ISOLATION, CHARACTERIZATION, ANTIBIOLOGICAL, ANTICANCER ACTIVITY OF TETRAHYDROBENZO CHROMONE GLYCOSIDE DERIVATIVE FROM LEAF OF *Melochia corchorifolia*

M.Vinoth^{1,2} and B. Natarajan^{1,✉}

¹Department of Chemistry, SRM Institute of Science and Technology, Kattankulathur 603 203, (Tamil Nadu) India

²Department of Chemistry, Dhanalakshmi Srinivasan College of Engineering and Technology, Mamallapuram 603 104, (Tamil Nadu) India

✉Corresponding Author: balanattunet@gmail.com

ABSTRACT

A new tetrahydrobenzo chromone glycoside derivative was isolated from the ethanolic extract of the dried powdered leaves of *Melochia corchorifolia* by using silica gel chromatography. The isolated compound was subjected to High-Performance Liquid Chromatography analysis for purity determination. Further characterization carried out by using FT-IR, UV-Visible, ¹H-NMR, ¹³C-NMR, DEPT-135NMR, ¹H-¹H COSY, HSQC NMR, and Mass Spectrometric methods. The pure isolated derivative was tested against human pathogenic microorganism and exhibited the maximum zone of inhibition against *Pseudomonas aeruginosa*, *E.coli*, and *Staphylococcus aureus*. The Anticancer activity MCF-7 cancer cell line against the compound showed the drug efficacy of half-maximal inhibitory concentration IC₅₀ value of 120.71 ± 3.46.

Keywords: *Melochia corchorifolia*, Spectroscopic Techniques, Antimicrobial, MCF-Cell Line, Anticancer Activity. RASĀYAN J. Chem., Vol. 14, No.2, 2021

INTRODUCTION

The medicinal plants of secondary metabolites are an excellent natural source of therapeutics and are subsequently used for the treatment of innumerable disease and infections¹. Human infection which is a major source of distress is frequently caused by pathogens such as bacteria and fungi². The problem of bacterial and viral infection becomes a crisis due to the progress of antibiotic resistance by the infecting bacteria and fungi³. Further, amongst the available antimicrobial drugs, the use of antimycotic drugs is limited by several factors including the emergence of resistant strains, poor solubility, low potency, and medication toxicity⁴. As a consequence, the rigorous search for novel disinfectants must continue and should be augmented with research on potential chemotherapeutic agents of plant origin considering that the distinctive pharmacological properties of plants vary and attributed to the unique mixtures of secondary metabolites that are species-specific and taxonomically distinct^{5, 6, 7}. *Melochia corchorifolia* is the most normally used in folk medication and the plant belongs to the *Sterculiaceae* family⁸. The plant leaves are edible, which is used to treatment of ulcer conditions, chest pains, ear-related problems, parasitic infections, blotting, snake bites, and used as fodder for cattle. The leaves are used as protein, dietary lipid, fibers, and mineral supplements⁹. The medicinal plant has shrub with hairy, thorny stems, and leaves that possess variable shapes. The flowers are of deep pink, terminal peduncle, and seeds are black¹⁰. There are pharmacologically active compounds derived from *Sterculiaceae* plants, which are widespread and grows fine in tropical climatic conditions. Since chromone glycoside derivative possesses pharmacological activities and can interact with cytochrome P⁴⁵⁰ and is useful for medicinal applications¹¹. The research report confirms the occurrence of alkaloids, pyridine derivatives, flavonol glycosides, flavonoids, an aliphatic compound like β-D-sitosterol, and ethanolic extract of this plant leaves is capable to inhibit MCF-7 cancer cell lines. The plant leaves also contain anti biological activity, anticancer property, antioxidant activity, and Anthelmintic activity¹². The existing study focused to

identify a novel pharmaceutically active compound derived from the ethanolic extract of *Melochia corchorifolia*, which includes isolation, structural elucidation, anti biological and anticancer activities.

