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NEAR EAST UNIVERSITY

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ANTICANCER AND ANTIOXIDANT EFFECTS OF *Lycium barbarum*

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BIOCHEMISTRY PROGRAM

MASTERS THESIS

ADVISOR

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I would like to first give thanks to God Almighty for strength, wisdom and the zeal to keep at it and finally finish my postgraduate studies.

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ABSTRACT

Eric Kiama. Anticancer and Antioxidant effects of *Lycium barbarum*. Near East University, Graduate School of Health Sciences, Graduation Thesis in Biochemistry Program, Nicosia, 2018

Cancer is one of the topreasons of demise in the world today.It is caused by uncontrolled growth and multiplication of cells. Reactive oxygen species are chemicals that can cause oxidation in an organism leading to damage of the cells. In this research, we study and report on the effect of phytochemical *Lycium barbarum.* L polysaccharide against both cancer and oxidation. *Lycium barbarum* has been present for many 2000 and has mostly been used as a traditional medicine to help in improving the well-being for individuals and as an anti-aging remedy.Today it is sold as a "super food" to most parts of the world. Despite little knowledge on the biochemical processes, it was observed that *Lycium barbarum* Polysaccharide (LBP)shows both antitumor and antioxidant properties. Phytochemical tests have been carried out on the plant and it has shown a reduction of tumour growth in cells via cell cycle arrest and apoptosis. This is brought about by the inhibition of cell cycle proteins namely cyclins and cyclin dependent kinases(CDKs)by gene regulation mechanisms. Apoptosis can also be brought about via the mitochondria pathways activated mostly by an apoptotic stimulus sent to the cell. *Lycium barbarum* hasshown various antioxidant properties when used on cells damaged by oxidation by increasing antioxidants such as glutathione and enzymes levels, which aid in combating free radicals.

Keywords: *Lycium barbarum* polysaccharide, anti-cancer, anti-oxidant, apoptosis, tumour,

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LIST OF ABBREVIATIONS

AA-2BG: 2-O-β-D-glucopyranosyl-L-ascorbic acid

Apaf-1:Apoptotic protease activating factor 1

BDMC : Bone marrow dendritic cells

CDKs:Cyclin Dependent Kinases

DC: Dendritic cells

DNA: Deoxyribonucleic Acid

GSH: Glutathione

GSH-Px: Glutathione peroxidase

H2O2: Hydrogen Peroxide

IFN-ɣ : Interferon-ɣ

IL-2: Interleukin 2

kDa: Dalton

LBP:*Lycium barbarum* polysaccharide

MCF-7 : Michigan cancer foundation 7

MDA: Malondialdehyde

Mw: Molecular weight

NF-kB : Nuclear factor kappa-light-chain-enhancer of activated B cells

Nrf2: Nuclear factor erythroid- 2 related factor 2

ROS: Reactive Oxygen species

SOD: Superoxide Dismutase

Sp: Species

TC:Total cholesterol

TG: Triglyceride

TNF-α: Tumour necrosis factor

Vit: Vitamin

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1. INTRODUCTION

The genus *Lycium* contains about 80 species of shrubs and small trees growing in subtropical and temperate areas. *Lycium barbarum* L. commonly identified as goji berry belongs to the Solanaceae family. It is normally found growing on sandy loam and alkaline soils.Mostly found in the Southwest and Central Asia and Mediterranean countries (Potterat,2010). It has been in use for thousands of years as a traditional Chinese medicine, source of food and in the making of beverages. With the progress of analysis methods; various chemical ingredients and pharmacological properties have been discovered. *Lycium barbarum* polysaccharides (LBP) are one of its main primary components. There are about 30 polysaccharides that have been identified so far. The bioactivities of the polysaccharides are affected by physiochemical properties and chemical structure.It is said to have varying biological and pharmacological activities such as anti-oxidant, anti-aging, anti-tumor, anti-fatigue and immunoregulatory. Other constituents include amino acids, trace elements,carotenoids, protein and flavonoids. Carotenoids mainly include β-carotene, β-cryptoxanthin and zeaxanthin.The objective of this study was to research and report on the anti-cancer and antioxidant effects of *Lycium barbarum.* Though various clinicals trials and research are still ongoing on *Lycium barbarum* and its chemical components, LBP has been used as a treatment for antitumor and it has been seen to cause cell arrest on the G0/G1 and S phases through apoptosis and this has an effect by reducing tumor size and growth. Antioxidant properties have effectively been shown by LBP whereby itincreases the levels of antioxidant enzymes such as SOD, catalase and GSH-Px in cells which are chief in fighting off free radicals.

2. GENERAL INFORMATION

Plants have been in existence for millions of years and are important inthe ecosystem. They are mostly a source of food giving muchnutrition needed. Over the last 3 decades, plant have been collected, investigated and evidence has shown that not only do they have nutritional value but contain active biologicalcompounds that are important. Phytochemical studies have indicated richness in various constituents such as polysaccharides, carotenoids, flavonoids, alkaloids, amides and so on. This has been used to create a pool of data where one can refer to while seeking knowledge about the plant kingdom. *Lycium barbarum* L.is commonly consumed as food,used in the manufacture of certain beverages and as herbal medicine (Wagner et al. 2011). It is also marketed as a superfood in Europe and some parts of N. America (Wolfe, 2010; Chang & So, 2015). When investigated using the various scientific procedures, biological active compounds were isolated from the plant (Qian et al. 2017; Yao et al. 2011.).Theyseem to be effective in treatment of numerous diseases such as cancer, diabetes and in the manufacture of synthetic drugs. Ongoing clinical trials basis of the plant are being used to show its full potential and help to discover newer active compounds that may be of use in science today.

2.1. ***Lycium Sp.***

2.1.1**Botany**

They are mostly shrubs, thorny and can grow upwards between 1-4 meters. Its leaves are narrow, fleshy and small and are alternately arranged on the branches. Flowers are mostly in clusters and are funnel or bell shaped with a corolla that is purple-white in colour. Fruits are found in 2 chambers and are orange-red or yellow in colour. They contain many seeds inside the fruits. *Lycium* sp are either monoecious with male and female parts or gynodioecious plants. It is part of the genus of flowering plants in the nightshade family of Solanaceae. They are distributed all over the world and are mostly found in continents with subtropical and temperate regions. There are about 80 known species of *Lycium*and mostly located in South America, North America & Southern Africa.*Lycium* sp was first printed by Linnaeus

and only 3 species (*L.europaeum*, *L.barbarum*&*L.afrum*) were known and described in species Planturum (Linnaeus, 1753). In 1932 Hitchcock published a systematic taxonomic study on 43 *Lycium* species. *Lycium* first originated in the Americana continent and later was dispersed to other parts of the world as investigated by (Olmstead et al.1999; Fukuda, 2001; Miller, 2002; Levin et al. 2009a).

