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NATURAL PRODUCT SUPPRESSORS OF MDM2 AS ANTICANCER AGENTS

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ABSTRACT

Cancer is always regarded as a large group of disease that generally involves rapid division of abnormal cells in various parts of human tissues and organs. Wherein targeting the interaction between E3 ligase Mdm2 oncogene and tumor suppressor p53 represents an attractive approach toward the treatment of various forms of cancer. The p53 tumor suppressor gene plays a vital role in regulating cell cycle arrest at G1/S phase, promoting cell proliferation, apoptosis, DNA repair and also provides protection against malignant transformation. Mdm2 oncogene has been observed to be a negative regulator of p53 as it is being involved in downregulating p53 levels and reducing cell stability. While the overexpression of Mdm2 has resulted in high mortality rate and poorer prognosis to cancer patients. Several in vivo and in vitro studies have been reported various natural products affecting Mdm2 to induce their diverse biological activities. Most of these agents have come into existence in order to decrease the levels of Mdm2 or targeting p53-Mdm2 interaction to activate p53 for induction of apoptosis. The natural agents which have been found to suppress Mdm2 oncogene are genistein, apigenin, curcumin, berberine, gambogic acid, platycodin D etc. They induce inhibitory effects on Mdm2 in order to induce various anti-cancer effects in growing tumour cells. This review focus on the various natural products or phytoconstituents which have been shown to suppress Mdm2 activity and up-regulate the p53 with significance potency for their anticancer activity. Moreover, it also describes their binding pattern to the target protein along with mechanism of action.

INTRODUCTION

Cancer is regarded collective disease that is identified by uncontrolled growth of abnormal cells in the body. This state occurs due to lack of contact inhibition property of a cell and decreased apoptosis of cell[i]. Recently, as per New Global Cancer Data Report (GLOBOCAN 2018), more than 18.1 million people were diagnosed with cancer while the death toll has risen up-to 9.6 million cases globally while the number of individuals who are being treated within the tenure of 5 years was estimated to be 43.8 million. While it has been reported that the proportion of cancer deaths in Asia (57.3%) is higher than the proportion of incident cases (48.4%) respectively due to poor prognosis and higher mortality rates [ii]. Human cancer and tumour progression are being directly associated with the alteration of oncogene and suppressor genes in different stages of cancer development. Most of the anti-cancer agents have a broader range of targets including Mdm2 Oncogene and cellular tumor antigen p53 [iii- iv]. It has been observed that dysregulation of apoptotic program is the main cause of various diseased conditions such as cancer, certain autoimmune and cardio-vascular disorders as well (p53 downregulation may reduce apoptosis and may enhance tumor growth) [v]. The p53tumor suppressor gene (potent anti-proliferative and pro- apoptotic protein) is a nuclear phosphoprotein that has significant role in regulating cell division, DNA repair, cell cycle

arrest and apoptosis [vi]. p53 contains 393 amino acids and is encoded by 20-kb gene accommodating 11 exons and 10 introns, which is located onto a small arm of Chromosome 17, which is implicated in wide range of human genetic diseases [vii-viii]. While Mdm2 Oncogene has been recognised as a negative regulator of p53,where human Mdm2 oncogene contain 491 amino acids with 3 major domains onto its structure: including a p53-binding domain, an acidic domain with a Zinc finger along with a RING domain [ix- x].Through autoregulatory feedback loop Mdm2 inhibits p53 activity by functioning as an E3 ubiquitin ligase and promote p53 ubiquitin mediated proteasomal degradation and thereby reducing its function and expression by inhibiting the p53-Mdm2 interaction[xi].Overexpression of Mdm2 is observed in varied forms of cancer including glioblastoma multiformis and breast cancer may lead to decline in p53 Expression[xii -xiii]. While it has been observed that overexpression and amplification of Mdm2oncogene correlates with poor response and poor prognosis to current cancer therapy [xiv].

A wide range of natural products have beenrecognized in order to target the Mdm2-p53Interaction as well as to inhibit Mdm2 oncogene including Sempervirine, Tricetin, Berberine, Platycodin D etc [xv]. In present review, we will provide extensive review on the mechanism of Mdm2 suppression along with their biological uses and critical analysis of Structural Activity Relationship for anticancer effects of different natural products.

1. A PERSPECTIVE ABOUT MDM2 ONCOGENE AND INTERACTION BETWEEN Mdm2 AND P53.

P53 was seen to stimulate the expression of Mdm2 oncogene at transcriptional level. Mdm2 binds to p53 and ubiquitinates it by promoting its rapid degradation through proteolysis pathway [xvi]. There are several strategies involved to activate the p53-mediated apoptotic pathway in tumors *via* inhibition of the Mdm2 protein including decrease in cellular concentration of Mdm2 with antisense oligodeoxynucleotides, mimicking the p14ARF (a negative regulator of Mdm2) along with the blockage of Mdm2-mediated p53 ubiquitination and inhibition of p53-Mdm2 interaction with small molecules that bind at the interface between these two proteins [xvii-xviii].

Murine double minute 2 oncogene (Mdm2) was identified along with two corresponding genes (including Mdm1 and Mdm3) in a locus amplified on double minute chromosomes in a spontaneously transformed mouse fibroblast. It was also observed that overexpression of Mdm2 can cause cell transformation [xix - xx].

The N-terminal p53- binding component of Mdm2 plays an integral function in binding with the tumour suppressor p53 protein and inhibits its transcriptional activity [xxi- xxii]. Mdm2 can be mono/poly ubiqitinase p53 contingent on the level of Mdm2 activity. Lin *et al.* demonstrated that Leu14, Phe19, Leu22, and Trp23 residues of p53 were of great significance in Mdm2-binding via various site-directed experiments and mapping with short p53-peptides [xxiii].

Under normal situations, Mdm2 is expressed in nucleus and then it translocates towards cytoplasm to mediate the degradation of certain of its targets using proteasome. The carboxylic terminal of the oncogene is accountable for its various function and promotes self-ubiquitination of target protein (p53 ‘regarded as cellular gatekeeper’), and commute p53 out of the nucleus and furtherly prevent the binding of p53 with transcriptional coactivators and recruit co-repressors (as hCtBP2 to p53) [23].

Mdm2 also has the ability for self-ubiquitylation and may act as the starting material of certain endocytic proteins, Retinoblastoma protein, Mdm4 as well [xxiv- xxv-xxvi]. Also, upon ubiquitylation, these homologs would move towards the nucleus and undergo disintegration which is generally expressed during the carcinoma of mammary gland which may lead to down-regulated expression of the TP53 and lead to poor prognosis. Different approaches have been put forth in order to affect the interaction between Hdm2 and p53, along with the ubiquitin ligase activity of Hdm2, for the evolution of variousMdm2 inhibitors for cancer therapy and prevention.

