuantiGene 2.0 Plex assay were used [20]. Aloe-emodin has upregulated the CD95 (Fas) expression in human breast cancer cell line (MCF-7) and apoptosis has been determined by flow cytometry and MTT assay. Apoptosishas been observed at an effective concentration of 100 µM for 72 hr treatment [21]. Effects of aloe-emodin on different breast cancer cell lines i.e. MCF-7, MDA-MB-231, MDA-MB-468, BT-474, and HCC-1954 were also studied. Western blot analysis and flow cytometry techniques have been used to determine the expression levels of different signaling proteins and apoptosis rate respectively. Aloe emodin (4 µg/ml) in combination with tamoxifen (9 µg/ml) enhances its cytotoxicity by supressing EGFR, ERα, Ras, ERK, c-Myc, and mTOR protein expression and blocking PI3K/mTOR pathways. The MCF-7 cells were found to be more sensitive [22].

3.2. Cervical Cancer Cells

Aloe-emodin treated cervical cancer cells (HeLa) showed growth inhibition in dose-dependent manner and caused cell-cycle arrest at G2/M phase. MTT assay and flow cytometry has been used to determine cell growth and cell cycle distribution, apoptosis rate respectively. Western blotting has been done to determine the expression levels of proteins. The decrease in cyclin A and CDK2 and increase in cyclin B1 and CDK1 proteins was observed. Alkaline phosphatase (ALP) activity was increased leading to the inhibition of proliferating cell nuclear antigen (PCNA) expression. Aloe-emodin has also suppressed the expression of PKCα and c-myc [23]. Aloe-emodin can

Table 1. Biophysical Studies on Aloe-emodin with DNA, Proteins and Enzymes representing the binding constants (K_b) and mode of binding or forces.

COMPLEX	TECHNIQUES USED	RESULTS				MODE OF BINDING	REFERENCES
Aloe-emodin:Calf Thymus DNA {AED-CT DNA}	Steady State Fluorescence Study	$K_b = 6.76 \times 10^4\,M^{-1}$ with n value 2.8				Might be Non-covalent Binding	[2]
	Competitive Dye Displacement Assay	AED-EtBr : $K_{sv} = 10.94 \times 10^3\,M^{-1}$ AED-Hoescht: $K_{sv} = 45.02 \times 10^3\,M^{-1}$ (EtBr: Intercalator Dye ; Hoescht: Groove Binder Dye)				Minor groove Binding	
	Isothermal Titration Calorimetric Study	T(K)	$K \times 10^4\,M^{-1}$	ΔH^0(kcal/mol)	$T\Delta S$(kcal/mol)	Minor Groove binding	
		288	7.29	-1.20	5.24		
		298	6.02	-1.70	4.83		
		308	4.70	-1.98	4.53		
	Differential Scanning Calorimetric Study	T_m (Ct-DNA) = 66.98 ^{0}C T_m (Ct-DNA-AED) = 73.50 ^{0}C ΔT_m = 6.52 ^{0}C (Stablization duplex DNA)				Minor Groove Binding	
AED-Herring Sperm DNA	Fluorescence, Resonance Light Scattering Study	$K_b = 5.765 \times 10^2\,M^{-1}$ with n value 0.7127				Intercalation Binding	[7]
Aloe-emodin:Calf Thymus DNA {AED-CT DNA}	Steady State Fluorescence Study	$K_b = 8.5 \times 10^5\,L\,mol^{-1}$				NR	[8]
Aloe-emodin:Calf Thymus DNA {AED-CT DNA}	Fluorescence, UV-Spectral Analysis	$K_q = 0.961 \times 10^4\,L\,mol^{-1}\,sec^{-1}$				NR	[10]
	Molecular Docking	Interaction Energy: -24.1616 Kcal/mol Binding Energy: -8.59 Kcal/mol				Minor Groove Binding	
Aloe-emodin:Guanine Rich Quadruplex DNA {AED-GDNA}	Fluorescence resonance energy transfer, Circular Dichroism Spectroscopy	$K_b = 2.58 \times 10^5\,L\,mol^{-1}$				NR	[11]
Aloe-emodin: Bovine Serum Albumin {AED-BSA}	Fluorescence, UV-Spectral analysis	$K_b = 0.24 \times 10^6\,L\,mol^{-1}$ with n = 1.2				NR	
	Molecular Docking	Interaction Energy: -34.12 Kcal/mol Binding Energy: -7.83 Kcal/mol				Hydrophobic Interactions and Hydrogen Bonding	[12]