2.1.2. **Traditional uses**

About 28 of the species plant parts have been used for medicinal purposes.They have been used to sustain body organs such as liver and kidney, improve eye sight, enrich the blood, improve sexuality and many more. Leaves, fruit, bark root and young shoots are consumed. The leaves and bark are used in making most medicines while the young shoots are consumed as food. The berries are commonly used in the making of teas or consumed as food. In today's society it is used clinically for its purposes which are anti-cancer, anti-oxidant, anti-aging, anti-diabetic, growth stimulant and many more. It is also useful in the brewing of certain beverages. *Lycium chinese* and *Lycium barbarum* have been marketed as "superfoods" and are consumed all over the world.

2.1.3**Biological activities**

Phytochemical investigation of *Lycium* species have showed that the fullness in constituents of diverseclasses such as polysaccharides, carotenoids, amides, flavonoids, terpenoids etc. gives it a variety of biological activities(Yao et al.2017). The table below is a summary:

Table 1: Biological activities and major compounds of *Lycium* sp. (Yao et al. 2017)

Bioactivity	**Compounds, extracts or plant constituents**	**Reference**
Antioxidant	Flavonoids, polysaccharides, pigments, fatty acids	Le et al.2007; Li & Zhou 2007; Bai et al.2008; Wang et al.2010
Anti-aging	Polysaccharides, vitamins,pigments	Bucheli et al.2011; Kim et al.1997; Tao et al.2008; Yi et al.2013
Immunomodulation	Polysaccharides-protein complex, polysaccharides, pigments	Zhang et al.2014; Chen et al.2012; Xie et al.2016; Gan et al.2004
Anti-tumour	Polysaccharides-protein complex, polysaccharides, scopoletin, AA-2Bg	He et al.2012; Cui et al.2012; Tang et al.2012; Hu et al.1994
Anti-microbial	Lyciumoside I, AcOEt-soluble fraction	Terauchi et al.1998; Lee et al.2005; Kim et al.2000
Anti-diabetic	Water extract, polysaccharides, organic acids, alkaloids	Ye et al.2008; Song et al.2012; Li et al.2004; Jia et al.2003; Luo et al.2004
Anti-fatigue	Betaine, Polysaccharides	Wu & Guo 2015; Kim & Back 2014
Neuroprotective	Alkaline extract, Polysaccharides, Water extract	Ho et al.2007; Chan et al.2010; Mi et al.2013; Wang et al.2014

2.2. *Lycium barbarum* L.

2.2.1 Origin and local name

Lycium barbarum was named by Carolus Linnaeus, a Swedish botanist in 1753 while its botanical name was designed and given by Philip Miller in 1768, a British botanist (Dharmananda, 2007). It was later included in the new taxonomy system set by the Department of Agriculture in the United States of America (ITIS 2007), (USDA 2010a). It grows naturally in parts of China (Bensky & Gamble, 1993). Goji berries are harvested during the summer and autumn where they are dried, and the pulp is removed for further processing (Zhu 2008). Some of the local names identified with *Lycium barbarum* include Chinese wolfberry, Barbary wolfberry, red medlar, lycii berry, lycii fruit, *Lycium* fruit.

2.2.2. Botany

Lycium barbarum L. is a shrubbery that is located in North West China, Tibet and other part of Asia. It grows up to 3 meters high. It has grey-green leaves that are alternate, lanceolate and gradually narrow to the petiole. It has 1-3 auxiliary flowers and the calyx and pistil are fused. Its corolla is funnel shaped with light purple violet with a 5-lobbed margin. It has a 4 stamen that is hairy at the base and its ovary has 2 chambers with 1 style (Amagose, Sun, & Borek. 2009). Its fruit commonly known as Goji berry or Wolfberry is 1-2 cm long, bright orange-red in colour and is an ellipsoid berry. Its leaves areovoidal with an apex of 6-20mm and a length of 3-8mm in diameter (Potterrat, 2010; Zhao et al, 2009).Photo of fruits and leaves are shown in Figure 1 while the photo of the fruit is shown on Figure 2.

Figure 1: Fruits and leaves of*Lycium barbarum* (Goji berry)

Figure2: Dried fruits of *Lycium barbarum* (Goji berry)(Tang et al.2011)

2.2.3**Traditional uses of *Lycium barbarum***

Traditionary it was used as food, medicine oralcohol brewing. Its fruits were dried up and soaked to form a local alcoholic drink. It was used as a medicine and nutritional purposesby locals around China and other neighboring countries (Bensky & Gamble,1993; Chang & But, 2001). Herbalists from Israel used it as a "magic" plant which was believed to adjust "yin & yang" and supplement blood energy (Wang, 2006; Dafni & Yaniv,1994). In Japan, its berries were used in the making of a formula that was used in the treatment of poor eye sight (JapanMinistry of health labour and welfare, Pharmaceutical and safety bureau, 2010a). The bark and root are also used in Japan to come up with a drug formula"Jikoppi"used in the treatment dry mouth and urination difficulties (Zhou et al.1996).

2.3. CHEMICAL COMPOUNDS OF *LYCIUM* SP

There are numerous investigations that have been carried out on the active ingredients of *Lycium sp.* Most of them have been on *Lycium barbarum* as it is the most commonly found in certain areas and seems to give most biological active compounds such as polysaccharides, flavonoids, vitamins and so forth(Tang et al.2011).

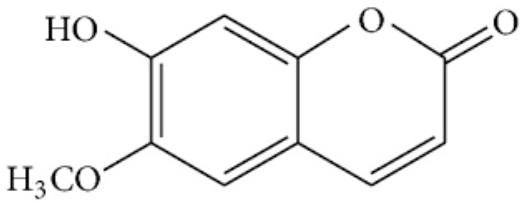
2.3.1. **Chemical compounds of *Lycium barbarum***

The following chemicalcompounds have been isolated and branded from *Lycium barbarum*. The most active ingredients isolated from *Lycium barbarum* are illustrated on the table below. The structures of the compounds are given on Figure 3 and 4

Table 2: Most active compounds of *Lycium barbarum* (Tang et al.2011)

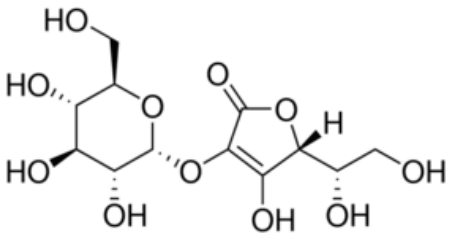
Classifications	**Compounds**	**MW**	**Molecular formula**	**Reference**
Proteoglycans	*Lycium barbarum* polysaccharides			Wanga et al. 2009; Wu et al. 2010; Zhu et al. 2010
Phytoalexin	Scopoletin	C10H8O4	192.17	Liu et al. 2000; Kim et al. 2005; Shawet al. 2003; Oliviera et al. 2001
Vitamin C analogue	2-O-β-D-glucopyranosyl-L-ascorbic acid (AA-2βG)	C12H18O11	338.27	Toyada-Ono et al. 2004; Zhang et al. 2010

