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Characterization and Cytotoxic Activity of *Dalbergia latifolia* Wood Extract

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Abstract: *Dalbergia latifolia* commonly known as the Indian rosewood, commonly found in association with Teak trees population in and around Western Ghat areas of Tamil Nadu and Kerala in India. From the heartwood of roots and trunk many phytochemical contents were investigated. A wide spectrum of flavanoids namely, flavones, isoflavones, neoflavones and chalcones have been obtained. Specifically, one of the flavonoids compound chalcone compound-Isoliquiritigenin (ILTG, 2',4',4'-trihydroxychalcone) shows various pharmacological properties, including cytotoxic activities. This study revealed the cytotoxic effect of Isoliquiritigenin, efficiently controls the invasive capacity of breast cancer cells MCF10A human mammary epithelial cell line *through* uplifting G1-phase cell cycle, even arrest at low concentration, also accelerate the extracellular signal regulated kinase signaling pathway to topup the proteins combined with apoptosis and arrest cancer. The cytotoxicity *invitro* of isoliquitrigenin against MCF10A ATCC cells was investigated by MTT assay. Apoptosis was evaluated by microscopy and flow cytometry. Isoliquiritigenin exhibited the most potent anticancer activity against MCF 10A ATCC with an IC 50 of 10.00 µg/ml. The methanolic extract expressed cytotoxic effect with an IC50 value of 20 µg/ml. The compounds were also tested for their toxicity on normal human cell lines-LO2 and were found to be nontoxic. Administering the wood extract approaches may help reduce side effects in patients under conventional chemotherapy. These potential molecule may exerts their co-active effects through various pathways and helpful for the development of novel drugs; also conservation of these important germplasm is mandatory.

Keywords: *Dalbergia latifolia*, Isoliquiritigenin, Breast Cancer Cell Line, Cytotoxicity, Conservation

1. Introduction

Dalbergia latifolia (Roxb) trees are important timber tree species valued for their decorative and valuable wood. Traditionallly used for various treatments like, diseases of the blood, diseases of the eye and nose, syphilis, stomach troubles, leprosy etc. The heart wood extract contain latinone, neoflavonoid dalcriodon and Latinone, and Isoliquiritigenin. Isoliquiritigenin is a chalcone derivative, which exerts anti-tumour promoting and anti-platelet activity. For effective utilization of the metabolites rich germplasm in order to deploy them in various improvement and utilization programmes, superior genetic stocks to be used by farmers and stake holders should be evaluated and documented. In connection with this there is a need for biochemical characterization of germplasms to give additive income along with wood to farmers. The study was designed to evaluate the phytochemical profiles in two different accessions, and cytotoxic activity of a methanolic extract from the wood of *Dalbergia latifolia* especially the component Isoliquiritigenin.

Dalbergia latifolia contains numerous flavonoids and

triterpenes. Isoliquiritigenin (ISL), one of the flavonoids many research findings explored about its anticancerous activity, by stimulating apoptosis, autophagy and causing DNA damage [1]. It was reported that this compound shown hindering effect on the spread of prostate and breast cancer cells [2]. Similarly this compound suppress imflammatory responses by defensive Toll-like receptor 4 signaling and inflammasome instigation methods [3]. In several reports it was reported that this compound terminate cancerous effect through immune regulation and the signalling pathways associated with this metabolism was also reported [4]. The molecules in the G2/M cell cycle of PC-3 and 22RVI cells and the expression of protein B1, cyclin-dependent kinase 1 (CDK1) disturbances in the case of prostate cancer was described [2].

Chalcones are sources of proton suppliers because of its phenolic hydroxyls frame, having the capacity to dissolve the oxidative damage by fusing with a radical [5]. Isoliquitrigenin a chalcone compound from *Dalbergia latifolia* having antioxidant capacity. The *in-vitro* anti-oxidative activity was investigated using DPPH assay, superoxide scavenging assay and cytotoxic activity was studied using cell viability (MTT) assay on MCF 10A ATCC breast cancer cell-line. Isoliquinitrigenin displayed prominent anti-oxidative and cytotoxic activity against MCF 10A ATCC cells.