Aloe-emodin: Human Serum Albumin {AED-HSA}	Fluorescence, UV-Spectral analysis	$K_b = 1.0. \times 10^5$ L mol^{-1} with n = 0.6			Electrostatic Forces	[13]
Aloe-emodin: Bovine Serum Albumin {AED-BSA}	Fluorescence UV-Spectral analysis	$K_b = 3.82 \times 10^4$ L mol^{-1} with n = 0.98			Electrostatic Forces	
Aloe-emodin: Human Serum Albumin {AED-HSA}	Fluorescence, Circular Dichroism Spectroscopy	T (K) / 296 / 303 / 310	$K \times 10^5$ M^{-1} / 1.754 / 1.650 / 1.542	n / 0.937 / 0.914 / 0.915	ΔG^0(KJ.mol^{-1}) / -29.720 / -30.257 / -30.793 → Hydrophobic Interaction	[14]
Aloe-emodin: Human Serum Albumin {AED-HSA}	Fluorescence Spectroscopy Study	$K_q = 0.11 \times 10^5$ L mol^{-1} sec^{-1}			NR	[15]
Aloe-emodin:Trypsin {AED- Trypsin}	Fluorescence, UV-spectroscopy Study	T (K) / 298 / 304 / 310	K / 1.0898×10^4 M^{-1} / 1.0507×10^4 M^{-1} / 6.5832×10^3 M^{-1}	n / 0.9849 / 0.9953 / 0.9698	ΔG^0(KJ.mol^{-1}) / -23.0324 / -23.4037 / -22.6607 → van der waals forces and hydrogen bonding	[16]
Aloe-emodin:Pepsin {AED-Pepsin}	Fluorescence, UV-spectroscopy Study	T (K) / 298 / 304 / 310	K / 2.0618×10^4 M^{-1} / 7.1679×10^3 M^{-1} / 6.2257×10^3 M^{-1}	n / 1.0442 / 1.0583 / 1.0523	ΔG^0(KJ.mol^{-1}) / -18.9072 / -16.5628 / -16.5822 → van der waals forces and hydrogen bonding	[17]
Aloe-emodin: Tyrosinase {AED-Tyrosinase}	Fluorescence, UV-analysis, Circular Dichroism spectroscopy	T (K) / 293 / 298 / 310	K / 1.287×10^4 M^{-1} / 1.110×10^4 M^{-1} / 8.683×10^3 M^{-1}	n / 0.8799 / 1.2152 / 0.8385	ΔG^0(KJ.mol^{-1}) / -21.87 / -22.69 / -23.37 → Electrostatic forces	[18]

Kq: Quenching Constant; n: Number of Binding Sites; Tm: Melting temperature; ΔG^0: Standard Gibbs Free Energy Change; ΔH^0: Standard Enthalpy Change; T (K): Temperature in Kelvin; ΔS: Entropy Change; NR: Not Reported

regulate apoptosis in human pappilomavirus (HPV) induced cervical cancer cells (HeLa and SiHa). Several techniques such as MTT assay, flow cytometry, real-time quantitative polymerase chain reaction (qRT-PCR) and western blot have been performed to determine the cell-growth, apoptosis and protein expression regulation respectively in these cell lines. Aloe-emodin inhibited the expression of HPV-related protein E6 and E7 and it also affected glucose metabolism by reducing GLUT1 expression [24].

3.3. Lung Cancer Cells

Human lung carcinoma cells (H460) were treated with aloe-emodin resulting into apoptosis via DNA damage through production of reactive oxygen species (ROS). A decrease in the mRNA of DNA repair enzymes such as hMTH1, hOGG1 and APE was observed by RT-PCR. DNA single strand breaks were observed using comet assay at an effective concentration of 40 µM of aloe-emodin [25]. In another study, two cell-lines i.e. human lung squamous carcinoma cell (CH27) & human lung non-small cell

carcinoma cell (H460) were used. Aloe-emodin induced apoptosis showing the involvement of protein kinase C (PKC) by western blotting. Downregulation in the expression of PKCδ and ε and caspase-3 was observed [26]. At an IC_{50} of 25 μM aloe-emodin induced apoptosis in human lung squamous carcinoma cell line (CH27) via Bax and Fas mediated pathway. Through western blot and flow cytometry techniques, alteration in the expression of Bcl-2 family proteins, such as BclXL, Bag-1, and Bak and activation of caspase-3, caspase-8, and caspase-9 has been found [27].