Figure 3: **Chemical structure of Scopoletin**



Scopoletin

Figure 4: **Chemical structure of AA-2βG**



2-O-β-D-Glucopyranosyl-L-Ascorbic acid

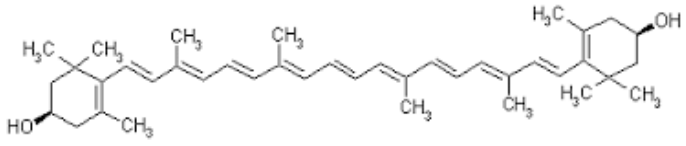
2.3.2 **Other compounds found in *Lycium barbarum***

Other compounds were also isolated from the *Lycium barbarum* and identified as shown the table 3.1. The structures of the compounds are given on figure 2.5-

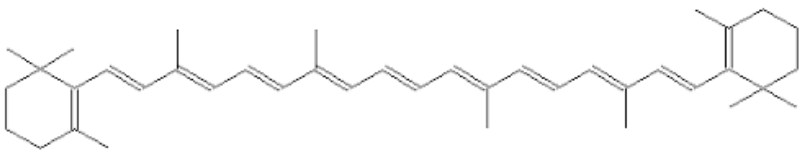
Table 3: Other compounds determined in *Lycium barbarum* (Tang et al.2011)

Types	Nutritional ingredients	References
Carotenoids	Zeaxanthin dipalmitate, β-cryptoxanthin palmitate, zeaxanthin monopalmitate, β-carotene	Weller and Breithaupt 2003; Peng et al. 2005; Inbaraj et al. 2005
Flavonoids	Myricetin, quercetin, kaempferol	Le et al.2007
Amino acids	10 non-essential, 8 essential amino acids	Yin and Dang 2008
Non-proteinogenic amino acids	Taurine, ɣ- aminobutyric acid, betaine	Qun et al. 1998; Cao et al. 2003
Essential oils and fatty acids	Hexadecanoic acid, Linoleic acid, myristic acid	Altintas et al.2006
Vitamins	B1, B2, B3, B6, C, E	Qun et al. 1998; Yin and Dang 2008
Others	B-sitosterol, Daucositerol, p-coumaric acid, lyciumide	Xie et al. 2001; Zou et al. 1999; Hiserodt et al. 2004