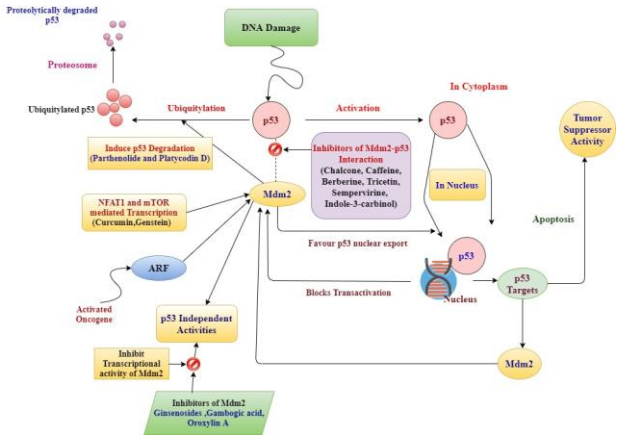

Figure1 Representative mechanistic pathway or interaction and role of Mdm2-p53 and the target site for inhibition of apoptosis by different molecules.

2. NATURAL PRODUCTS AS SUPPRESSORS OF MDM2

Different approaches have been employed in order to target p53-Mdm2 pathway which can be classified into three major categories:

(a) Natural products that block/inhibit Mdm2 expression (p53 -dependent or independent pathways)

(b) Natural products that regulate or affect the ubiquitylation of TP53

(c) Natural products that would inhibit p53-Mdm2 binding [xxvii]

Natural products contributed with their diverse sources, complicated organic chains and structurally diverse scaffolds have an integral and valuable role in evolution of anti-neoplastic compounds and for the instance in targeting interaction between p53-Mdm2 [xxviii-xxix-xxx]. There are almost half of the anti-metabolite agents which have been indorsed in the last fifties of the 20th century which were either obtained from natural origins or these are the counterfeit derivatives of these products [xxxi]. Chemical agents obtained from plant or animal origin will include natural products and the derivatized products or counter-parts of naturally obtained components, and synthetic Analogues based upon the carbon pharmacophores identified in natural products [xxxii]. The rationale for establishing the new natural products as Mdm2 inhibitors is certainly based upon two crucial components including different sets of natural compounds exhibiting Mdm2 inhibition properties irrespective of p53 effect onto the cell or and that natural products may possess massive chemical divergence or a significant chemical stability and "drug-like" properties. Natural products exhibit greater firmness than synthetic products because of their highly stable stereo-chemical structures [xxxiii]. Other than these natural products often exhibit less toxicity than the synthetic products, as they are being isolated from dietary vegetables, fruits, or medicinal herbs. These are the reasons because of which natural products are opted as a major source for curing majority of disease since long time and are regarded as traditional or folk medicines [xxxiv]. Additionally, natural products are often reported with long lasting target effects and fewer side effects. Recently, a wide variety of natural products of considerably varying properties possessing considerable Mdm2 binding affinity at micro/nano molar concentrations ranging with different values, which lead to the development of Mdm2 inhibitors based upon natural origin.

GENISTEIN

Genistein (Source- *Glycine angustifolia / Apios americana)* is an isoflavone, majorly found in soy and soy-based food products which has been variedly used for the prevention and suppression of tumorigenesis [xxxv]. While, numerous studies have established that the significant anti-neoplastic effect of genistein can be observed both in prominent cultures as well as in animal models [xxxvi- xxxvii].

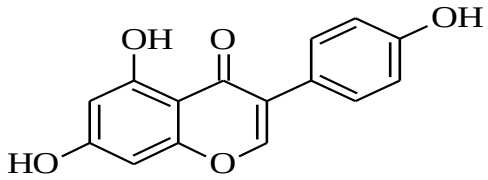

Figure 2: Chemical structure of Genistein

It is regarded as a plant secondary metabolite that consists of the 3-phenylchromen-4- one nucleus composed of two aromatic rings (A and B), where the rings are linked to another carbon pyran ring (C). Other than this it consists of basic functional moieties available in basic carbon skeleton is at C2–C3 double bond and an oxo group at C4 position of ring C. Additionally, there are three hydroxyl groups at C5,7and 4 ′ positions of ring A and ring B, respectively.

Table1: Mdm2 suppressing natural products (various natural products suppressing Mdm2 and expressing p53

S. No.	Compounds	Sources	Mechanism	References
1.	Platycodin D	*Platycodon grandiflorus*	Decrease the protein levels of Mdm2, MdmX and mutant p53	[80]
2.	Berberine	*Berberis vulgaris* *Berberis aristata*	Increases Mdm2 self-ubiquitination by disrupting its interactions decreases Mdm2 protein level	[xxxviii - xxxix]
3.	Parathenolide	*Tanacetum parthenium*	Promote ubiquitination of p53 bound Mdm2 and cause p53 activation	[xl]
4.	Origanum majorana extract	*Origanum majorana*	decline the protein expression level of mutant p53	[xli]
5.	Sempervirine	*Gelsemium sempervirens*	Inhibits Mdm2 E3 ligase activity	[xlii]
6.	Indole-3-carbinol	*Brassica oleracea*	Inhibits Mdm2-p53 binding and induces p53 phosphorylation	[xliii]
7.	Tricetin	*Eucalyptus globulus*	Inhibits Mdm2-P53 binding and may induce p53 phosphorylation	[xliv]
8.	Caffeine	*Thea sinesis*	Changes the mutation profile of p53 in epidermal patches	[xlv]
9.	Curcumin	*Curcuma longa*	Inhibits Mdm2 transcriptional activity through the mTOR pathway	[xlvi]
10.	Chalcone	*Glycyrrhiza glabra*	Inhibits Mdm2-p53 binding	[xlvii]
11	Gambogic acid	*Garcinia hanburyi*	Inhibits Mdm2 transcription and promote its ubiquitination	[xlviii]
12	Genistein	*Glycine angustifolia (G. max)* *Apios americana* *Cicer arietinum*	Inhibits NFAT1 mediated Mdm2 transcription and induction of self-ubiquitination	[xlix]
13	Oroxylin A	*Scutellaria baccalensis* *Oroxylum indicum*	Decrease Mdm2 protein expression level	[l]
14	Apigenin	*Apium graveolens*	Inhibits Mdm2 phosphorylation causing decreased level of Mdm2 protein.	[li]