EXPERIMENTAL

Extraction of Plant Leaves

The plant *M. corchorifolia* was spotted and collected from the Reserve forest area, Vandalur, Thiruporur, Kanchipuram Dt. The plant was recommended taxonomically by the Director of the Institute of Herbal Botany, Chennai, Prof. P. Jayaraman. The collected leaves were washed and shaded as well as dried to eliminate chlorophyll content. The dried leaves were finely powdered by using a mechanical blender. The 100g of powdered leaves extracted with ethanol solvent in Soxhlet apparatus.¹³

Isolation of the Compound by Silica Gel Column Chromatography

Column chromatography was performed for the active crude sample with 100-200 mesh size silica gel using petroleum ether as a solvent. Around 5g of the crude was taken for silica gel chromatography and purified using petroleum ether and ethyl acetate (9:1).¹⁴ From the TLC plate, the major fractions were spotted, isolated, and stored under ambient temperature.

Instrumental Methods

Spectral Analysis

Using Shimadzu-FTIR Infrared spectrometer, FT-IR spectrum was recorded in KBr ($\nu_{\max}$ in 4000-400 cm^{-1}). A ^1H NMR spectrum was observed using a Bruker Avance II 500 MHz spectrometer and CDCl_3 as solvent. The Mass spectrometric analysis was performed using the Thermo Scientific Orbit Rap Elite Mass spectrometer with the Data system is a high resolution, double-focusing instrument^{15, 16}. A UV-Visible spectrum was recorded using Shimadzu, Japan with a scan range between 200 and 800 nm that was used to catalog UV-Vis spectra. Ethanol was used as a blank.¹⁸

HPLC Chromatography Analysis

A Shimadzu LC solution with a quaternary pump was used to conduct HPLC analysis. The column which helped in this study was C18250 $4.6 \times 5 \mu\text{m}$. The mixture of toluene, ethyl acetate, and ethanol (7:2:1) mobile phase solvent. The flow rate set to 1 ml/min, and the total run time 20 min.¹⁷

In-vitro activity-Antibiological Studies

Test-microorganisms

In-vitro biological activity is done with the Test type strains of *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 404), *Pseudomonas aeruginosa* (MTCC 2453), *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441) (IMTECH, Chandigarh, India).¹⁹

The agar well diffusion method was used for antimicrobial activity analysis. Exactly, about 25ml of potato dextrose agar media for antifungal studies and molten Mueller Hinton agar media for antibacterial studies were used for biological analysis. Then agar medium (Himedia, Mumbai, India) is poured into a sterile Petri plate and the OD value was adjusted to 0.6 of the 18 hours grown test microorganisms, and 100 μl of each was plated onto the solidified media and spread using a sterile L-rod. After 5 min, each well was ready with a sterile cork borer. In sterile saline, the test samples dissolved and further loaded into the well with specific concentrations at 25, 50, 75, 100 mg/ml separately and the solvent saline loaded well used negative control, Streptomycin (30 $\mu\text{g/ml}$) and Clotrimazole (30 $\mu\text{g/ml}$) act as a positive control. Culture samples are incubated (37°C) by bacteriological incubator for 24 hours and 48-72 hours fungi²⁰. The antimicrobial activity was performed, and the inhibition zone of diameter formed around the well was computed.

Cytotoxicity Assay

The MCF-7 cell line in Dulbecco's Modified Eagle's Medium cultured, about 25ml of DMEM with 10% FCS added to disaggregated cells. The cells are suspended then homogenized and in addition to the different sample concentrations (3.12-400 $\mu\text{g/ml}$), then 1 ml homogenized cell suspension added to culture plates and incubated at 37 °C in a humidified 5% CO_2 incubator. The cytotoxicity assay was

performed with 3-(4, 5-Dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide. The well was added with MTT assay after that 48 hrs of incubation and leave at room temperature for 3 hrs. The well is taken out by using a pipette 100 μ l in DMSO and the solutions were applied and dissolve with formazan crystal the absorbance was read in (Read Well Touch) microplate recorder at 570nm.^{21, 22}

RESULTS AND DISCUSSION

Silica Gel Column Chromatography and TLC

In column chromatography, five fractions were eluted with petroleum ether and ethyl acetate, which yield 90 mg of the greenish-yellow color solid. The Major fraction 1 showed an Rf value of 0.87 with a single spot on the TLC plate. The isolated compound was finally subjected to spectroscopic analysis Table-1.