2. Materials and Methods

2.1. Plant Materials Collection Area

The wood of *Dalbergia latifolia* was collected (Figures 1 to 3) from Tamil Nadu (Bargur, Krishnagiri, Tamil Nadu and Walayar and Nelliampathy Western Ghat forest areas of Kerala of India.

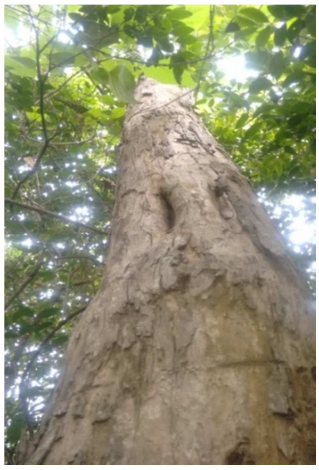

Figure 1. *Dalbergia latifolia germplasm.*

(a) (b)

Figure 2. *a and b Dalbergia latifolia root collection.*

(a) (b)

Figure 3. *Hard and soft wood of Dalbergia latifolia root.*

2.2. Preparation of Wood Extract

The dried wood chips were pulverized and powdered wood of *Dalbergia latifolia* was extracted using 99% methanol by cold percolation method. The powdered material was soaked The methanolic extract (200 g) was purified by reverse-phase flash column chromatography on silica gel with gradient elution using MeOH/H2O; 25: 75, 50: 50, 75: 25 and 100: 0, V/V, each 300 mL (each collection was 50 mL) in the order of decreasing polarity. The eluents were collected, monitored in methanol (1:4) for 48 hours at 37ºC. Then the filtrate was filtered and distilled for recovering the solvent and then it was evaporated under reduced pressure at 50ºC. Phytochemical screening Phytochemical evaluation were performed for all the extracts as per the standard methods.

2.2.1. Fractionation of Active Compound

The methanolic extract (200 g) was purified by reverse-phase flash column chromatography on silica gel with gradient elution using MeOH/H2O; 25: 75, 50: 50, 75: 25 and 100: 0, V/V, each 300 mL (each collection was 50 mL) in the order of decreasing polarity. The eluents were collected, monitored by TLC. The solvents were evaporated to dryness in vacuum to provide 6 major fractions. Fraction 3 was further purified by RP-HPLC. Elution was conducted initially with

50 % MeOH/H2O up to 100 % MeOH (for 40 min, flow rate 1.5 mL min–1, UV detection at 252 nm) to give 10 subfractions-compound 1, 2.0 mg, tR = 20.2 min). Subfraction-2 (4 mg) was purified by RP-HPLC using the same conditions to give 4 subfractions (3 mg) was subjected to further RP-HPLC purification (65 % MeOH/H2O up to 100 % MeOH) to afford compounds (1.5 mg, tR = 10.7 min) and (0.50 mg, tR = 15.0 min). Fraction F4 (20 mg) was purified by RP-HPLC using gradient elution from MeOH/water (80: 20) to 100 % MeOH to afford compounds F2 (1.0 mg, tR = 10.5 min) and F3 (1.0 mg, tR = 14.8 min).

2.2.2. (2, 2-diphenylpicrylhydrazyl (DPPH) Activity)

Scavenging activity on DPPH free radicals by the extract was assessed using the eluted fractions of HPLC. Each fraction was analyzed in triplicate. The percentage of inhibition was calculated against blank: where is the absorbance of the control reaction (containing all reagents except the test compound) and is the absorbance of the test compound. This radical is used in the DPPH radical scavenging capacity assay to quantify the ability of antioxidants to quench the DPPH radical. The dark purple color of DPPH will be lost when it is reduced to its nonradical form stable organic nitrogen centered free radical with a dark purple color which when reduced to its nonradical form by antioxidants becomes colorless. DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. When the DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm. 200 mg of sample was taken in centrifuge tube (in triplicate). Two hundred microliter distilled water was taken in blank instead of the sample. Then 1 mL of DPPH (8 mg/100 mL of ethanol) solution was added to the sample and the blank. This setup was left at room temperature for 30 minutes with shaking at times. Tubes were then centrifuged at 4000 rpm for 10 min. After that, 0.5 mL supernatant was poured in fresh tubes containing 1 mL of ethanol and the absorbance was taken at 517 nm against the ethanol by using UV spectrophotometer (Analytik jena, Germany) [25].