15	25-OCH3-PPD and 25-OH-PPD	*Panax notoginseng*	transcriptionally down-regulating the Mdm2 protein	[lii]
16	Flavopiridol	*Dysoxylum binectariferum*	Transcriptionally down-regulates Mdm2 mRNA level	[75]
17	Makaluvamines	family of sponges of genus *Zyzzya.*	downregulate the expression level of MDM2 in concentration dependent manner	[91]
18	Matrine	*Sophora flavescens* *Radix sophorae*	Inhibit expression level of Mdm2 upon down-regulating Mdm2 mRNA synthesis	[96]
19	Melatonin	*Agaricus bisporus*	inhibit Mdm2 phosphorylation at transcriptional levels	[98]

It can be synthesized using trihydroxy benzoin, which, is obtained via acylation of phloroglucinol, with substituted phenyl acetonitrile using Hydrochloric acid and anhydrous Zinc Chloride in dry ether as catalyst.

Genistein is found to down regulate the Mdm2 oncogenes at both the transcriptional and posttranslational levels in a dose and time dependent manner where it is found to reduce Mdm2 levels in varied Human cell lines (HCT116 and MCF-7 cells) at a varied concentration of around 50µmol/L or with IC_{50}value of 24.8 ± 0.5µM (promptly depending upon time and concentration dependency) [liii].It was also observed that the NFAT transcriptional sites in the region between 132-33 in Mdm2 protein promoter which was receptive to Genistein. At Post transcriptional level, it may induce ubiquitination of Mdm2, certainly leading to elevated levels of p53 and p21 due to Mdm2degradation. It was found that Genistein certainly decreases the expression level of Mdm2 and Mdm2 oncogenes via p53 independent pathway [liv].

Additionally, genistein is found to decrease Mdm2 expression level without involving the tyrosine kinase inhibitory activity of the product, and consequently up-regulating p21 levels. Then by means of Subsequent In vivo studies it was observed that genistein's anti-metabolite actions are linked with its forbidding effects on Mdm2 expression [lv]. While the synthetic genistein and its 7-O-modifiedderivatives have been observed to have astounding cytotoxic effects in the *in vitro* studies therefore, these components have not been used for their forbidding effects for the oncogene [lvi].

Other than this, it may also exhibit a wide range of Biological and Therapeutic properties as antioxidant, anti-inflammatory, antiangiogenic, pro-apoptotic, and anti-burgeoning activities, all of which confers chemo-therapeutic or anti-neoplastic potential of genistein.

3.1 APIGENIN

Apigenin(4,5,7-trihydroxyflavone) is a plant derived non mutagenic flavonoid generally obtained from Apium species in the Apiaceae Family (celery, carrot or parsley family). Structurally, it possesses hydroxyl groups at C5 and C7 positions of ring A and C4 position of ring B [lvii]. It is a yellow-coloured crystalline powder and usually soluble in dimethyl sulfoxide or hot ethanol. Naturally, Apigenin is found as an essential component in chamomile tea, and also being obtained from dried flowers of *Matricaria chamomilla* with a concentration of 0.8-1.2%. or 3000-5000ug/g [lviii- lix]. It is a yellow-coloured crystalline powder It is also found in Chinese celery, vine spinach, oregano and artichokes in a good quantity. Apigenin has been found to inhibit HIF-1 and VEGF through p53 in A2780 or CP70 cells. It may cause increased level of p53 protein expression in a dose- and time-dependent manner.

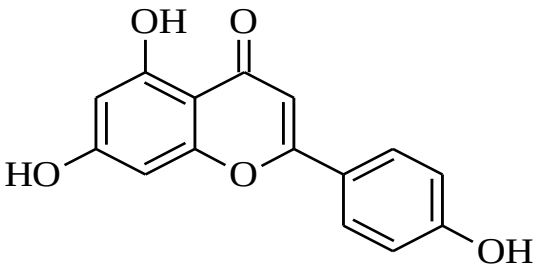

Figure 3: Chemical structure of Apigenin

Hdm2 consulate p53 self-ubiquitination by binding with it and energizing the addition of ubiquitin to the carboxyl group of p53 for its degradation [lx]. Therefore, the consequence of apigenin on expression of Hdm2 in A2780/CP70 cells were observed which impaired or downregulate the expression of Hdm2. It was observed that apigenin induce p53 through the decrease expression levels of Hdm2 and certainly inhibits the phosphorylation of Mdm2 by AKT in ovarian cancer cell lines (CP70 cells). In A2780/CP70 cells it was depicted that the incorporation of LY294002 prohibited phosphorylation of AKT and Hdm2. The total Hdm2 protein was declined whereas p53 expression was increased within a concentration 0f 10-80uM. It regulates Hdm2 probably via PI3K/AKT signalling. Hdm2/p53 signalling might be involved in the management of VEGF expression in ovarian cancer cells. It was seen that overexpression of HDM2 on the VEGF promoter reporter activities in A2780/CP70 cells [lxi]. Hdm2 adjusts VEGF transcriptional initiation through expression of HIF-1 protein. Other than this as per In vivo and in vitro studies Apigenin is found to induce cell cycle arrest by acting onto p53 target gene in Renal cell carcinoma (in cell lines like ACNH,786-0 and Caki-1) at a concentration of 20µM(30mg/kg) and evenly induce autophagy in leukaemia cells [lxii].

Traditionally it was used for the treatment of insomnia and anxiety (have anxiolytic activity), induction of anti-inflammatory, anti-toxicant and anti- cancer activity as well. It is also used as potent therapeutic agent in diseases such as auto-immune disorders, rheumatoid arthritis, Parkinson's disease and Alzheimer disease as well.

3.2 OROXYLIN A

Oroxylin A is a naturally occurring flavonoid which is being extracted from *Scutellariae radix* and dried roots of *S. baicalensis* and structurally possesses the 2,3-double bond and 5,7- hydroxyl groups [lxiii]. It was firstly extracted by *Shah.at.al.* using an effective method from the roots and bark of *Oroxylum indicum* where the roots were initially grinded and the content of the drug was extracted in alkaline solution along with 40-70% ethanol for elution purpose at constant temperature conditions

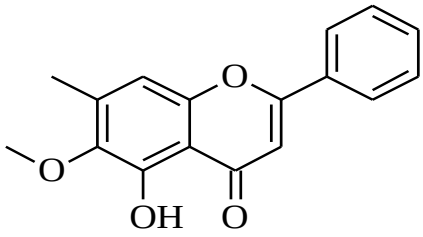

Figure 4: Chemical structure of Oroxylin A

. Then the concentrate was poured onto polyamide column for the purpose of purification and furtherly being eluted with a mixture of ethyl acetate and methanol and crystallised by means of petroleum ether and filtered to obtain oroxylin A crystals but in nowadays being extracted by means of Soxhlet extraction method.