Figure 5: Carotenoids

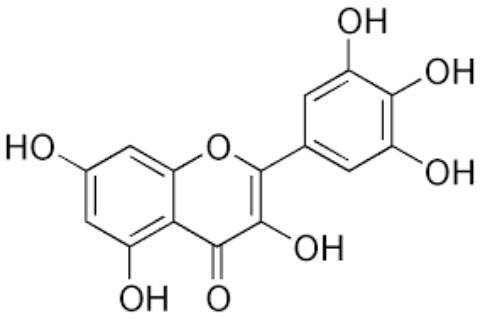


Zeanxathin

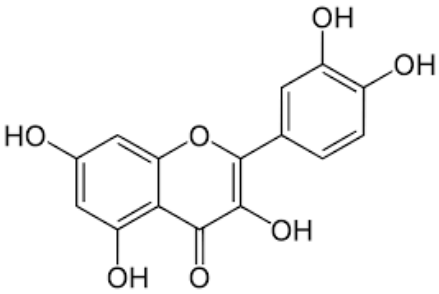


β-carotene

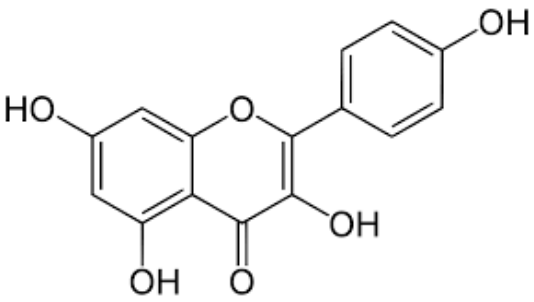
Figure 6: Flavonoids



Myricetin

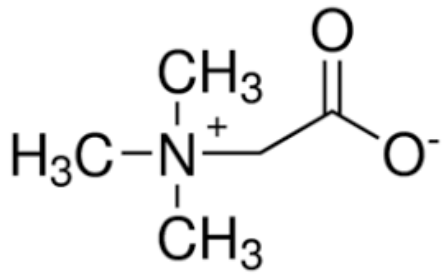


Quercitin

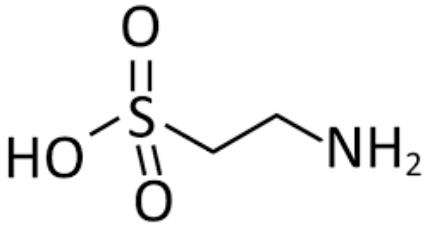


Kaempferol

Figure 7: Non-proteinogenic amino acids

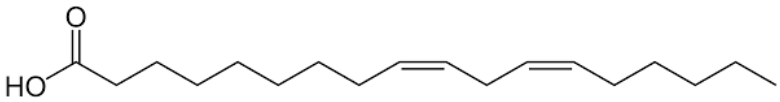


Betaine

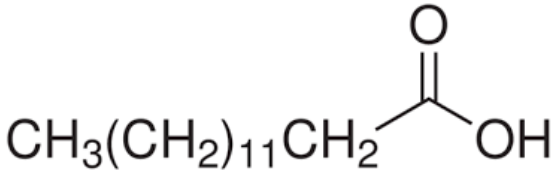


Taurine

Figure 8: Fatty acids

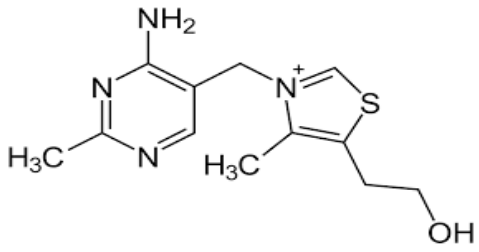


Linoleic acid

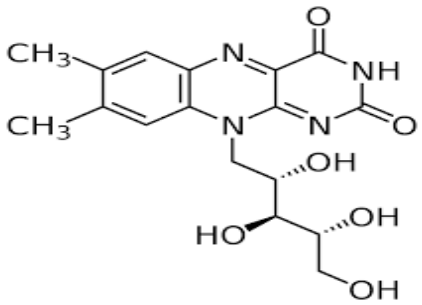


Myristic acid

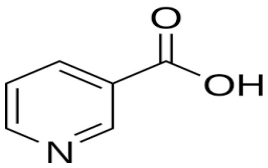
Figure 9: Vitamins



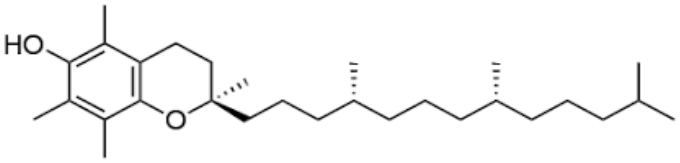
Vitamin B1



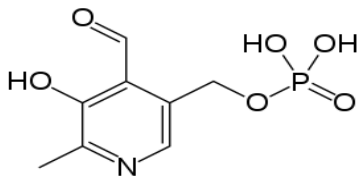
Vitamin B2



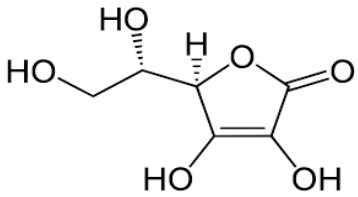
Vitamin B3



Vitamin E

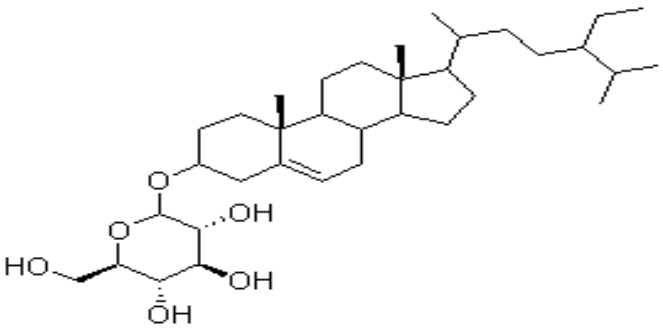


Vitamin B6

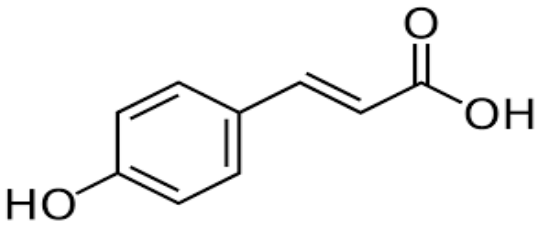


Vitamin C

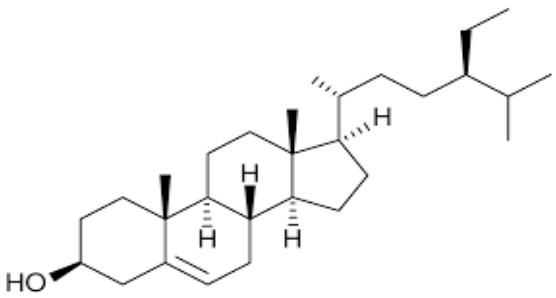
Figure10: Others Compounds



Daucositerol



p-coumaric



β-sitosterol

2.3.3. **Polysaccharides of *Lycium barbarum***

Polysaccharides are a continuous chain of repetitive monosaccharide subunits. LBP is a polar polysaccharide which is made up of the following monosaccharides: Rhamnose, Xylose, Mannose, Arabinose, Glucose and Galactose. Together this mixture of monosaccharides makes up *Lycium barbarum* polysaccharide. It makes up about 6-8% of the dry fruit. *Lycium barbarum* polysaccharide isolation from goji berry yielded the below constituents on table 4

Table 4: Polysaccharides of *Lycium barbarum* (Tang et al.2011)

Glycoconjugate	**MW (kDa)**	**Carbohydrate %**	**Monosaccharide (molar ratio or %)**	**Reference**
LBGp2	68.2	90.71	Ara, Gal (4:5)	Peng and Tian 2001
LBGp3	92.5	93.6	Ara, Gal (1:1)	Huang et al.1998
LBGp4	214.8	85.6	Ara, Gal, Rha, Glc (1.5:2.5:0.43:0.23)	Huang et al. 1998 Peng et al. 2001a
LBGp5	23.7	8.6	Rha, Ara, Gal, Man, Glc (033:0.52:0.42:0.94 :0.85:1)	Huang et al. 1998
LBGp5B	23.7		Rha. Ara, Glc, (0.1”1:1.2:0.3); Galu (0.9%)	Peng et al. 2001b
LBP3p	157	92.4	Gal, Glc, Rha, Ara, Man, Xyl (1:2.12:1.25:1.10:1. 95:1.76)	Gan et al. 2003

LBPA3	66			Zhao et al. 1997
LBP1a-1	115		Glc	Duan et al. 2001
LBP1a-2	94		Glc	
LBP3a-1	103		GalA	
LBP3a-2	82		GalA	
LBPF1	150	48.2		Chen et al. 2008
LBPB1	18			
LBPC2	12			
LBPC4	10		Glc	
LBPF1	150	48.2		Chen et al. 2008
LBPF2	150	48.2		
LBPF3 LBPF4 LBPF5	150 150 290	34.5 20.3 23.5		Chen et al. 2009b

2.4. *Lycium ferocissimum*

It is a member of the Solanaceae family, *Lycium* genus. It is mostly found in South Africa, New Zealand and Australia as a shrub. It is mostly known as "Boxthorn" and was recently declared a weed. (Yao et al.2017)

2.4.1 **Chemical compounds of *Lycium ferocissimum***

Scientists have been able to isolate certain biological active compounds from *Lycium ferocissimum.* Active compounds of *Lycium ferocissimum* is given on Table 5.

Table 5: Active compounds of

Part of plant	**Compound**	**Reference**
Leaves	Flavonoids, Physalins	Dong, Lu, & Wang, 2009
Berries	Carotenoids, Monoterpenes, Polyphenolic compounds, glycoconjugates, glycopeptides	D. Donno, et al Luo, Yan & Zhang, 2000
Flowers	Lutein	Dharmananda, 2007

2.5. BIOLOGICAL ACTIVITIES OF *LYCIUM SP.*

Several clinical trials have been carried out to determine the biological activities of *Lycium sp*. Despite evidence of minimal results shown, more trials need to be carried out to determine the validity of the compounds.

2.5.1 Biological activities of *Lycium barbarum*

2.5.1.1.**Anti-cancer Properties**

Cancer is one of the principal roots of life loss in our society today. Cancer can be described as the abnormal and uncontrolled growth of cells that spread throughout the body of an organism. *Lycium barbarum*is known to contain compounds that are useful in the reduction of tumour cells and has been used to investigate on its anticancer properties. Some of the results are as shown on the Table 6.

Table 6: Anticancer activities of *Lycium barbarum*

Cancer	**Observation**	**Reference**
Breast	It was observed that LBP could reduce the proliferation of estrogen receptor cells and activate extracellular signal-regulated kinase leading to inhibition of MCF-7 cells.	Li et al.2009
Prostate	LBP could inhibit the PC-3 cells and DU-145 cells by causing DNA breaks leading to apoptosis in the cancer.	Luo et al.2009
Gastric	LBP was able to stop the advancement of cells via cell cycle arrest at the G0\G1 phase	Miao et al.2009
Hepatoma	It found that LBP could be used as a treatment remedy for human hepatoma	Zhang et al.2013

	cancer as it caused an inhibition on the tumour cells through cell arrest and apoptosis was later induced.	
Colon	Invested the use of LBP on colon cancer cells and found that a dose dependent treatment on the cancerous SW480 and Caco-2 cells lead to a cell arrest cycle inhibiting their growth.	Mao et al.2009
Cervical	LBP was investigated on cervical cancer and it was observed that its Vit C analog (AA-2βG) was able to cause cell cycle arrest and apoptosis through stabilizing p53 protein.	Zhang et al.2010

2.5.1.2.**Anti-oxidant Properties**

Oxidative stress is mostly caused by free radicals and antioxidants are important as they are necessary to minimize the damage caused. The table below describes some of the effects of *Lycium barbarum* when it was investigated for its antioxidant properties:

Table 7: Antioxidant properties of *Lycium barbarum*

Subject	**Observation**	**Reference**
Human	LBP was investigated on humans and it was discovered that it elevated SOD and GSH-Px levels which are vital for fighting oxidative mediated damage.	Amagase et al.2009
High fat diet	LBP has an influence on lipid metabolism in the blood where it was seen to rise the antioxidant activities of GSH-Px, GSH and catalase while lowering the levels of TC, TG and low-density lipoprotein which help protect the body against lipid oxidation brought about by free radicals from a high fat diet meal.	Cui et al.2011, Wu et al.2010, Ma et al.2009
Mice with exhaustive skeletal muscles	LBP was used to test its capabilities on mice that had undergone a physical activity inducing muscle exhaustion and it was observed that it elevated glycogen and antioxidant enzymes (SOD & GSH) while decreasing MDA levels and creatine kinase action in the muscles of the mice.	Shan et al. 2011 Niu et al. 2008
Chicken	LBP activities in vitro were studied on 2-week-old chickens that were injected with it and it was observed that there was a decrease in malondialdehyde level which an increase was seen on SOD and GSH-Px levels	Shulei et al.2013

Wistar mice	Investigated the outcome of LBP on exercise induced oxidative stress in skeletal muscle of Wistar mice and found that when administered in a dose dependent manner, it would decrease MDA levels and increase the levels of GSH-Px and SOD in the muscle.	Niu et al.2008
Alcohol induced rats	investigated the effects of LBP on alcohol induced oxidative stress in rats and observed that it enhanced the antioxidant functions by eliminating the free radicals brought about by alcohol and by activating the antioxidant enzymes that were used to treat any liver damage that might be caused by alcohol.	Daye Cheng and Hong Kong
Mice with liver oxidative injury	Investigated LBP on its inhibition against liver oxidative injury in high fat mice and concluded that there was an increase in the amount of antioxidant enzymes with a fall in the mice MDA level.	Hua et al.2010
Aged mice	LBP was investigated on aged mice that had age related oxidative stress and it was seen that it caused an elevation in the antioxidant enzymes leading to a reduction of the lipid peroxidation MDA levels.	Li et al.2007
Human endometrial stromal cells	LBP effect was investigated on damage to human endometrial stromal cells induced by H2O2. It was observed that it could stop death of cells caused by H2O2, increase SOD levels while significantly reducing the MDA levels in the cell.	Tieying et al.2016

Kunming mice	Effects of LBP were tested on Kunming mice spermatogenesis impaired by using cyclophosphamide. Cyclophosphamide was observed to elevate nitrite oxide levels which reduced the protein and SOD levels. LBP was able to alter this by increasing levels of SOD while decreasing NO levels leading to an increase in protein biosynthesis.	Li Qian &Sijiu Yu 2016
Dry eye diseased rats	LBP was used in the treatment of rats that had dry eye disease. It was perceived to obstruct the generation ROS, reduce the levels of MDA and increase the functional abilities of antioxidants.	Liang et al.2011 Li et al.2007

2.5.1.4.Immunomodulating Properties

Immunomodulators are chemical agents that are used to improve immune response or the functioning of the immune system. *Lycium barbarum* has been investigated on immunomodulating effects. Its effects are as shown on the Table 8.

Table 8: Immunomodulating effects of *Lycium barbarum*

Subject	**Observation**	**Reference**
Mice	Investigated LBP immunomodulating effects in mice and found out that it enhanced transcription factors T-cells, activator protein-1, CD25 cells that are important in the immune system. LBPF4-OL was investigated on its effects on mice splenocyte, T cells, B cells and macrophages and it was seen to induce proliferation of the splenocyte but not proliferation on induced T and B lymphocytes.	Chen et al.2008 Zhang et al.2011
Mononuclear cells	On peripheral blood mononuclear cells, it was seen that LBP amplified the expression of IL-2 and TNF-α, which is useful in effective immune responses.	Gan et al.2003
Macrophages	LBP effect was investigated on macrophages and seen to improve the endocytic and phagocytic capacities in vivo. It was also seen to improve transcription factors and enhance TNF-α production in a dosage dependent manner. LBP was also seen to have useful qualities in the treatment of neurodegenerative diseases.	Chen et al,2009 Kang et al.2013

Natural killer cells	LBP was observed to help the cytotoxicity of the cells by amplifying IFN-ɣ and perforin secretion and adding to its receptor expression showing it was useful as an immune regulator.	Huyan et al.2014
Dendritic cells	On dendritic cells, LBP was shown to aid both in the phenotypic and functional maturation of murine BMDCs in vitro, proved that LBP could grow the expression of DCs via NF-kB signaling pathway.	Zhu et al. 2007 Chen et al.2009
Helper T cells	Effect of LBP on follicular helper T cells was probed and shown to inflate T-cell dependent antibody responses by being supportive towards T helper cells generation	Su et al.2014

2.5.2. **Biological activities of *Lycium ferocissimum***

The active compounds in *Lycium ferocissimum* have been observed to have effects such as anti-cancer, antioxidant properties. Clinical trials are still ongoing to authenticate this information and show its full effects as a drug against certain diseases. The summary of its biological activities is shown on the table below:

Table 9: Summary of biological activities of *Lycium ferocissimum*

Activity	**Summary**	**Reference**
Antioxidant	It was observed that it had inhibitory activity of superoxide scavenging ability mediated by peroxyl free radicals on mice. On humans, it was seen to increase serum levels of superoxide dismutase and glutathione peroxidase which are important in fighting body free radicles	Amagase et al.2009 He et al.2012 Li et al.2007
Anti-Cancer	It was seen to increaseT cells in tumour infiltrating lymphocytes to relieve the immunosuppression and enhance the anti-tumour functions in the immune system. In prostate cancer cells, it was observed to inhibit the growth of cells by causing breakage of DNA strands of these 2 lines. In gastric cancer cells, it inhibits the growth of cells, with cell cycle arrest at G0/G1 and S phase. It was also seen to have an apoptosis effect on human breast carcinoma cellsthrough induction of cell cycle arrest at G0/G1 phase.	Wu et al. 2006 Miao et al.2011 Luo et al.2009
Eye Care	It was tested on rats and observed to reduce the loss of ganglia cell on the retina while	Liu, Li & Tso, 1995

	it did not alter the intraocular pressure. Increase of this pressure may lead to loss of the retina ganglion cell.	Cheng et al. 2005

3. MATERIALS AND METHODS

To obtain articles on *Lycium* species, I sourced from the internet through the academic sites such as Google Scholar, PubMed, Science Direct and Scopus. Keywords were important in searching for articles. "*Lycium* species" text was used on Science Direct and Google scholar to look up articles or citations. On PubMed, texts such as "*Lycium barbarum*" and "*Lycium barbarum* polysaccharides" were used to generate various published articles that were important for this research. Texts such as "Goji berry and cancer", "Goji berry and antioxidants" and "Goji berry and immunomodulators" were also used as research methods to generate articles from the academic sites. Online articles and journals published on the internet through independent sites such as Journals of Biochemistry, Food Chemistry and the likes also aided in generating information used on this thesis. About 60 articles were used and were divided based on characteristics such as phytochemicals.