2.2.3. Superoxide Radical Scavenging Activity

The eluted fraction were analysed for its capacity to inhibit the formazan formation upon photochemical reduction of nitroblue tetrazolium (NBT). Concisily, each 3 mL reaction mixture (0.01 M phosphate buffer (pH 7.8), 130 mM methionine, 60 μM riboflavin, 0.5 mM EDTA, NBT (0.75 mM) with 0.5 mL extract/$CuSO_4$ solution; positive Control). These tubes were kept in front of fluorescent light for 6 minutes and absorbance was taken at 560 nm. The nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system generates superoxide radicals, which reduce NBT to a purple formazan. The decrease in absorbance at 560 nm with the plant extract and the reference compound quercetin indicates their abilities to quench superoxide radicals in the reaction mixture. Identical tubes were kept in the dark and served as blanks. The results were expressed in percent inhibition as compared to control [26].

2.2.4. Cell Viability Assay

The cell viability after treatment with Isoliquiritigenin from wood of *D. latifolia* was assessed using the MTT-dye reduction assay, based on the bioconversion of the yellow dye 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide to a violet formazan product via the mitochondrial succinate dehydrogenase in viable cells. The capability of cells to endure toxic concentration is the principle behind all cytotoxicity assays [14]. This assay is based on the theory that dead cells or their products do not reduce tetrozolium. The MTT assay depends on the mitochondrial activity per cell and amount of cells present.1 × 10^4 cells were plated in 96-well plates to check toxicity of extracts towards MCF 10A ATCC breast cancer cell-line. After adherence of cells, the medium was removed and replaced by media having the wood extracts and Isoliquiritigenin. In 5% CO2 incubator the plates were incubated for 24h at 37°C. Colorimetric assay with the tetrazolium salt MTT was used to determine cell viability. Absorbance of the formed purple formazan was measured at wavelength of 570 nm. Results were expressed as percentage cellular viability of the extracts. % Cytotoxicity = O. D of control sample-O. D of treated sample/O. D of control sample ×100.

3. Results and Discussion

Dalbergia latifolia is a precious timber species. Which are highly priced and commonly called as the Indian Rosewood.

The population of these species is on a decline stage in the natural forest areas. we have identified several natural populations of these species in the forest areas of Tamil Nadu and Kerala (Figures 1, 2, 3). This wood is having very high commercial value because of its colour, decorative nature and holding fragrance and enriched with aromatic oils. Traditionallly used for various treatments like, diseases of the blood, diseases of the eye and nose, syphilis, stomach troubles, leprosy ailments. The heart wood extract contain neoflavonoid, dalcriodon and Latinone, and Isoliquiritigenin [6]. Isoliquiritigenin is a phenolic chemical compound and a chalcone derivative and it's having aromatic ketone and enone. This kind of chalcone implements anti-tumour promoting and anti-platelet activity [7].

Natural plant originated medicine nowadays people's choice to treat cancer. Nearly 95% of this globe depends on plant based medicine for their preliminary first aids. From the renowned research it's proved that herbals created compounds doing potential anticancer drugs. The important aim of the valuation of cytotoxic activity from the crude plant extracts are either to isolate bioactive agents that could lead molecule for anticancer drugs therapy or to develop the crude plant extract itself to become standardized herbal medicine that can be used along with chemo preventive agents. In the order of, to assure the pharmaceutical importance of plant origin medicine, proper identification and quantification of active molecules, proper purity analysis and quality measurement of the starting materials are an essential need to obtain the safety and potency of plant origin medicine [8].

A mobile phase consisting of MeOH: Water: in reverse-phase flash column chromatography on silica gel with gradient elution using MeOH/H2O; 25: 75, 50: 50, 75: 25 and 100: 0, V/V, each 300 mL (each collection was 50 mL) was used for the analysis which gave sharp and well resolved peak of Isoliquiritigenin. Best resolution and sensitivity of the method was obtained at 285 nm with retention factor of 0.15 (Figure 4).