It is found to induce programmed cell death in HepG2 hepatocellular carcinoma cells and evenly stabilize the p53 expression level at post translational level in order to induce apoptosis. This was brought into action by downregulating the expression levels of Mdm2 oncogene and also by interfering with Mdm2-modulated proteasomal degradation of p53 [lxiv]. It also forbided the cellular expansion of wild type-p53 cancer cells including MCF-7 and HCT116 cell lines. In the concentration of 100mg/kg, oroxylin A was found to downregulate Mdm2 expression level and increase p53 expression levels in MCF-2 cell lines. It also inhibited the glucose uptake and lactate generation in both the cell lines at a concentration of 100-200 µM concentration. It also possesses a wide range of therapeutic properties including anti-cancer, anti-inflammatory, neuroprotective and anti-coagulation effect as well.

3.3 FLAVOPIRIDOL

Flavopiridol is a semi-synthetic flavonoid which is structurally similar to naturally derived alkaloid which is isolated from *Dysoxylum binectariferum* which is indigenous

to India [lxv]. It may inhibit gene expression and lead to apoptosis by inhibiting cyclin-dependent kinases that usually governs cell cycle. It induces p53 by inhibiting MDM2 levels transcriptionally which may result in superinduction of p21 and MDM2.and accumulation of p53 at a concentration of about 100 nM and certainly inhibit cellular expansion and may also cause programmed cell death in A549 and PC3M cancer cell lines.

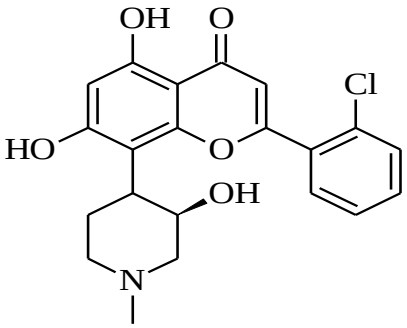

Figure 5: Chemical structure of Flavopiridol

Its IC_{50} value may range from 50 - 500 nM. It may arrest at G2/M phase and induce cell apoptosis and certainly down-regulating levels of Mdm2 mRNA level p53 independently [lxvi]. It is used in the treatment of oesophageal, leukaemia, lung carcinoma. It is also help in therapy of non-Hodgkin lymphoma and also possess anti-inflammatory function as well. While it may possess side effects like secretory diarrhoea and pro-inflammatory syndrome associated syndrome as well.

3.4 GINSENOSIDES

Ginsenosides are the significant class of steroidal compounds which are being obtained out from various Panax species which may have a diverse pharmacological activity as for anti-cancer, anti-inflammatory and anti-diabetic activities [lxvii]. They may evenly improve the antioxidant and blood circulation. Ginsenosides have been identified to suppress the Mdm2 activity and induce the apoptosis through upregulation of p53Such as 25-OCH_3 –PPD [lxviii].

It is a novel ginsenoside which was being obtained from the dried leaves of *Panax notoginseng* and considerably possesses cellular-toxicity effect against twelve cancer cell lines. As per the *in-vivo and in-vitro studies,* it is found to be effectual against human prostate, pancreatic and lung cancer [lxix]. It exhibits its properties by transcriptionally down-regulating the Mdm2 protein levels and certainly up-regulating the p53 expression level in a dose-dependent manner that is usually lying between 5-10 mg/kg.

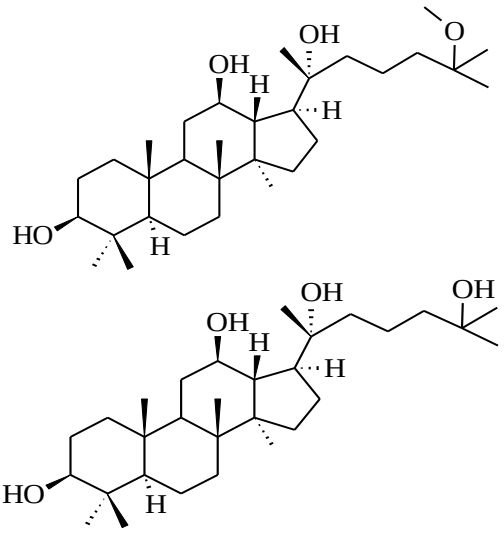

Figure6: Chemical structures of 25-OCH3-PPD and 25-OH-PPD

It is promptly found to exhibit such properties by of prompting Mdm2 ubiquitination and degradation. It is found to inhibit gall bladder cancer cellular multiplication, cell quiescence at G1 phase and evenly promoting the cell senescence and caspase-mediated cell death by down regulating Mdm2 protein levels [lxx].

Ginsenoside 25-OH-PPD has been also found to possess such effects in a dose dependent manner at a comparatively low IC_{50} values of 10-60 µM and induce apoptosis, proliferation and evenly the cell cycle progression *via* down-regulating the Mdm2 protein levels [lxxi].These ginsenosides are widely used to address cardiovascular risk factors including hypertension and hypercholesterolemia and evenly be useful in strengthening the immune system, enhance brain functions, in fatigue and also helps in improving the symptoms of erectile dysfunction.

3.5 PLATYCODIN D

Platycodin D a traditional Chinese Medicinal herb commonly termed as Jiegeng or Balloon Flower. It is widely areas of found in China and Japan. This Triterpenoid Saponin (Bio-Active Agent) was isolated from the roots and earthly

parts of *Platycodin grandiflorum* and was determined to be 0.2-0.018% of its dry weight respectively. It can evenly be obtained from platycoside E and D3 by means enzymatic modification. While the yield of platycodin D by Enzymatic method is bi-formed in contrast with the direct isolation and purification techniques from methanolic extract of roots [lxxii].

Platycodin D would inhibit triple negative breast cancer and the growth of MDA-M231 cancer lines *in vitro* along with the xeno-graft tumor growth *in-vivo* which may have certain restrictive effects on Mdm2 and MdmX as well and certainly upregulates the expression level of p21 and p27. It may also decrease the levels of mutant p53 in the given cell lines. It has been shown to decrease cellular viability by inducing cellular apoptosis at IC_{50} value of 7.77± 1.86µM.