Table-1: TLC profiling of the purified compounds from *Melochia corchorifolia* ethanol extract

S. No.	Compounds	Rf Value
1	1	0.87
2	2	0.61
3	3	0.70
4	4	0.82
5	5	0.50

*Rf=Retention Factor

Spectroscopic Characterizations of the Isolated Glycoside Derivative

FT-IR Spectral Analysis

The FT-IR spectrum of a glycoside derivative showed the peaks of functional groups such as Hydroxyl group (3557), Aliphatic CH (2925), Methylene symmetric vibration CH (2855), Aromatic ring C-C stretch (1623), C-O-C stretch (1063), and Methyl bend (1460) the spectrum is showed Table-2 Fig.-1.

Table-2: FT-IR Spectrum-Glycoside Derivative

S. No.	Group Frequency Wavenumber (cm ⁻¹)	Functional Groups	Possible Origin of Functional Groups
1	3557	-O-H (Internally bonded OH stretch)	Alcohol
2	2925	-C-H (Sym/Asym)	Aliphatic
3	2855	-C-H (Sp ³ stret)	Methylene
4	1063	-C-O-C (stret)	Ether
5	1623	-C-C (ring stret)	Aromatic
6	1460	-CH ₃ (sp ³ ,bend)	Methyl

Fig.-1: FT-IR Spectral Analysis Spectrum of Isolated Compound

Structure Elucidation of Glycoside Derivative by Spectral Analyses

The ^1H NMR Spectrum of an isolated compound is shown Fig.-2. ^1H NMR (500 MHz, CDCl_3) δ 7.21 (t, $J=5\text{Hz}$, 1H), 7.45 (t, $J=5\text{Hz}$, 1H), 6.97 (d, $J=5\text{Hz}$, 1H), 6.55 (d, $J=10\text{Hz}$, 1H), 7.06 (d, $J=5\text{Hz}$, 1H), δ 7.65 (t, $J=5\text{Hz}$, 1H), 5.77-5.70 (m, 1H), 5.29-5.26 (m, 1H), 5.05 (bs, 3H), 4.86 (t, $J=10\text{Hz}$, 1H), 4.93 (d, $J=20\text{Hz}$, 2H), 4.52-4.47 (m, 1H), 4.24 (t, $J=5\text{Hz}$, 2H), 4.02 (d, $J=10\text{Hz}$, 2H), 1.97 (s), 1.61 (s), 1.53 (d, $J=5\text{Hz}$), 1.33-0.79 (m) ppm.

The ^{13}C NMR Spectrum of isolated compound in CDCl_3 is shown Fig.-3. ^{13}C NMR (125 MHz, CDCl_3) δ 167.68, 152.05, 139.19, 135.17, 132.40, 130.89, 128.84, 125.03, 123.38, 115.85, 114.08, 71.77, 68.13, 65.54, 47.82, 39.37, 37.44, 34.38, 33.82, 32.79, 32.21, 31.93, 31.64, 31.43, 30.57, 30.18, 29.71, 29.69, 29.52, 29.37, 29.16, 28.96, 27.97, 27.72, 27.20, 26.41, 24.80, 23.43, 22.69, 19.74, 19.15, 15.99, 14.12, 13.72, 10.96 ppm.

The DEPT 135 NMR Spectrum of isolated compound in CDCl_3 is shown Fig.-4. DEPT 135 NMR (125 MHz, CDCl_3) δ 139.19, 130.88, 128.84, 125.03, 123.91, 115.85, 114.08, 71.77, 68.12, 65.54, 39.72, 39.37, 37.43, 34.37, 33.82, 32.20, 31.93, 31.63, 31.42, 30.57, 30.18, 29.80, 29.70, 29.62, 29.52, 29.37, 29.16, 28.95, 27.19, 26.41, 25.62, 25.50, 24.79, 23.75, 23.43, 22.69, 19.74, 19.18, 19.15, 15.98, 14.12, 13.72, 10.95 ppm.

The HSQC NMR Spectrum of an isolated compound is shown Fig.-5. The ^1H - ^1H COSY NMR Spectrum of the isolated compound in CDCl_3 is shown Fig.-6.