The DPPH scavenging activity was increased as concentration was increased. These results provide a comparison of the antioxidant activity directly with ascorbic acid. *D. latifolia* exhibited the highest scavenging activity (IC_{50}; 70±2.55 µg/ml). These activities were based on scavenging of DPPH radical was supposed to be owed to their hydrogendonating capacity. In the presence of oxygen radicals formed ($ROO^{\cdot}$, HO., $O_2^{\bullet-}$) are extremely reactive species vary in their lifespan and organic properties.

Superoxide ($O_2^{\bullet-}$) radical is known to be very dangerous to cellular components as a precursor of the extra reactive oxygen species, cause damage to tissue and because of that various diseases occur. isoliquitrigenin (200±2.02 µg/ml) showed strong activity.

The free radical scavenging ability proves that this compound owe higher antioxidant activity. Fractions, when tested expressed that it has capacity to fight against oxidative damage, for its DPPH activity and superoxide scavenging activity.

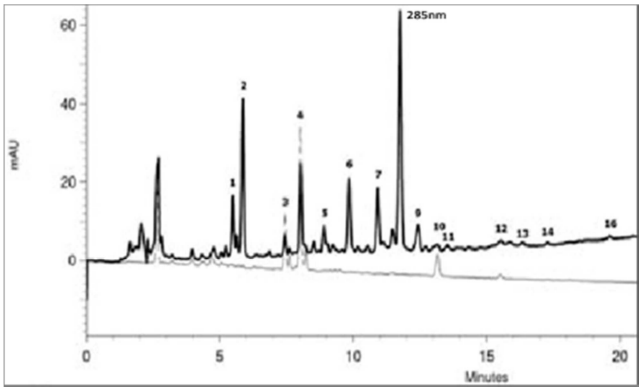

Figure 4. *Characterization of methanol extracts of D. latifolia root wood powder through HPLC recorded at 285nm.*

In the case of this cell line Isoliquiritigenin caused IC 50 at 20 µg/mL concentration, *in vitro* cytotoxicity was 56.22±0.47 against positive control drug Doxorubicine was 38.29±0.39 (Figure 5). This concentration induced a cytotoxic effect densely comparing other concentrations viz., 10, 15, 20, 100, 125, 150, 250 µg/mL of Isoliquiritigenin for 24 h. (Table 1).

Table 1. *Isoliquiritigenin Induces Cytotoxicity in MCF10A human mammary epithelial cell line in vitro.*

S. No	Concentration µg/ml	Dilution	*invitro* cytotoxic activity	Doxorubicine+control
1	1000	-	10.10±0.53	10.34±0.65
2	250	1.1	12.60±0.62	14.10±1.0
3	150	1.2	14.20±1.20	16.26±1.20
4	125	1.4	19.13±1.45	16.56 ±1.25
5	100	1.6	20.34±0.60	18.78±0.56
6	20	1.5	56.22±0.47	38.29±0.39
7	10	1.2	58.34±0.65	40.10±0.47
8	5	1.1	90.12±0.1.22	60.29±1.20
9	Cell control	-	100	100

Breast cancer cells were processed with cells with different concentrations 5,10,15,20,100,125,150,250 µg/mL of Isoliquiritigenin for 24 h exhibited a characteristic DNA pattern that portrayed sub-G1, G1, S, and G2/M phases of the cell cycle. Dispensed cells showed greater G1 population (75%) compared with 60 % in the control when dispensed with 10 µg/mL compound. Notable decrease of cells from control (15%) to treated MCF10A cells (10%) showed a synergistic decrease in the percentage of cells in G2/M phase (Figure 5).

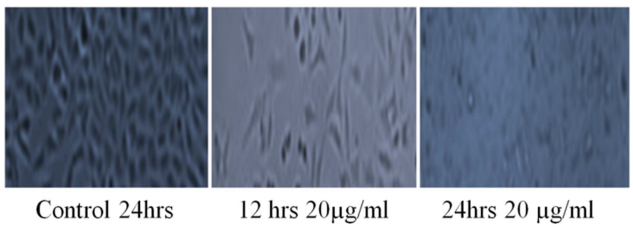

Figure 5. *Cytotoxicity test.*

In contrast, the percentages of sub-G1 phase (apoptotic cells) were remarkably topped up after cells were treated with 125 and 100 µg/mL Isoliquiritigenin up to 45 and 65% respectively compared with 6% in the control. This study signified that Isoliquiritigenin induces G1-phase cell cycle arrest at low concentration (10µg/mL). Consequently, Wood extract treatment at higher concentrations (125µg/mL) induced cell death in MCF10A cells. Isoliquiritigenin treatment stimulates the extracellular signal regulated kinase signaling pathway to enhance the proteins associated with apoptosis.