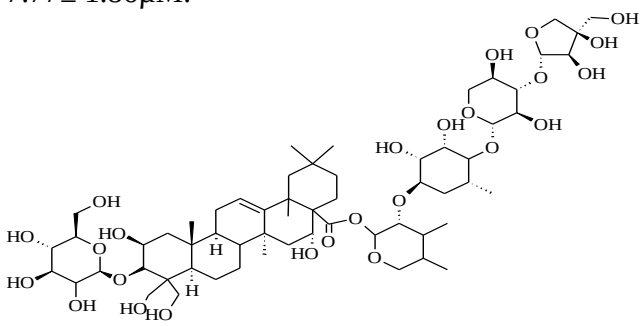

Figure 8: Chemical structure of Platycodin D

Further platycodin D cause significant decrease cell proliferation of MDA-MB-231 cell lines in a concentration dependent manner (concentration > 5µM) to induce apoptotic effect. It may induce cell cycle arrest in Go/G1 phase evenly cause apoptosis, autophagy and angiogenesis, invasion and metastasis by targeting multiple signalling pathways [lxxiii]. It also possesses a wide variety of biological properties including anti-atherosclerotic, spermicidal and contraceptive activity, antiviral, anti-inflammatory, immune-regulatory, anti-hepatotoxicity and anti-metabolite activities along with hypocholesterolaemia activity as well.

3.6 BERBERINE

Berberine is a (organic hetero-pentacyclic compound) plant iso-quinoline alkaloid with therapeutic application as in both Ayurvedic and Chinese medicine. It is certainly available in varied plant species such as *Hydrastis Canadensis*, *Coptis chinensis*, *Berberis aquifolium*, *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). The berberine alkaloid can be obtained from the roots, rhizomes, and stem bark of the plant species [lxxiv].

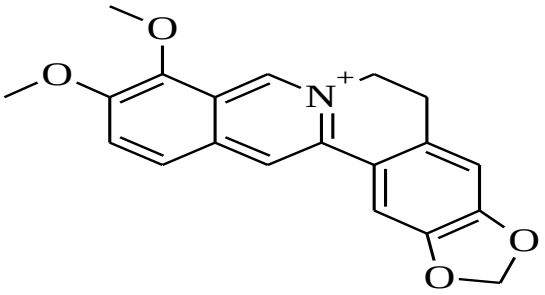

Figure 9: Chemical structure of Berberine

Berberine has been found to induce apoptosis in acute lymphoblastic leukaemia (ALL) cells lines including EU cell lines (pre-B cell leukaemia EU1-EU-6) at a concentration of 50umol/L by decreasing the oncoprotein expression level. The apoptotic actions of the compound were associated with both the Mdm2 expression levels and p53 protein level in ALL (leukemic) cell lines. Berberine strongly induced persistent downregulation of Mdm2 at a post-translational level via modulation of DAXX, which would have a deep influence on the Mdm2–DAXX interactions and thereby promoted Mdm2 self-ubiquitination and degeneration followed by a steady-state activation of p53 suppressor gene. Numerous berberine derivatives and analogs including palmatine, coralyne and sangiuinarine have been found to have remarkable cell-toxicity against varied types of cancer cell lines [lxxv- lxxvi].

Berberine have significant antimicrobial activity against a various type of organisms including bacteria, viruses, fungi, protozoans, helminths etc. Berberine can evenly be used against several disorders including metabolic, neurological and cardiological problems. It can also be used as an antilipidemic, hypoglycemic, an antioxidant, a potassium channel blocker, an antineoplastic agent as well [lxxvii].

3.7 PARTHENOLIDE

Parthenolide (*Tanacetum parthenium* / Extract of *Magnolia grandiflora*) is a sesquiterpene lactone belonging to germacranolide class which occurs naturally in feverfew plant *Tanacetum parthenium*. Its major concentration

is available in the flowers, leaves and fruit with 0.1-2% of its dry weight, respectively [lxxviii]. Significantly, parthenolide exhibits its cancer specific activity where its non-toxic to normal cells making it an appealing candidate for drug development. However, the high lipophilicity of the compound limits its bioavailability, requiring the synthesis of analogues with improved solubility with much needed hydrophilic character [lxxix].

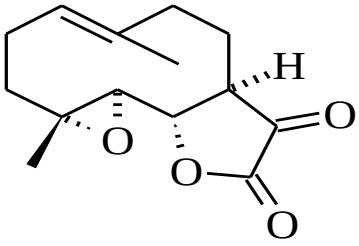

Figure 10: Chemical structure of Parthenolide

Parthenolide treatment in mammary cells (ZR-75-1 cell lines) with a concentration of 15µmol/L causes accumulation of MDM2, mRNA induced self-ubiquitination and proteasomal mortification of MDM2 in an ATM-dependent manner, much like in HDAC1, which resulted in the activation and rapid increment of p53 protein in a concentration and time-dependent manner [lxxx]. This traditionally used medicinal plant, has been used for treating inflammation, stomach ache, fever, and rheumatoid arthritis and migraines since long time and now be well known for its antitumor activity. The compound antitumor effects can be seen against various types cancer cell lines including AML, ALL, brain, breast, colon, pancreas, prostate, and skin. It's well known analogue dimethyl-amino-parthenolide has found to be an effective anti-metabolite agent in models of leukaemia, lung, bladder, and pancreatic and breast cancers[lxxxi].

3.8 MAKALUVAMINES

Makaluvamine belongs to the class of marine pyrrolo-imino-quinolone alkaloids which are being isolated from family of sponges of genus Zyzzya. Almost fourty makaluvamine analogues have been found to possess anti-neoplastic effect [lxxxii]. As per *in-vitro* and *in-vivo* studies, it is found to have inhibitory effects on topoisomerase II and by means of reductive activation it may be involved in DNA damage also [lxxxiii]. Six of its novel analogues including FBA TPQ (IC_{50}-0.1-1.8 µM); PEA-TPQ (IC50-0.1-2.5 uM); MPA-TPQ (IC50-0.6-4.9 uM); DPA-TPQ (IC50- 0.3-24.4uM); TCBA-TPQ (IC50-0.39-1.41uM) and BA-TPQ (IC50-0.1-0.4uM) have been reportedly found to up-regulate the level of tumor suppressor gene and evenly downregulate the expression level of MDM2 at a concentration of 1.0µM in both In-vivo and In-vitro models of breast cancer and evenly lead to the activation of MDM2- p53 feedback loop in ovarian cancer cell lines[lxxxiv].