^1H NMR spectrum of glycoside derivative showed aromatic methine proton was observed at 7.65 (t, $J=5\text{Hz}$, 1H), 7.45 (t, $J=5\text{Hz}$, 1H), 7.21 (t, $J=5\text{Hz}$, 1H), 7.06 (d, $J=5\text{Hz}$, 1H), 6.97 (d, $J=5\text{Hz}$, 1H) and 6.55 (d, $J=10\text{Hz}$, 1H) ppm respectively. Pyran and Furan ring attached methylene protons which were observed at 4.86, 4.52 ppm. Sugar attached methine, methylene protons were observed at 4.52, 4.86, 4.93 and 4.24 ppm respectively. The alkene protons were observed at 5.29 and 5.77 ppm. Hydroxyl groups were observed at 5.09 broad singlets.

^{13}C NMR of the glycoside derivative aromatic methine carbons was observed at 130.88, 128.84, 123.91, 114.08, and 123.38 ppm respectively. The Pyran and Furan ring attached methylene carbons were observed at 65.54 and 77.07 ppm. Sugar attached methine and methylene carbons were observed at 63.12, 100.84, and 71.77 ppm respectively. The alkene carbons showed at 128.09 and 139.19 ppm. Quaternary aromatic carbons were observed in the range at 132.40, 123.38, 167.68, and 135.17 ppm respectively.

Fig.- 2: ^1H NMR Spectrum Glycoside Derivative - CDCl_3

Further, the DEPT 135 NMR was confirmed by primary, secondary, and tertiary carbons. The aromatic methane of tertiary carbons is 130.88, 128.88, 123.91, 114.08 and 123.38 ppm respectively. The aliphatic methane showing the tertiary carbons is 31.42, 33.83, 32.20, 31.93, 78.02, 68.12, 65.54, 100.84, and

71.77 ppm respectively. The aliphatic methylene (secondary carbon) is 29.80, 65.54, 29.70, 30.18, 47.82, 70.82 and 63.12 ppm.

Fig.-3: ¹³C NMR Spectrum Glycoside Derivative - CDCl₃

Fig.-4: DEPT 135 NMR Spectrum Glycoside Derivative - CDCl₃

Fig.-5: HSQC NMR Spectrum Glycoside Derivative - CDCl₃

The investigation of the correlations in the ^1H - ^1H COSY spectrum of aromatic H1 coupled with H2 proton and H5 coupled with H6. Furthermore, the HSQC NMR was confirmed the anomeric H-atoms connected with carbon and contributed the glycoside moiety shown in Fig.-7.

Fig.-6: ^1H - ^1H COSY NMR Spectrum Glycoside Derivative - CDCl_3

Fig.-7: HSQC NMR – Glycoside Derivative

S-3_1412231544 #116 RT: 1.12 AV: 1 NL: 1.70E6
T: FTMS + c ESI Full ms [150.00-2000.00]

Fig.-8: Mass Spectrum Glycoside Derivative

From the mass spectral analysis Fig.-8, the glycoside derivative showed the most intense base peak around 566.2880 is considered as molecular ion peak, the molecular mass of a compound is agreed with the theoretical mass m/z calculated as 566.6818. From HR-MS, the molecular formula of the compound deduced is $C_{33}H_{42}O_8$, and the name of the compound is 6-(((5-(3-benzyl-4-(2, 3, 3a, 6-tetrahydrobenzo[de]chromen-3-yl) butan-2-yl) tetrahydrofuran-3-yl)oxy) methyl) tetrahydro-2H-pyran-2, 3, 4, 5-tetraol have been reported with the spectral studies^{23,24} Fig.-9.

Fig.-9: Glycoside Derivative: 6-(((5-(3-benzyl-4-(2,3,3a,6-tetrahydrobenzo[de]chromen-3-yl)butan-2-yl)tetrahydrofuran-3-yl)oxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetraol.

HPLC Analysis

The isolated and purified glycoside derivative was spotted as a single peak with a Retention Time of 6.218 min with a sharp peak. The peak area is 45214 with peak area coverage of 100% showed Fig.-10.