Variance between cell proliferation and cell death due to cell cycle disruption may topup cancer upliftment. Therefore, the cell cycle could serve as a target for anticancer agents to arrest the unbounded proliferation of cancer cells and stimulate their cancerous cell death. Particularly, Isoliquiritigenin nullifies the breast cancer cell line cells through suppressing β-catenin an ATP binding subfamily G2 signaling was reported [14]. It was also reported that this having the efficiency to enhance the cell death of bladder cancer cells through toxicity induction in cells [13].

Biocomponents from trees are globlaly glimpsed to notify its accessability in breast cancer affected patients as an alternative therapy. From curcumin the biocompound does inhibitory activity on MDA-MB-231 breast cancer cells by

shooting up regulation of p12 protein and Bax-to-BCl ratio [11, 12, 15].

In connection with this research results, Han li et al. expressed that treatment of MDA-MB-231 cells with Isoliquiritigenin uplift the ratio of of Bax to Bcl-2 also causes apoptosis [16]. Further in lung cancer and endometrial cancer also this treatment was showed induction of apoptosis [9-10]. Antiapoptotic protein reduction along with the proapoptotic protein Bax uplifement and caspase-3 and PARP molecules downstream activity was noticed The expression of the anti-apoptotic protein Bcl-2 was reduced, whereas expression of was increased. Furthermore, the downstream molecules were active. With licorice isoliquiritigenin it was reported that pretreatment of mice showed least chances rate of getting cancer attack [17]. Also consequently, there was a reduction in cell proliferation and also the induction of Ki-67 protein which also reduces the cell proliferation and the proteins like caspase-3 and p62 proteins formation were reduced [20-24]. This denotes that the cancerous cells were underwent autophagy. Also it was reported VEGF protein induction was very much hindered, when mice treated with isoliquiritigenin [18-20].

This research revealed the cytotoxic effect of Isoliquiritigenin, potentially controls the invasive capacity of breast cancer cells MCF10A human mammary epithelial cell line. Along with it, flow cytometry analysis expressed that Isoliquiritigenin caused sub-G1 or G2/M phase arrest. Therefore, Isoliquitrigenin is a potential anticancer molecule that is believed to selectively induce apoptosis in cancer cells and arrest proliferation. Furthermore, *in vivo* studies using animal models are necessary to confirm the validity of this molecule strategy for the treatment of breast cancers and possibly other types of cancers.

4. Conclusion

D. latifolia is one of the potent forestry resource enriched with Isoliquitrigenin an anticancer compound, due to various biotic and abiotic stresses, evacuating forest lands and drastic climatic changes brings down the population from nature. Also farmers are not likely to plant these long rotation period trees. Nowadays traditional knowledge and old therapy combinations is very much utilized in this cutting edge technology era. Ultra chemotherapy deploys numerous active components of tree origin. In village area also in urban are because of the unsustainable economic situation people prefer to consume natural cure products in combination therapy. It is assured that plant derived compounds in combination of modern medicament not affecting normal cells. Also it was proved those side effects caused by drugs are nullified in certain amount. In this study characterization and evaluation of different zones germplasm displays that all having this anti-cancerous compound. For future scientific prospects, its mandatory to conserve these anticancers enriched characterized and evaluated germplasm, to conserve the genetic variation, its diversity and its valuable traits. Valuable germplasm is a profit and source of all the traits this has to be conserved for generations. Hence, making awareness among farmers about the importance of this species and encouraging cultivation in farmland and conservation, of the biocomponents rich germplasm is mandaotry to provide alternative therapy along with chemotherapy.

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References

[1] Changliang Chen, Anitha K. Shenoy, Ravi Padia, Dongdong Fang, Qing Jing, Ping Yang, Shi-Bing Suand Shuang Huang. (2018). Suppression of lung cancer progression by isoliquiritigenin through its metabolite 2, 4, 2', 4'-Tetrahydroxy chalcone. Journal of Experimental & Clinical Cancer Research 37: 243.