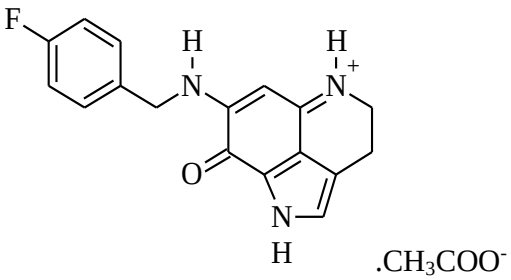

Figure 11: Chemical Structure for Makaluvamine FBA-TPQ

Other than that, it has been found to possess cytotoxicity effect in almost 13 cancer cell lines and work in a dose- dependent manner in order to inhibit the cell expansion so as to induce programmed cell death and cell cycle arrest and cell augmentation, independent of p53 status. Other to this these agents have been found to have downregulating effect on Mdm2, CycD1, Cdk2 and 4, Cdk4, and E21 expression level as well upregulating the p53 expression levels in MCF-7 cells and MDA-MB-468 cells[lxxxv].

3.9 MATRINE

It is a plant based quinolizidine alkaloid which was firstly being obtained from the roots of traditional Chinese herb *Sophora flavescens* or from *Radix sophorae* species which has been found to inhibit expression level of Mdm2upon down-regulating Mdm2 mRNA production and evenly be involved in etoposide- induced apoptosis in liver cancer cell lines usually independent of p53 status [lxxxvi]. It has also been reported to inhibit the cell proliferation and induce cell cycle metastasis in K562 leukemic cell lines as well as in hepatic cell lines (SMMC-7721) at a considerable time and dose dependent manner [lxxxvii].

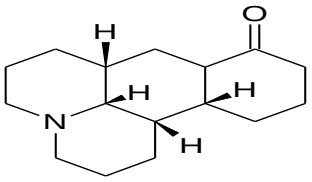

Figure 12: Chemical structure for Matrine

3.10 MELATONIN

Melatonin is a mono-amine alkaloid that has been widely found in various types of foods from fungi (*Agaricus bisporus*) or from animals and plants products including eggs and fish whereas in plant obtained diets ground-nuts, cereals and germinated legumes have the richest content of melatonin in them [lxxxviii]. It has been observed that its amount in human serum could significantly increase after consumption of melatonin rich diets.

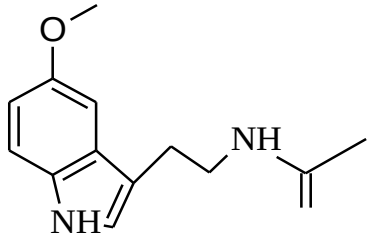

Figure13: Chemical structure for Melatonin

Melatonin was found to inhibit Mdm2 phosphorylation at transcriptional levels in MCF-7 breast cancer cells and evenly enhance p53 acetylation and evenly results in disruption of p53-Mdm2 binding and p53 stability while their effects are generally observed in *in-vivo* cancer models[lxxxix].Melatonin exhibits different therapeutic and pharmacological responses such as anti-oxidant activity and anti-inflammatory characteristics, anti-neoplastic activity, cardiovascular preventing activities, neuroprotective and anti-aging activity, anti-diabetic, anti-obese as well as immunity-booster properties[xc].

3.11 SEMPERVIRINE

Sempervirine is a reddish-brown alkaloid obtained from *Gelsemium sempervirens* roots (Carolina-jasmine), family Gelsemiaceae, native-America: Honduras, Guatemala, Belize, Mexico and south eastern and south-central United States. Almost all parts of the plant contain the toxic strychnine related alkaloids gelseminine and gelsemine and should not be ingested without treatment [xci]. Despite having significant safety concerns, the root and underground stem (rhizome) of gelsemium are used to produce medicine. Sempervirine preferentially inhibits Mdm2 auto-ubiquitination in a dose-dependent manner with IC_{50} value of 8 ug/ml and accumulates p53 and Mdm2 in cells. p53 accumulates in SN-treated RPE cells preferentially RPE-E1A and RPE cell. These were observed by means of western blotting technique. SN settle down the Mdm2 in p53/Mdm2 MEFs as well in order to observe the apoptotic effect [xcii].

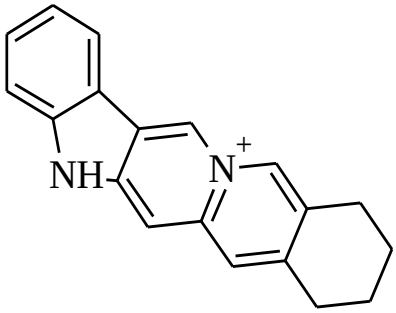

Figure 14: Chemical structure for Sempervine

Sempervirine preferentially induced apoptosis in transformed cells expressing wild-type p53.It possess anti-coagulant, anti-microbial, anti-parasitic, insecticidal, wound healing, anti-neoplastic, estrogenic as well as anti-oxidant effect also [xciii].

3.12 ORIGANUM MAJORANA EXTRACT

Origanum marjorana is a cold-sensitive perennial herb with sweet pine and citrus flavours and widespread worldwide (family Lamiaceae or Labiatae). It is native to Cyprus, Turkey, Mediterranean, Western Asia, Arabian Peninsula and the Levant [xciv]. It is highly rich in phenolic compounds such as thymol, carvacrol, luteolin, arbutin, oleanolic acid etc.

Preparation of the Extract: -

Dried leaves (5g) were crushed into fine powder along with 100ml of absolute ethanol (70%). Then this mixture was kept into dark room at 4degree C for about 72 hours in a refrigeration chamber without stirring.

Then this content was filtered through a glass funnel in-order to collect the filtrate which was then evaporated at room temperature using a rota-vapour.

Then a green coloured residue was obtained and collected which was even kept under vacuum for about 3 hours and molecular weight of the product was recorded and finally obtained it percentage purity [xcv].

It has been observed the effect of *Origanum majorana* extract on the expression of pro-apoptotic protein p53 in MDA-MB-231 cell lines. It was observed that at low concentrations of 150-300 mg/mL of the extract, there was a slight rise in the protein levels of mutant p53 while at a slight higher concentration of around (450-600 mg/mL of majorana extract) absolute enervation of mutant p53 level in MDA-231 cells were observed. As mutant p53contribute with cancer cells more resistance to anticancer drugs, terminating the effect of mutant p53 may there-after offering a promising perspective for cancer treatment and prevention [xcvi].