4. RESULTS

The use of natural compounds extracted from plants to make drugs has been increasingly adopted over the years. Natural products have well defined 3-D structures with functional groups that have been fine turned into a precise spatial formation and for this reason contain good specific and potent molecules as compared to artificially designed drugs. Systemic characterization of active compounds in medicinal herbs has been used to determine their potency and efficiency in transformation of the herbal medicines to evidence-based medicines. The active principles of herbal extracts are important aspects of biological activities. Phenolic compounds and carotenoids have a strong antioxidant activity. Polysaccharides found in most herbal plants have an immune modulating or immune stimulating properties (Paulsen, B. S 2001). Dependent on recent results on anticancer and antioxidant activities, *Lycium barbarum* was investigated using phytochemical, in vivo and in vitro methods and by use of clinical trials.

Lycium barbarum

Clinical trials are ongoing for the use of *Lycium barbarum* as a drug-based herb to investigate its effect on various diseases. Despite this result explained below, more clinical trials are required to shed more light on the use of *Lycium barbarum* as a drug-based plant. Scientists and numerous research institutes have been extracting and using its biological active compounds as methods of treatments for various diseases we have today. Its phytochemical compounds have been investigated on various basis and data collected.

To find out about its antioxidant properties, (Chien etal.2017) used rats that were treated to different dosages of GBE an aqueous extract from *Lycium barbarum* over a period of 21 days. Atropine was injected to the rats to cause their eyes to dry and after 7 days the first dosage of GBE was injected except for the control group. Tests and reading were taken, and it was observed as shown that the eye dryness in the rats were minimally reduced at the different dosages. Schirmer's test which is used as a measurement of tear production was taken and it was observed that with each dosage level, there was a reduction of eye dryness symptoms. Tear break-up-time test is a test taken to measure the dry eye severity and it shows the overall tear

quality. It was observed that when treated with GBE, at different dosages it was observed that there was a reduction of tear evaporation and a more stable tear film in the eye

Wu et al.2010 investigated 50 three-months old Kunming mice which were divided into groups of 3 (Control group, model group & LBP group) and fed on a high fat diet. They were also dose dependently fed with LBP once each dayduring the 2 months investigation. It was seen that LBP could dose dependently significantly hikedantioxidant levels and GSH levels while lowering the levels of MDA in mice groups (3-5) as compared to the model group.

Gan et al.2014 studied on the sarcoma in mice and how it is affected by LBP.They showed that under different dosages, LBP was able to enhance the level of macrophages in the mice inhibiting the growth of the sarcoma. This was under the medium dosage. They also found a reduction in lipid peroxidation in the mice.

High fat diet is linked with ailments such as obesity, diabetes, hypertension and oxidative stress in some cases. LBP was used to investigate on mice that were kept on a high fat diet. It was revealed that it had effects on blood sugar, lipid breakdown and oxidative stress. LBP could increase the antioxidant enzymes such as SOD, catalase and GSH-Px while lowering the levels of TC, TG and blood glucose. This shows that LBP can inhibit free radical formation (Cui et al.2011; Wu et al.2010; Ma et al.2009.)

LBP was investigated on Endometrial stromal cells and seen to induce apoptosis via the mitochondrial pathway by downregulating the Bcl-2 family proteins (Shan et al.2015). LBP can reduce H2O2- induced ESC damage thereby reducing the mRNA expression of Bcl-2 proteins blocking the levels of cytochrome c. This causes an increase in expression of mRNA caspace-3 protein that can trigger apoptosis in cells. It is also seen to reduce the MDA levels in the cells caused by lipid peroxidation while improving SOD activity.

Shan et al.2011; Niu et al.2008 investigated the role of LBP on exercise driven rats. Physical exercise increases the uptake of oxygen leading to the creation of ROS. It is also known to help prevent conditions such as cardiovascular diseases, hypertension and obesity. They observed that LBP lowered the levels of MDA in the rats while elevating the levels of antioxidant enzymes showing a positive effect on preventing oxidative stress.

Li et al.2007; Liang et al.2011 revealed that LBP had an effect of aged mice where it increased levels of SOD, CAT and GSH-Px to fight off free radicals caused by oxidative damage by aging of mice. Lipid peroxide levels were reduced by LBP showing an affirmative effect on oxidative stress.

Luo et al.2006 investigated on LBP effects on oxidative damage brought about by heat exposed to rat testis. The oxidative damage caused by the heat was inhibited by an increase in SOD levels in the rat's testis. There was also an increase in sexual hormone levels that offered protection to the testis.

Qiu et al.2013 investigated on the actions of selenylated LBPas an antioxidant on different mice for 28 days. LBP was modified by addition of sodium selenite which is a salt. There were 3 groups used in the experiment: sLBP, LBP and blank control. It was observed that levels of SOD and GSH-Px increased until day 21 before a slight drop was seen afterwards while MDA levels were shown to decrease.

LBP has also been used severally to investigate on its anti-tumour properties. Though clinical trials are still ongoing, a few characteristics have been reported for this study:

Miao et al.2009 studied the effects of LBP on gastric cancer cells and it was observed that after 5 days of treatment under different concentrations, there was an inhibition in their growth mediated by cell cycle arrestatthe S phase. This was achieved via cell apoptosis induced by decline of cyclin D, E and CDK2.

Mao et al.2010 explored the effects of LBP on colon cancer cells. Cell lines (SW 480 and Caco-2) were dose dependently treated with LBP and it was seen to decrease the growth of cancer cells. There was an S phase arrest that lead to the cell cycle arrest mediated by a reduction in level of cyclin D, E and CDK in the colon cancer cells.

Zhang et al.2013 investigated the effects of LBP on human hepatoma cells using 5 different fractions of LBP and it was seen to decrease the level of growth. LBP was able to incite cell apoptosis to stop cell growth through a G0/G1 arrest. This was brought about by damage of the DNA in S phase or by the damaging of proteins that are required for progression to G2 phase.He also saw that Ca 2+ could be useful in the apoptosis process as LBP was seen to improve its cellular concentration which is used as a signal transduction pathway of apoptosis whereby it affects the sensitivity of tumour cells to cancer agents.

Luo et al.2009 investigated the use of LBP on prostate cancer cell lines PC-3 and DU-145 and showed to encourage apoptosis of the cells. There was evidence of a reduction in the expression of Bcl/Bax protein expression after the cells were dose dependently treated with LBP. It was also observed that in vivo treatment resulted in the obstruction of PC-3 tumour growth in some mice that resulted to a reduced tumour volume and growth.

Li et al.2009 investigated the effects of LBP on breast cancer and found that under a dosedependent manner, it was able to reduce the growth of the cells by altering the metabolic pathways of estradiol. It was revealed that LBP could stop the spread of the receptor of the MCF-7 cells by modulation of estrogen metabolism which switched off the metabolic pathways.Shen and Du.2012 also found that LBP dose dependently inhibited MCF-7 cells via cell cycle arrest at the S phase. This took place through the activation of extracellular signal regulated kinase which is involved in the expression of p53.