3.4. Gastric Cancer Cells

Inhibitory effect of aloe-emodin has been demonstrated on human gastric cancer cell line (MKN45). At an effective concentration of 0.05 mM, aloe-emodin has induced cell death. Cell growth arrest has been observed in G_0/G_1 phase by flow cytometry. An analysis of DNA fragmentation by agarose gel electrophoresis has been done which was observed in aloe-emodin treated cells [28]. In another human gastric cancer cell line (MGC-803), dose-dependent inhibition occurs determined by MTT assay. Reduction in alkaline phosphatase activity and increase in S-phase was observed by dynamics assay and flow cytometry respectively [29]. In aloe-emodin treated human gastric carcinoma cells (AGS and NCI-N87), apoptosis occurs in time and dose-dependent manner. Apoptotic pathway involves the release of cytochrome-c from mitochondria, activation of caspase-3 that leads to nuclear shrinkage. Downregulation of casein kinase-II activity in time-dependent manner was observed. From XTT viability assay, the IC_{50} value for AGS and NCI-N87 are observed to be >0.07 mM and 0.15-0.19 mM respectively (Table 2) [30]. Human gastric carcinoma cells i.e. MGC-803 and SGC-7901 when treated with aloe-emodin showed downregulation in the expressions of protein kinase C and c-myc that showed anti-proliferation effect of aloe-emodin. G_2/M phase arrest was observed in

SGC-7901 cells. It inhibited the growth and migration of gastric cancer cells. Crystal violet assay, western blotting, flow cytometry and scratch wound healing motility assays has been performed [31].

3.5. Liver Cancer Cells

Two human liver cancer cell lines i.e. Hep G2 and Hep 3B were studied and have shown different antiproliferative mechanism. Reduced cell proliferation and apoptosis has been induced by aloe emodin in these cell lines. Bax expression has been observed and Bcl-2 family proteins were not detected in western blot analysis. In HepG2 cells, induced p53 and p21 expression and G1 phase arrest inhibited cell proliferation. While in Hep 3B cells which are p53 deficient induction of p21 expression caused apoptosis but not cell cycle arrest. From XTT viability assay, the IC_{50} values for Hep G2 and Hep 3B are observed to be 11.77 μg/mL and 15.67 μg/mL respectively [32]. Molecular targets of aloe-emodin on hepatocellular carcinoma cell line HepG2 were investigated. Aloe emodin exhibited anticancer action through multiple pathways by affecting various protein targets. From proteomic and biochemical studies, it was observed that aloe emodin induce oxidative stress via increasing the levels of intracellular reactive oxygen species (ROS) and by the oxidation of redox sensitive proteins such as peroxiredoxins (PRDX) and DJ-1 determined by flow cytometry. Upregulation of PRDX and DJ-1 has been found. The upregulation of CDK4 inhibitor p16 and inhibition of Rb phosphorylation reduced the DNA formation in aloe-emodin treated cells. The upregulation of metastatis inhibitor (nm23) reduced the cell migration showing anti-migration activity. Cell cycle analysis showed the G2/M phase arrest and apoptotic cell death as determined by MTT assay [33]. Human normal liver HL-7702 cell was treated with aloe-emodin to determine its cytotoxic effects. Viability of HL-7702 cell was reduced in dose and time dependent manner

as observed by CCK-8 assay. Up-regulated the levels of Fas, p53, p21, Bax/Bcl-2 ratio and cleaved caspase-3, -8, -9 as determined by western blot analysis. The apoptosis was caspase dependent mediated through Fas death and mitochondrial pathway. Through flow cytometry S and G2/M phase arrest was observed. Hence, aloe-emodin can be risky for human exposure [34].

3.6. Neuroectodermal Tumours

Aloe-emodin has been found to have a specific activity against neuroblastoma cell line. There is involvement of p53 tumor suppressor gene in anti-neuroectodermal tumours. Inhibition activity occurs through apoptosis. It shows both in-vitro and in-vivo activity against these tumours [35, 36].