<Chromatogram>

1 Det.A Ch1 / 254nm

Peak Table

Peak#	Ret. Time	Area	Area %	Name
1	6.218	45214	45214	-
	Total	45214	100.00	-

Fig.-10: HPLC Analysis Glycoside Derivative

UV-Vis Spectroscopy

The isolated compound of UV-Visible Spectrum is shown Fig.-11. The spectrum shows an absorption maximum at a wavelength of $\lambda_{\max}$ 229 nm and 279 nm indicate carbonyl group with $n-\pi^*$ transition.

In-vitro Antimicrobial Activity

To evaluate *In-vitro* studies, Streptomycin used as (30 μ g/ml) positive control against bacterial strains and produced a zone of inhibition range is 19 - 26 mm, While Clotrimazole (30 μ g/ml) served as the positive control for fungal strains and zone of inhibition range is 24 - 26 mm. The four different concentrations of 25, 50, 75, and 100 μ g/ml compound were taken to evaluate anti biological activity to measure the

minimum inhibitory concentration. The test compound was active against both bacterial and fungal species with inhibition activity. Out of four concentrations, 25 µg/ml of the sample did not show antibacterial activity against *Bacillus subtilis*, *Candida albicans* (50 and 75 µg/ml), and *Aspergillus niger* (50 and 75 µg/ml). The concentrations tried at 75 and 100 µg/ml exhibited the maximum antimicrobial activity against *Escherichia coli* (15.98±0.25mm and 18.10±0.15mm, respectively) and *Staphylococcus aureus* (14.97±0.03 and 17.24±0.14mm, respectively). Anti-fungal activity against *Aspergillus niger* and *Candida albicans* showed the least activity (09.10±0.12 and 08.36±0.23mm in 100 µg/ml concentrations)²⁵ Table-3.

Table-3: Antibacterial & Antifungal activity of Glycoside derivative by agar well diffusion assay method

S.No.	Name of Bacterial and Fungal microorganism	Antibacterial activity				
		STD drug	Test sample (mg/ml) / Zone of inhibition (mm)			
		30 mg/mL	25	50	75	100
1.	<i>E.coli</i>	26.05±0.32	12.08±0.07	14.10±0.25	15.98±0.25	18.10±0.15
2.	<i>P. aeruginosa</i>	24.03±0.06	9.21±0.12	11.21±0.05	13.25±0.09	15.05±0.09
3.	<i>B. subtilis</i>	19.20±0.11	0.00±0.00	08.13±0.06	10.06±0.10	12.16±0.03
4.	<i>S. aureus</i>	23.14±0.17	11.42±0.09	13.08±0.14	14.97±0.03	17.24±0.14
5.	<i>C.albicans</i>	26.19±0.09	0.00±0.00	0.00±0.00	00.00±0.00	09.10±0.12
6.	<i>A. niger</i>	24.14±0.01	0.00±0.00	0.00±0.00	00.00±0.00	08.36±0.23

* Streptomycin; # Clotrimazole ZOI: Zone of inhibition

Fig.-11: UV-Visible Spectrum Glycoside Derivative

Table-4: *In-vitro* cytotoxicity Compound - MCF-7 Cell Viability

Sample Concentrations (µg/ml)	MCF-7 (%)
	Compound
0	100.0± 00.00
3.125	89.80± 1.18
6.25	83.94± 1.46
12.5	75.58± 0.88
25	62.81± 1.13
50	41.12± 0.77
100	27.39± 0.69

200	19.36± 0.92
400	9.48± 0.40
IC50	120.71± 3.46

Fig.-12: Anticancer Activity of the Glycoside Derivative

Anticancer Activity

The MCF-7 cell line of the compound showed better cell viability at a concentration range from 3.125 µg/ml to 400 µg/ml at 48 hours (89.80% and 9.48%) showed in Table-4. The IC50 value of the compound was 120.71 µg/ml against MCF-7 cell lines which induced apoptotic cell death²⁶ Fig.-12.