[2] Biyan Zhang, Yun Lai, Yufeng Li, Nan Shu, Zheng Wang, Yanping Wang, Yunsen Li Zijun Chen. (2018). Antineoplastic activity of isoliquiritigenin, a chalcone compound, in androgen-independent human prostate cancer cells linked to G2/M cell cycle arrest and cell apoptosis. Europian Journal of Pharmacology. Volume 821, 15 February Pages 57-67.

[3] H. Honda, Y. Nagai and T. Matsunaga. (2012) Glycyrrhizin and isoliquiritigenin suppress the LPS sensor toll-like receptor 4/MD-2 complex signaling in a different manner, Journal of Leukocyte Biology, vol. 91, no. 6, pp. 967–976.

[4] Nagisa Nosrati, Marica Bakovic, Gopinadhan Paliyath. (2017). Molecular Mechanisms and Pathways as Targets for Cancer Prevention and Progression with Dietary Compounds. Int J Mol Sci, Sep 25; 18 (10): 2050.

[5] Phamhuy, L. A. H. He, and C. Phamhuy (2008) Free radicals, antioxidants in disease and health, InternatIonal Journal of Biomedical Science, vol. 4, no. 2, pp. 89–96.

[6] Mohammad Khalid, H. H. Siddiqui and Sheeba Freed (2011). In-vitro Assessment of Antioxidant Activity of Dalbergia latifolia Barks Extract Against Free Radicals American-Eurasian Journal of Scientific Research, 6 (3): 172-177, 2011 ISSN 1818-6785.

[7] Sheu-Meei Yu and Sheng-Chu Kuo (1995). Vasorelaxant effect of isoliquiritigenin, a novel soluble guanylate cyclase activator, in rat aorta. British J of Pharmacology 124, 1587-1594.

[8] Muhammad Zubair, Hilde Nybom, Christina Lindholm, Johanna M Brandner, Kimmo Rumpunen. (2016). Promotion of wound healing by Plantago major L. leaf extracts–ex-vivo experiments confirm experiences from traditional medicine, Natural product research, Volume 30,5, 622-624.

[9] Sheu-Meei Yu and Sheng-Chu Kuo (1995). Vasorelaxant effect of isoliquiritigenin, a novel soluble guanylate cyclase activator, in rat aorta. British J of Pharmacology 124, 1587-1594.

[10] Houghton, P., Photiou, A., Uddin, S., Shah, P., Browning, M., Jackson, S., Retsas, S (1994) Activity of extracts of Kigelia pinnata against melanoma and renal carcinoma cell lines Planta med 60 (05), pp. 430-433.

[11] Nutan V Badgujar and Kinnari N Mistry (2017) In-vitro antioxidative, cytotoxic activity and phytochemical screening from stem bark of Kigelia pinnata" International J of Biotechnology and Biochemistry ISSN 0973-2691 Volume 13, Number 1 pp. 11-18.

[12] Liping Ye 1, Junjie Zhang 2, Yu Zhang 1, Binbin Gu 1, Hongyuan Zhu 1, Xinli Mao. (2020) Isoliquiritigenin Suppressed Esophageal Squamous Carcinoma Growth by Blocking EGFR Activation and Inducing Cell Cycle Arrest. Biomed Res Int. 2020 Mar 8; 2020: 9259852.

[13] Ewelina, S.; Zenon, P. C.; Bogdan, M.; Andrzej, P.; Wojciech, K. (2010) "Chalcones and Dihydrochalcones Augment TRAIL-Mediated Apoptosis in Prostate Cancer Cells" Molecules 2010, *15*, 5336–5353.

[14] Khushbu N. Thakkar, A K Prasad, Jyotiranjan Nayak, Satish V. Iyer, Shankulkumar (2014). Antioxidant and in vitro cytotoxic activity of extracts of aerial parts of Cocculushirsutus (L) using cell line cultures (breast cell line). The Journal of Phytopharmacology; 3 (6): 395-399.