3.7. Leukemia Cells

The anticancer effect of aloe-emodin has been demonstrated on human promyelocytic leukemia HL-60 cells. The cell line has been assayed for proliferation, cell cycle arrest and apoptosis. G2/M phase arrest and apoptosis was determined by flow cytometry and DNA fragmentation gel electrophoresis. Immunoblot analysis has been employed for monitoring the levels of protein expression. Aloe-emodin enhanced the levels of cyclin B1 and A, cyclin E level remain unchanged; cyclin dependent kinase levels in which CDK1 levels were increased while CDK2 levels remain unchanged. Enhancement in p27 levels caused the G2/M phase arrest. At an effective concentration of 10 µM casapase-3 levels were increased [37]. Another study on human monoblastic leukemia U937 cell line had been performed where aloe-emodin has been found to be a differentiating and specific agent for leukemia cells. An increase in transglutaminase activity has been observed which may be responsible for growth arrest. Transglutaminase activity assay, nitro blue tetrazolium (NBT) assay, etc. has been performed [38].

3.8. Glioma Cells

Effects of aloe-emodin on rat and human glioma cell lines i.e. C6 and U251 respectively were studied. MTT assay, flow cytometry, cell-based ELISA assays have been performed. It reduced the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in C6 cell lines. In U251 cell line, G2/M phase arrest has been observed by flow cytometry and active form of ERK1/2 was inhibited as shown by MTT assay. Cell death is mediated through apoptosis or autophagy [39]. In human U87 glioblastoma cell line, aloe-emodin has altered expression of various genes for example- SHARPIN, BCAP31, FIS1, RAC1 and TGM2, etc. as determined by quantitative real-time PCR. The expression level of genes has also been investigated. Out of 28,869 genes, changes in 8,226 genes have been found at an effective concentration of 58.6 µg/mL after 24 hr treatment. Among them, statistically major alteration was observed in 34 genes out of which 22 genes were upregulated while 12 genes were downregulated on treatment with aloe emodin [40].

3.9. Melanoma Cells

In human malignant melanoma cells A375.S2, effect of aloe-emodin on enzymatic activity and gene expression of N-acetyltransferase (NAT) at mRNA and protein levels has been investigated. Aloe-emodin reduced the NAT1 activity in dose dependent manner as determined by western blotting. From PCR and cDNA microarray, mRNA levels were examined and it has been found that aloe-emodin reduced the mRNA expression levels [41]. Another study was conducted on mouse B16 melanoma and human A375 melanoma cell line through MTT assay, propidium iodide staining method for cell cycle analysis, immunoblot analysis for determination of protein levels. In B16 melanoma cells, increase in melanin production and tyrosinase

activity accompanied with H_2O_2 production was found. This activity was associated with rapid p53 accumulation and increased expressions of cyclins D1 and D3. In A375 melanoma cells, apoptosis was caspase mediated which was connected with Bcl-2 downregulation. Aloe-emodin has induced resistivity in both cells against doxorubicin and paclitaxel induced killing in dose dependent manner [42]. Another human malignant melanoma cells (SK-MEL-28 and A375) when co-treated with aloe-emodin, a significant inhibition in cell proliferation was observed and induced cell differentiation. Aloe-emodin has shown immunomodulatory, antiproliferative, antineoplastic activities against these melanoma cell lines. Immunomodulatory activity has been confirmed through production of GM-CSF and IFN-γ. These all are BRAF-mutated melanoma cells [43]. In B16-F10 melanoma cells, increase in transglutaminase activity was found associated with cell adhesion and aggregation. The IC_{50} value was found to be 60 µM (Table 2) as determined by MTT assay and trypan blue exclusion tests [44].

3.10. Bladder Cancer Cells

Aloe-emodin treated human bladder T24 cells caused apoptosis. Cell line has been assayed for cell viability, cell cycle analysis and apoptosis by flow cytometry analysis. Whereas the levels of proteins and enzymes has been investigated by western blotting analysis. Aloe-emodin reduced the cell proliferation and caused G2/M arrest. Reduction in the levels of cyclin dependent kinase 1 and cyclin B1 and enhancement in the levels of Wee1 and cdc25c was found causing cell cycle arrest. Activation of p53, p21, Fas/APO-1 receptor, caspase-3 and Bax while downregulation of Bcl-2 expression is responsible for apoptosis [45].