Zhu and Zhang 2013 inspected the effects of LBP on the human cervical cancer Hela cells and revealed that after a 4-day treatment there was a decrease in the number of cells at the G0/G1 phase citing a cell arrest cycle stopping the growth of cells.Hu et al.1994 used LBP and garlic to treat mice bearing the human cervical U14 cancer cells and found that there was a cell cycle arrest at the G0/G1 phase. It was revealed that the mitochondria of the cells were injuredleading to the enlargement and degranulation of the ER.

5. DISCUSSION

There is no clear biochemical procedure on how LBP affects the cancer cells. LBPshindersthespreadofseveraltypesofcancercellsthroughcellcyclearrestattheG$_0$/G$_1$,S,orG$_2$/Mphase. The phases are governed by proteins called cyclins and are responsible for movement through each "checkpoint". Cyclins A, D and E are required by the cell. A complex between cyclin and cyclin dependent kinases are needed for the movement. LBP is thought to inhibit these proteins via the activation of gene regulators such as p53. p53 is an oncogene that activates p21; a cyclin dependent kinase inhibitor causing arrest at the G0/G1 phase. p21 inhibits their functions by phosphorylating the retinoblastoma tumour suppressor genes which leads to an increase in the cyclin suppressor genes inducing cells to settle at the G1 phase. p21 is known to inactivate cyclin E-CDK2 complexes and cyclin D associated kinase activity. LBPsgovernstheexpression of Bcl-2 and Bax to induce tumor cell apoptosis via increasing intracellular Ca^{2+} concentration and mitochondrial pathway. Bcl-2 are an anti-apoptotic protein block found on the outer membrane of the mitochondria. They are known to be inhibitors of cytochrome c that is part of the mitochondria pathway that leads to apoptosis. Ca2+ is important as it controls signaling pathways. With the Bcl-2 and Bax proteins regulates, the mitochondria through an apoptotic stimulus can produce cytochrome c to the cytosol. It will then bind to apoptotic protease factor 1 (Apaf-1) with ATP. This leads to the activation of caspase 9. These are cysteine proteases known to induce apoptosis. Caspase 9 then activates caspase 3 that induces apoptosis in the cell. This is summarized in figure 5.1. Caspase activation is summarized in Figure 11 below.

Figure 11:Possible mechanisms for the anticancer actions of LBPs. (Cheng et al.2018)