[15] Muhammad Sulaiman, Zubair, SyarifulAnam, SubehanLallo (2016). Cytotoxic activity and phytochemical standardization of *Lunasiaamara* Blanco wood extract. Asian Pacific Journal of Tropical Biomedicine, Volume 6, Issue 11, November 2016, Pages 962-966.

[16] Jiabing, W. Lili, H. Chanchan, C. Ge, L. Jingwen, X. Mengya, S. Qian, C. Wulan, L., Wenfei, H. Peihong, Q. (2019) Design, synthesis and biological evaluation of chalcone analogues with novel dual antioxidant mechanisms as potential anti-ischemic stroke agents. Acta Pharm. Sin. B 2019, *9*, 335–350.

[17] Elfi, S. V. H.; Widiastuti, A. E. S (2018) A green synthesis of chalcones as an antioxidant and anticancer. IOP Conf. Ser. Mater. Sci. Eng. (2018), *299*, 012077.

[18] Cuendet, M., Guo, J., Luo, Y., Chen, S.-N., Oteham, C. P., Moon, R. C., Van Breemen, R. B., Marler, L. E., Pezzuto, J. M. (2010) Cancer chemopreventive activity and metabolism of isoliquiritigenin, a compound found in licorice. Cancer Prev. Res. 2010, 3, 221–232.

[19] Wang, K.-L.; Hsia, S.-M.; Chan, C.-J.; Chang, F.-Y.; Huang, C.; Bau, D.-T.; Wang, P. S. (2013) Inhibitory effects of isoliquiritigenin on the migration and invasion of human breast cancer cells. Expert Opin. Ther. Targets 2013, 17, 337–349.

[20] Chen, H.-Y.; Huang, T.-C.; Shieh, T.-M.; Wu, C.-H.; Lin, L.-C.; Hsia, S.-M. (2017) Isoliquiritigenin Induces Autophagy and Inhibits Ovarian Cancer Cell Growth. *Int. J. Mol. Sci.* 2017, 18, 2025.

[21] Po-Han Li, Yi-Fen Chiang, Tzong-Ming Shieh, Hsin-Yuan Chen, Chun-Kuang Shih, Tong-Hong Wang, Kai-Lee Wang, Tsui-Chin Huan, Yong-Han Hong, Sing-Chung Li and Shih-Min Hsia (2020) Dietary Compound Isoliquiritigenin, an Antioxidant from Licorice, Suppresses Triple-Negative Breast Tumor Growth via Apoptotic Death Program Activation in Cell and Xenograft Animal Models Antioxidants 2020, 9, 228; doi: 10.3390/antiox9030228.

[22] Peng, F. Tang, H. Liu, P Shen, J. Guan, X.-Y. Xie, X. Gao, J. Xiong, L. Jia, L. Chen, J. (2017). Isoliquiritigenin modulates miR-374a/PTEN/Akt axis to suppress breast cancer tumorigenesis and metastasis. Sci. Rep. 2017, 7, 9022.

[23] Wang, Z.; Wang, N.; Han, S.; Wang, N.; Mo, S.; Yu, L.; Huang, H.; Tsui, K.; Shen, J.; Chen, J. 2013. Dietary Compound Isoliquiritigenin Inhibits Breast Cancer Neoangiogenesis via VEGF/VEGFR-2 Signaling Pathway. PLoS ONE, 8, e68566.

[24] Zhang, X.; Zhu, P.; Zhang, X.; Ma, Y.; Li, W.; Chen, J.-M.; Guo, H.-M.; Bucala, R.; Zhuang, J.; Li, J. (2013) Natural Antioxidant-Isoliquiritigenin Ameliorates Contractile Dysfunction of Hypoxic Cardiomyocytes via AMPK Signaling Pathway. Mediat. Inflamm. 2013, 390890.

[25] L. L. Mensor, F. S. Menezes, G. G. Leitao (2001)Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, Phytotherapy Research, vol. 15, pp. 127–130, 2001.

[26] Martinez CA, Loureiro ME, Oliva MA, Maestri M. (2001) Differential responses of superoxide dismutase in freezing resistant *Solanumcurtilobum*and freezing sensitive *Solanumtuberosum*subjected to oxidative and water stress. Plant Sci 2001; 160: 505-15.