3.11. Prostate Cancer Cells

Mammalian target of rapamycin complex 2 (mTORC2) regulates the cell proliferation in human prostate cancer cells. mTORC2 is a protein kinase complex consists of mTOR, rictor, mSin1, mLST8/GbL and PRR5 components and functions as phosphorylating agent. Aloe emodin can inhibit its enzymatic activity and can bind to mTORC2 as shown by pull-down assay and in vitro kinase assay. The cell line used for this study is PC3 cells in which aloe emodin reduced cell proliferation and anchorage-independent growth. The downstream substrate of mTORC2, AKT and PKCα was reduced upon treatment with aloe emodin. Hence, aloe-emodin can markedly reduce the prostate cancer growth resulting in cancer prevention [46]. Yuan et al., (2012) reported the IC_{50} values (Table 2) for human ovarian cancer and human nasopharyngeal carcinoma cell line using cell-counting kit-8 (CCK-8) assay [47].

3.12. Squamous Cancer Cells

Researchers have worked on human tongue squamous carcinoma SCC-4 cells treated with aloe-emodin. Cell cycle analysis have shown the S-phase arrest via the activation of p53, p21 and p27 while reduced the levels of cyclin A, E, thymidylate synthase and Cdc25A at 30 µM of aloe-emodin. The S-phase arrest and apoptosis has been induced in a dose and time dependent manner. The release of some factors such as apoptosis inducing factor (AIF), endonuclease G (Endo G), pro-caspase-9 and cytochrome c from the mitochondria was accompanied with enhancement in Bax/Bcl-2 ratio followed by the activation of caspase-9 and caspase-3. The mediated pathway for apoptosis was Fas/death receptor, mitochondria and caspase cascade pathways [48].

3.13. Colon Cancer Cells

Human colon cancer cell line WiDr has been investigated on treatment with aloe-emodin for examining the molecular mechanisms involved in inhibiting cell-growth. G2/M phase arrest and reduction in cyclin B1 level was found to be a factor in inhibiting cell growth. On activation of

caspase 9/6, aloe-emodin induced the cell death causing apoptosis. Different methods have been used such as MTT assay, flow cytometry, western blot analysis, RT-PCR, etc. [49].

3.14. Oral Cancer Cells

The cell line used for studying human oral cancer cell was KB which was assayed for its cell proliferation activity, cell cycle arrest etc. on treatment with aloe-emodin. Aloe-emodin inhibited cell proliferation in a dose-dependent manner. G2/M phase arrest was observed at the concentration ranging from 10-40 µM. Cell has also been assayed for alkaline phosphatase (ALP) activity which was found to be increased upon treatment with aloe-emodin. MTT assay, flow cytometry, DNA fragmentation analysis have been used in this study [50].

3.15. Stomach Tumour Cells

The effect of gamma-irradiated (doses ranging from 0 to 150 kGy) aloe-emodin was studied on stomach tumour cell (AGS). Cell line was assayed for its viability by MTT assay, for cell cycle arrest, cell morphology, signaling pathway by immmunoblot analysis and flow cytometry. Gamma irradiated aloe-emodin markedly enhanced the cytotoxic action in AGS cells. At an effective dose of 150 kGy, aloe-emodin enhanced the expression of Bax, cytosolic cytochrome c, PARP cleavage and activation of caspases-8, caspase-9, caspase-3, Bid, and Bcl-2. It also induces DNA fragmentation, ROS production, changes in cell morphology and migration of AGS cells. Enhancement in sub-G1 phase and depolarization of mitochondria membrane potential in ROS dependent manner was observed [51].

Table 2. Some IC_{50} Values of Aloe-emodin with different cancer cell lines

	Aloe-emodin	
	Cell-Lines	**IC_{50} (µM)**
Cancerous Cells	**ER** + breast cancer cells [19] **{MCF-7}]**	104 (24 hrs) 125 (48 hrs) 80 (72 hrs)
	Human lung squamous carcinoma cell line [27] **{CH27}**	25
	Human Gastric carcinoma cells [30] **[AGS]** **[NCI-N87]**	70 150-190
	Human liver cancer cell lines [32] **{Hep G2}** **{Hep 3B}**	43.6 58.04
	Melanoma cells [44] **{B16–F10}**	60
	Human Ovarian Cancer [47] **{SK-OV-3}**	39.7
	Human Nasopharyngeal Carcinoma [47] **{CNE}**	55.6
	Human Breast cancer cells [57] **{MCF-7}**	192