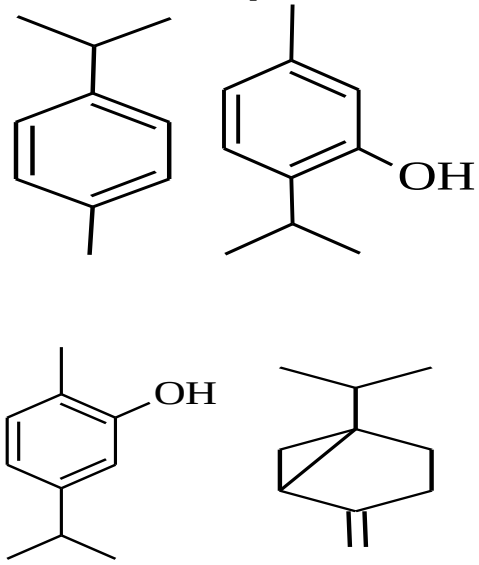

Figure 15: Chemical structures of compounds found in *Origanum majorana* extract [p-cymene(A); thymol(B); carvacrol(C); sabinene(D)]

It exhibits antimicrobial activity. It is used as a home remedy for various conditions such as chest infections, rheumatic pain, stomach and nervous disorders and in cardiovascular disease as well. Some of the studies have even reported that it may be used to forestall kidney and liver damage as well as genotoxicity generally caused due to lead acetate. It may evenly be used to impede platelet production and aggregation [xcvii].

3.13 TRICETIN: Tricetin (Source-*Eucalyptus globulus of Myrtaceae family*) is a flavonoid derivative obtained from Myrtaceae pollen and Eucalyptus honey [xcviii]. Its content may range from 202 - 769.9µg/100g of honey obtained from E. globulus, while its content would vary with different species Tricetin-3-O-glycoside is one of the major constituent present in eucalyptus honey. It is a triglyceride which is obtained on acetylation of three hydroxy groups of glycerol [xcix].

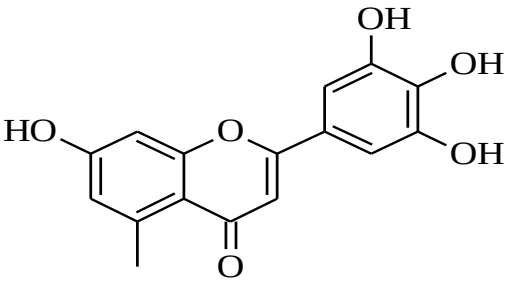

Figure 16: Chemical structure for Tricetin

Human breast adeno-carcinoma cells (MCF-7 cells) were used to report the molecular mechanisms of tricetin for accessing its anti-proliferative activity. It would cause cell cycle arrest at the G2 or M phase and observed to induce an apoptotic response. Some of the studies have found the stability of p53 is majorly regulated by phosphorylation of various cell sites. It was observed that its treatment with p53 has resulted in the amplification of p53 DNA binding activity with a IC_{50} value of 37.12µM or at a significant concentration varying from 10µM-60µM. The enhancement of p53 transcriptional activity is generally related with the phosphorylation of p53 at its Ser392 sites. Furthermore, the p53-MDM2 interaction declined in a time-dependent manner, which correlates with the phosphorylation of p53 at Ser15 sites as well [c]. It presents a potent anti-inflammatory activity. It has fungistatic properties as well and hence being used for topical treatment of minor dermatophyte infections. It has a major role as a plant-metabolite, an adjuvant, a food additive carrier, a food emulsifier and humectant as well. Though it has activity as a fuel additive and a solvent as well [ci].

3.14 GAMBOGIC ACID AND MANGOSTIN

These are the prenylated xanthones which are naturally obtained from the fruit of *Garcinia*

mangostana of family Clusiaceae and can also be obtained from the resins of *Garcinia hanburyi* [cii]. These have been observed to have potent anti-neoplastic effect against various cancer cells. While their Mdm2 inhibitory activity has been observed in yeast cells accompanying the MCF-7 tumour cell lines. At a lower concentration of 10µM they have been found to have inhibitory effect on Mdm2 at transcriptional levels and certainly lead to a rise in the Bax protein levels and along with p53 expression [ciii]. This way they will induce apoptosis and cause cell cycle arrest at G2 and M phase in tumour cell lines concealing wild type p53. Along with this Gambogic acid is found to inhibit the growth of tumour in Breast cell line (MCF-7) and non-small cell lung cancer (H1299 cells) at a IC50 value of 3.5uM. As whole found to cause Mdm2 transcription and promote its ubiquitination and degradation [civ].

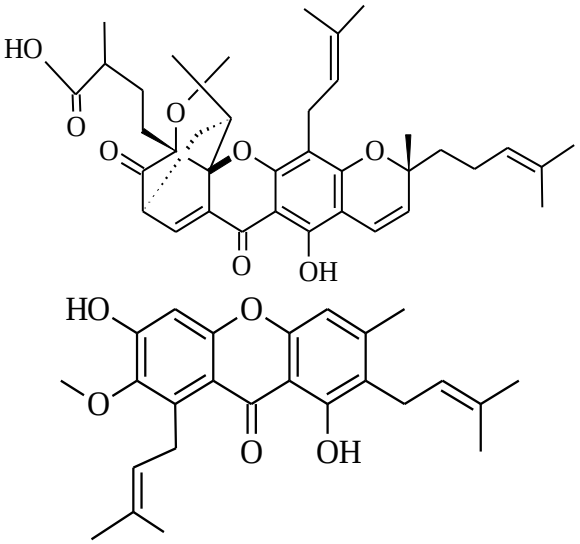

Figure 17: Chemical structures of Gambogic acid and α-Mangostin

GA sensitizes AML, gastric cancer, pancreatic and prostate cancer along with renal carcinoma and multiple myeloma. It usually exhibits its chemotherapeutic role by synergistically acting with other drugs like mangostin, cisplastin, doxorubicin, fluorouracil and other proteasome inhibitors.

3.15 CURCUMIN

Turmeric is a rhizomatous herbaceous flowering plant (Source- Curcuma longa) belonging to Zingiberaceae Family and is native to tropical South Asia. Its major constituent Curcumin(diferulomethane) is a dietary polyphenol [cv].