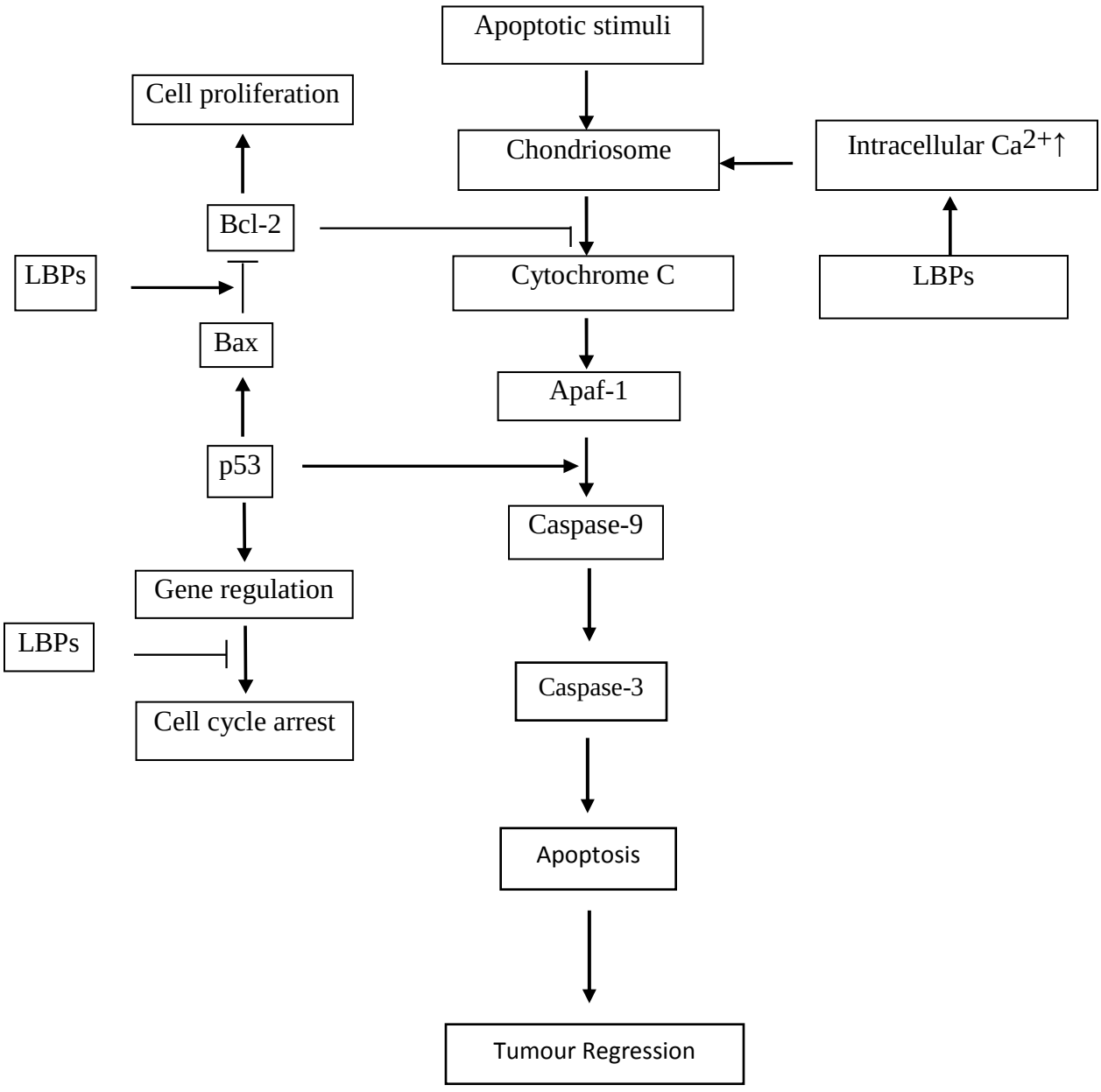
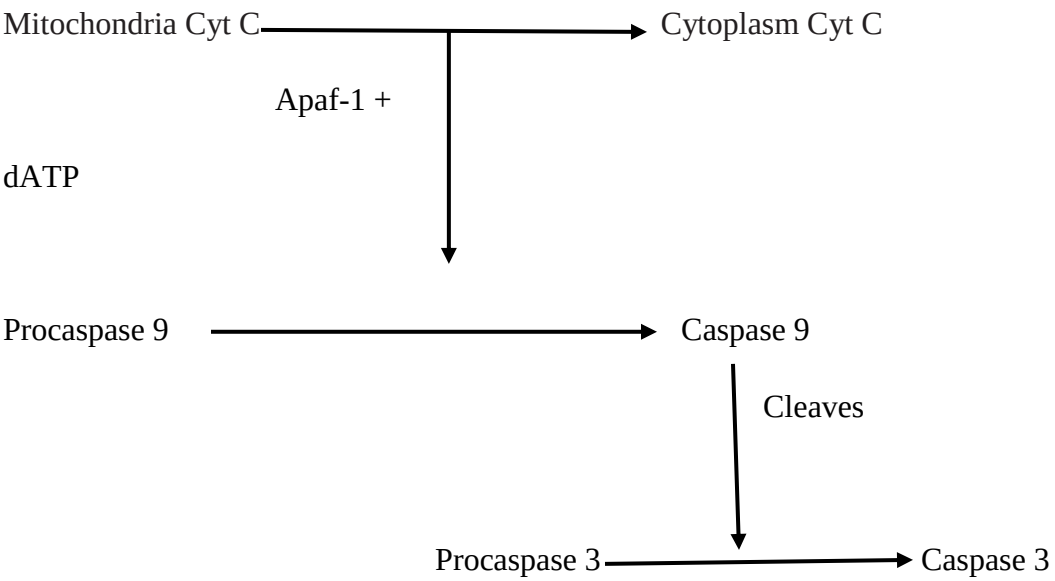
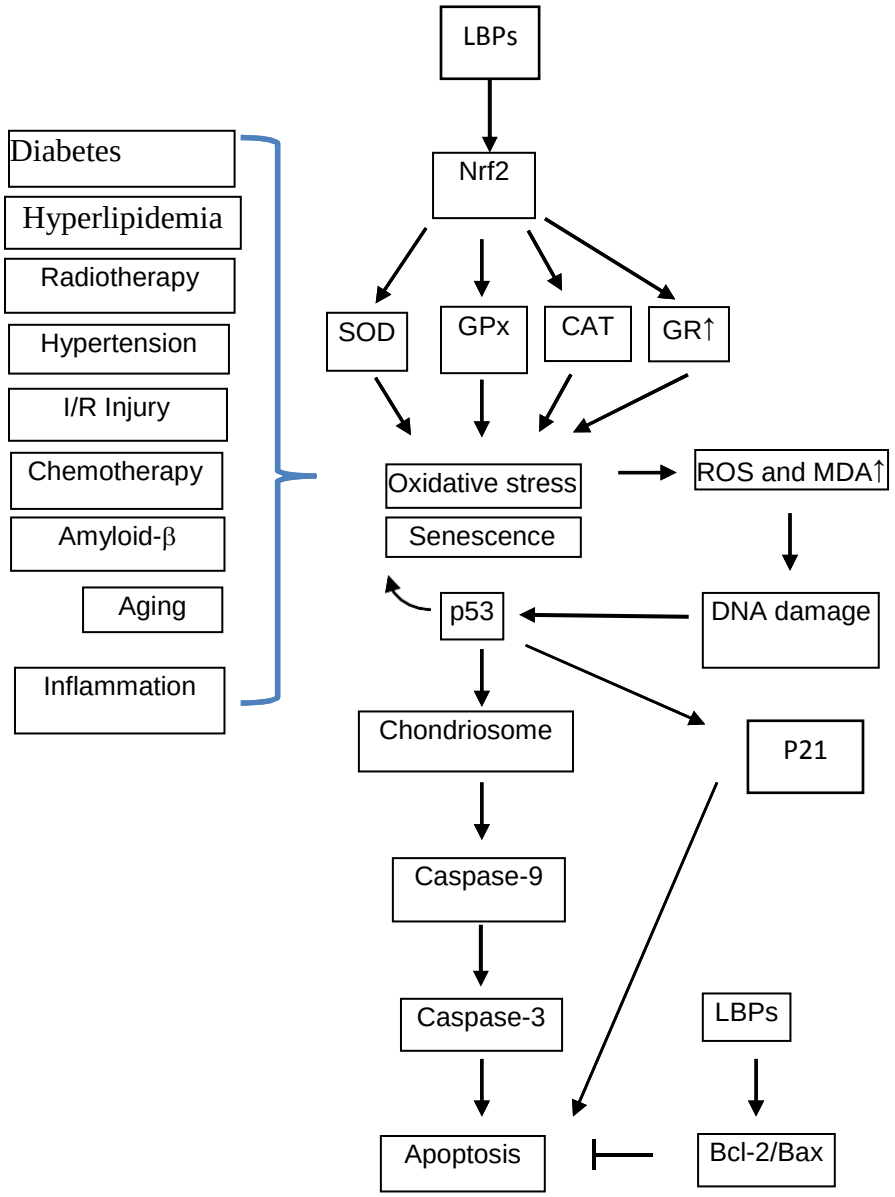


Figure 12: Caspase activation



LBPs increase SOD and CAT activities, thereby preventing oxidative stress-induced damage. LBPs boosts oxidative stress-induced cellular apoptosis. Oxidative stress is the imbalance between free radical manufacture and their deprivation by antioxidant systems which leads to accumulation of reactive oxygen species. ROS includes superoxide anion (O2-), hydrogen peroxide (H2O2) and hydroxyl radicals (OH-). ROS cause oxidative damage to proteins, lipids and DNA and can cause genotoxicity, cytotoxicity and carcinogenesis. Defensive mechanisms against it includes the use of enzymes such as catalase, peroxidase and superoxidase dismutase or use of antioxidants in the cells such as glutathione, tocopherols and ascorbic acid. LBP is thought to activate nuclear erythroid 2-related factor (Nrf-2); a protein that regulate the expression of antioxidant proteins that protect against oxidative damage. Expression of these proteins causes an increase in enzymes required to fight oxidative stress. ROS can cause an increase in malondialdehyde (MDA) brought about by a final product of polyunsaturated fatty acid peroxidation in the cells. DNA damage by ROS activates p53 gene regulators that leads to cell destruction. This leads to activation of p21 that activates apoptosis. P53 regulates the expression of Apaf-1 in the mitochondria and may influence the activation of mitochondria pathways activating caspase proteins that cause apoptosis.

Figure 13: Possible mechanisms for the antioxidant actions of LBPs (Cheng et al.2018)



6. CONCLUSION

Despite the few observed clinical results from various scholars, more investigations are needed before *Lycium barbarum* can be fully accepted as a drug effective as an anti-oxidant, anti-cancer. There is some unclear information on how *Lycium barbarum* works on target cells used to investigate it. There is also little knowledge on the biochemical process that take place inside the cells to show or express how LBP interferes with normal cell activities to be effective. More research and trials will be needed on *Lycium barbarum* to support the current available literature.

Lycium ferocissimum belongs to the same genus as *Lycium barbarum.*It has similar phytochemicals as those found in *Lycium barbarum*. Clinical trials done on it also reveal similar results when tested on different cell lines or organisms. Results collected from different investigations show a potential of it being used to manufacture a drug or used as herbal medicine Despite being no explanation as to how it effects any change, it should be investigated and researched to give a clearer review.

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