4. Anti-bacterial Activity

Aloe-emodin and aloin A are the two major components found in aloe species such as Aloe vera, Aloe ferox. However, in a study the antibacterial activity of aloe emodin extracted from Aloe excelsa species against gram positive and gram negative bacteria were examined and respective minimum inhibitory concentrations were calculated using microplate dilution method (Table 3). These studies proved the potential application of Aloe excelsa species to be used as an antimicrobial agent. It can be used in various treatments such as skin treatment, stomach diseases and as a laxative agent [52]. Antibacterial activity of aloe-emodin against Staphylococcuss aureus was investigated. However, aloe-emodin lacks the anti-Staphylococcuss aureus activity therefore its effect on α-toxin which is a virulence factor secreted by Staphylococcuss aureus and responsible for multiple infections was

examined. Aloe emodin has the potential to inhibit the hemolytic activity of α-toxin. The mechanism involve in this inhibition was determined by oligomerization assays, molecular dynamics simulations and fluorescence quenching analysis. Mechanism study suggested the interaction of aloe-emodin with K110, T112 and M113 of the toxin results in restriction of oligomerization of α-toxin responsible for reduction of hemolytic activity. These studies suggest that aloe-emodin can acts as an antibacterial agent against S. aureus infections [53].

Xiang et al., displayed that the aloe-emodin significantly reduces the proliferation of Staphylococcus aureus biofilm in dose-wise manner [54]. Rhubarb species are the major source of anthraquinones such as aloe-emodin, emodin, chrysophanol, rhein, etc. and their antibacterial activity against Aeromonas hydrophila were examined. The identification of these major bioactive components in Rhubarb species were done by ultra performance liquid chromatography (UPLC).

The MIC values against Aeromonas hydrophila were calculated using two fold microdilution broth method and found to be in the range of 50-200 μg/mL. For aloe-emodin, it was found to be 50 μg/mL [55].

Aloe-emodin extracted from roots of Rheum ribes was examined for its antibacterial activity against Staphylococcus aureus. Different extracts were investigated by spectroscopic analysis for identification of biologically active compounds present. The major bioactive components were aloe-emodin, emodin, chrysophanol, physcion. The MIC value for aloe-emodin found to be 500 μg/mL against this species. However, the compounds did not show activity against Pseudomonas aeruginosa and Eschirichia coli even at the maximum concentration of 4000 μg/mL and 250 μg/mL respectively [56].

Table 3. Bacterial strains with respective MIC values using Microplate Dilution Method

Bacteria	Minimum Inhibitory Concentration (μg/mL)
Bacillus subtilis	125
Micrococcus kristinae	250
Bacillus cereus	62.5
Staphylococcus aureus	125
Staphylococcus epidermidis	250
Escherichia coli	62.5
Proteus vulgaris	62.5
Enterobacter aerogenes	125
Shigella sonnei	250

5. Anti-viral Activity

Aloe-emodin has been exploited for its antiviral activity by various researchers. Aloe-emodin isolated from petroleum ether extract of Cassia roxburghii species examined for its antiviral activity against influenza virus-A. It exhibits IC_{50} value of 2 μg/mL and CC_{50} value of 0.47 μg/mL. These values were determined by MTT method. These Cassia sps. are major source of anthraquinone derivatives, flavanoids, etc. [57]. Another study on influenza virus-A in MDCK cells has been done using novel influenza A H7N9 and H591 viruses. Aloe-emodin has inhibited replication and showed dose-dependent effect on cytotoxic path induced by virus with IC_{50} value of >0.05 μg/ml. Upregulation of galectin-3, thioredoxin and downregulation of nucleoside diphosphate kinase A was examined by western blot analysis in MDCK cells. Anti-influenza virus action is due to the upregulation of galectin-3 expression responsible for virus replication inhibition confirmed by quantitative PCR [58].

In a study, anthraquinones were isolated from hot glycerin extracts of variety of species such as Rheum officinale, Aloe barbadensis, Rhamnus frangula, Rhamnus purshianus

and Cassia angustifolia and investigated for their antiviral activity against herpes simplex virus type 1. All the plant extracts has shown antiviral activity. Aloe-emodin was also tested against different viruses listed below with their ID_{50} values (concentration of aloe-emodin that reduced virus plaque formation or infectivity by 50%) by plaque reduction assay (Table 4). Only adenovirus and rhinovirus remain unaffected on aloe-emodin treatment while all other enveloped viruses were inactivated [59].