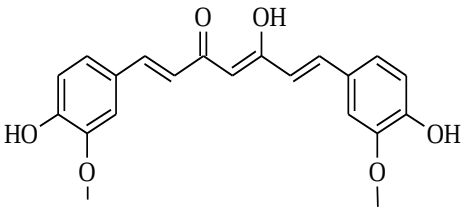

Figure 18: Chemical structure of Curcumin

It exerts its anti-cancer activities by down-regulating the levels of Mdm2 transcriptionally and p53 independently through MTOR/ ETS2 pathway. It inhibits tumour cell growth in PC3 cells or xeno-type models and enhance anti-tumour effect of gemcitabine [cvi]. It will induce cell cycle arrest at various phases of cancer development by upregulating ATF3 level as well as stabilizing p53 expression level at a concentration of 15-30µmol/L and certainly lead to apoptosis by inhibiting cell proliferation or colony formation. It is useful in the management of oxidative and inflammatory conditions, anti-mutagenic anti-microbial and anti-cancer activities along with therapeutic activities as in metabolic syndrome, arthritis, anxiety and hyperlipidaemia as well [cvii].

3.16 INDOLE-3-CARBINOL

Indole-3-carbinol (Source-*Brassica oleracea*) belongs to the class of compounds that is indole-glucosinolate, which is derived from the degradation of glucobrassin, a component which is usually found in cruciferous vegetables belonging to *Brassica* genus including broccoli, cabbage, cauliflower, kohlrabi, Brussels sprouts etc [cviii]. These vegetables are usually rich source of sulfur-containing compounds (glucosinolates) which have a bitter taste and produce a pungent smell well.

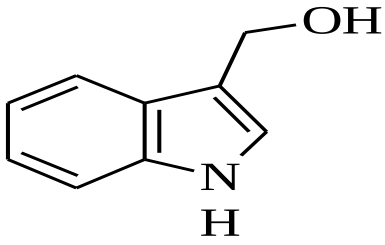

Figure 19: Chemical structures of Indole-3-carbinol

Indole-3-carbinol is found to inhibit the Mdm2- p53 binding and may also induce p53

phosphorylation. During studies it was observed that its treatment with a concentration of 300µM in MCF10A breast cancer cell lines may cause G1- cell cycle arrest by the activation of ATM signalling pathway [cix]. It was observed that the activated ATM phosphorylates p53 at serine 15, and thereby disrupts the interaction between Mdm2 and p53. This will certainly lead to the stabilization of p53 and upregulates p21 expression and furtherly cause cell arrest. It is also used for the prevention of different types of cancer, in supporting the immune system, fibromyalgia, laryngeal papillomatosis, cervical dysplasia and also for balancing certain hormone levels and for the detoxification of liver and intestines as well [cx].

3.17 CAFFEINE

Caffeine is a naturally occurring methylxanthine alkaloid that is found in fruits, leaves and seeds of tea (*Thea sinensis*), coffee (*Coffea arabica*), cocoa and guarana (*Paullinia cupana*) plants and is native to Africa, East Asia and South America [cxi]. It is a central nervous system stimulant which is extensively used as a psychoactive drug.

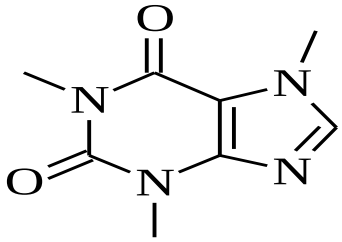

Figure 20: Chemical structures of Caffeine

Caffeine is also found to have an inhibitory effect by affecting the Mdm2-p53 interaction. It was found that caffeine with a concentration of 50µM in A549 cells (Adenocarcinoma human alveolar basal epithelium cells) [cxii]. Initially may cause a decline in p53 protein levels due to proteasomal – mediated interaction but after a 4-8hours treatment with the drug the levels of p53 will steadily upregulates which is due to ATM/ATR activation which certainly leads to the stabilization of p53 by phosphorylating it at Ser18 sites [cxiii}.

3.18 CHALCONES

Chalcones are plant derived polyphenolic compounds belonging to Flavonoids family having a wide range of biological activities [cxiv]. It is abundantly found in fruits (citruses like lemon and oranges, apples, various vegetables like tomatoes, beans and potatoes along with spices like Liquorice (*Glycyrrhiza glabra*). Chemically these are α, β- unsaturated ketones which usually consists of 2 aromatic rings connected with the unsaturated carbonyl system. Chalcone is a biosynthetic product of plant obtained via shikimate pathway. These can also be synthesized chemically by means of Aldol Condensation (Benzaldehyde + acetophenone react in presence of a base (KOH/ C_2H_5OH) at a temperature of 25degree Celsius. It is regarded as the precursors of various flavonoids and iso-flavonoids [cxv].

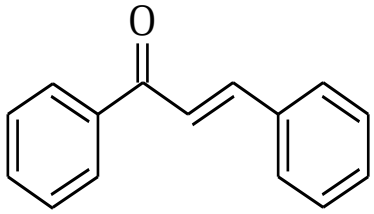

Figure 21: Chemical structures of Chalcone

It has been observed that chalcone and its derivatives gets bound to Mdm2 at p53 transactivation domain and results in the release of p53 p53-Mdm2 DNA bound complex along with Mdm2-p53 complex . In one case it has been demonstrated that the carboxylic acid bound chalcone derivative when placed near the base of K51 lysine leads to the formation of salt bridge and finally lead to dissolution of Glut25 interaction present within mdm2-p53 complex [cxvi].

It has wide range of biological and therapeutic activities including anti-microbial, anti-cancer, anti-leishmanial, anti-viral, anti- oxidant, anti-inflammatory and anti-mycobacterial effect, cardiovascular diseases as well.

3. CONCLUSION

The p53-Mdm2 autoregulatory loop pathway is usually disturbed in cancer initiation, progression, and metastasis where tumor suppressor role of p53 and the oncogenic role of Mdm2 are well characterized in various cancers. So, targeting the p53-Mdm2 pathway can be a novel approach for the development of various compounds for cancer treatment and prevention. Therefore, number of natural Mdm2 suppressing agents have been developed

to target the p53-Mdm2 pathway via various mechanisms including inhibiting Mdm2 expression, protein stability or E3 ligase activity of Mdm2, targeting the p53- Mdm2 interaction, or even reactivating the wild-type functions of mutant p53. These natural products have major potent chemo-preventive and chemotherapeutic activity in various in vivo and in vitro studies with least toxic effects.

A number of natural products (example include genistein, curcumin, makaluvamines, chalcone, parthenolide and semperavine) have been identified to target Mdm2, p53 or the p53-Mdm2autoregulatory pathway, and certainly identified to induce cell apoptosis, cell proliferation via upregulating p53 expression levels.

Conflict of interest: Authors declare no conflict of interest.

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