Aloe-emodin has been tested for its antiviral activity against poliovirus type-3. However, compared to chrysophanol it was less active against poliovirus type 3. The EC_{50} value was found to be 0.54 µg/ml determined by poliovirus yield reduction assay. The CH_2OH and H (at C3 and C6 position) groups in aloe emodin structure are responsible for activity against poliovirus [60]. Methanol extract of Rheum palmatum species was investigated for its antiviral activity against Japanese encephalitis virus (JEV). Methanol and water extracts were prepared however former was found to show more potent inhibitory action than latter one. By plaque reduction assay, the IC_{50} value was found to be 17.39 µg/mL for aloe-emodin. With quantitative real time RT-PCR, the inhibition of JEV yields in infected cells were examined resulting in 90% inhibition of JEV yields [61]. Activity of aloe-emodin against human cytomegalovirus (HCMV) strain AD-169 in MRC-5 cells was examined. The ID_{50} value was calculated by plaque reduction assay which was found to be >37 µM [62].

Table 4. ID_{50} Values of Aloe-emodin against Different Viruses

Viruses	ID_{50} Values (µg/ml)	Method
Herpes Simplex Virus Type-1 (HSV-1)	1.6	
Herpes Simplex Virus Type-2 (HSV-2)	1.5	
Varicella-Zoster Virus (VZV)	5	
Pseudorabies Virus (PSV)	6	Plaque Reduction Assay
Influenza Virus A (INF)	4.5	

The pharmacological activity of aloe-emodin has been represented schematically in Fig.4. illustrating the anti-cancer, anti-bacterial, anti-viral and other properties of aloe-emodin reported in literature.
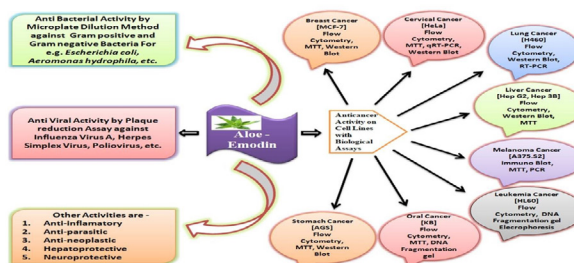

Fig. 4. Flowchart Representing Pharmacological activities of Aloe-emodin

6. Discussion

This mini-review on biophysical and biological profiles of aloe-emodin has shown its binding behaviour and biological relevant activities. The spectroscopic studies suggested that the binding of aloe-emodin with DNA, proteins or enzymes is environmental dependent factors. Generally, with DNA the binding constants value were found to be in the range of 10^3-10^5 M^{-1} whereas with proteins or enzymes, it was found to be in order of 10^4-10^6 M^{-1}. Hence, aloe-emodin showed higher binding affinity towards DNA, proteins or enzymes. Molecular modeling of aloe-emodin using molecular

docking technique gives an insight about the binding and interaction energy. Different docked structure illustrates the binding site of aloe-emodin with respective substrate at a specific location.

Aloe-emodin due to its established anti-cancer behaviour also regarded as a therapeutic molecule. Herein, the findings suggested the pharmacological advantages of aloe-emodin like anti-bacterial, anti-viral and anti-cancer effects. Also, aloe-emodin can be a therapeutic option for treatment of different diseases. Biological assays revealed that aloe-emodin strongly reduced the proteins and genes expression such as c-myc, ERα, NAT, FAK, etc. It also affect different signaling pathways. In-vitro and in-vivo application of aloe-emodin is well reported in literature. Regarding cancerous cells, the in-vitro activity against human skin fibroblasts, HepG2, HL-7702 and HepaRG cells via different mechanistic pathways are present. Studies on anti-bacterial activity of aloe-emodin suggested a decrease in NAT activity and extracellular proteins production. Anti-viral activity is facilitated through the upregulation of thioredoxin and galectin-3. However, some adverse effects of aloe-emodin such as phototoxicity, nephrotoxicity, hepatotoxicity can limit its clinical use. Therefore, further studies regarding its detailed mechanism, oral-suitability, side-effects needs to be explored for human therapeutic use and clinical administration.

Disclosure Statement

The authors have declared that there is no conflict of interest.

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