CONCLUSION

The new tetrahydrobenzo chromone glycoside derivative was isolated from the ethanolic extract of *Melochia corchorifolia* leaves and compound isolated from silica gel chromatography by solvents lower polarity to higher polarity then characterized through spectral analysis from that molecular formula of the compound deduced is C₃₃H₄₂O₈ and name of the compound is 6-(((5-(3-benzyl-4-(2,3,3a,6-tetrahydrobenzo[de]chromen-3-yl)butan-2-yl)tetrahydrofuran-3-yl)oxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetraol conformed both theoretically and experimentally. The data on biological activity provide scientific evidence for the conventional use of the plant *Melochia corchorifolia*. It can be a promising candidate as a potential source of the antibacterial agent against contagious diseases caused by the bacteria species *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Furthermore, the compound showed a better inhibitory effect on MCF-7 cell line growth and proliferation. The results specify that the isolated compound might be effective antimicrobial agents in the control of human diseases and residual toxicity. Future study structurally elucidated the remaining isolated bioactive compounds and will be analyzed their biological activity.

ACKNOWLEDGEMENT

We Acknowledge DST-FIST (fund improvement-S&T infrastructure) financial assistance for the Department of Chemistry, SRM Institute of Science & Technology, No.SR/FST/CST-266/2015(c).

REFERENCES

1. K. Gurning, W. Haryadi and H. Sastrohamidjojo, *Rasayan Journal of Chemistry*, **14(1)**, 248(2021), DOI:10.31788/ RJC.2021.1416077
2. R. Cavicchioli, W. J. Ripple, K. N. Timmis and N. S. Webster, *Nature Reviews Microbiology*, **17(9)**, 569(2019), DOI:10.1038/s41579-019-0222-5

3. M. Frieri, K. Kumar and A. Boutin, *Journal of Infection and Public Health*, **10(4)**, 369(2017), DOI:10.1016/j.jiph.2016.08.007
4. M. J. Salvador, P. S. Pereira, S. C. França, R. C. Candido , I. Y. Ito and D. A. Dias, *Brazilian Journal of Microbiology*, **35(1)**, 131(2004), DOI:10.1590/S1517-83822004000100022
5. A. C. Abreu, A. J. McBain and M. Simões, *Natural Product Reports*, **29(9)**, 1007(2012), DOI:10.1039/c2np20035j
6. P. Saranraj and S. Sivasakthi, *Global Journal of Pharmacology*, **8(3)**, 316(2014), DOI:10.5829/idosi.gjp.2014.8.3.83194
7. Donald P. Briskin, *Plant Physiology*, **124(2)**, 507(2000), DOI:10.1104/pp.124.2.507
8. V. Harini, M. Vijayalakshmi, C. Sivaraj and P. Arumugam, *International Journal of Science and Research*, **6(1)**, 1310(2017), DOI:10.21275/ART20164305
9. K. J. Umar, L. G. Hassan, S. M. Dangoggo, M. Inuwa and M. N. Almustapha, *International Journal of Biological Chemistry*, **1(4)**, 250(2007), DOI:10.3923/ijbc.2007.250.255
10. T. Pullaih, Ethnobotany, *International Research Journal of Pharmacy*, **5(7)**, 543(2014), DOI:10.7897/2230-8407.0507109
11. D. K. Sharma, *Journal of Scientific and Industrial Research*, **65(6)**, 477(2006).
12. Ivan Nielsen, G .J .H. Grubben and O. A. Denton, *Nordic Journal of Botany*, **23(3)**, 298(2008), DOI:10.1111/j.1756-1051.2003.tb00397.x
13. Sarvesh Kumar, Vijay Jyoti Kumar and Ranjit Singh, *Rasayan Journal of Chemistry*, **13(3)**, 1637(2020), DOI:10.31788/RJC.2020.1335911
14. R. Devika and J. Koilpillai, *International Journal of Pharmaceutical Sciences and Research*, **6(2)**, 762(2015), DOI:10.13040/IJPSR.0975-8232
15. S. Lakshmi Pillai and R. Bindu Nair, *Journal of Pharmacognosy and Phytochemistry*, **2(6)**, 120(2014)
16. K. Selvaraju and A. Manimekalai, *Rasayan Journal of Chemistry*, **10(1)**, 25(2017), DOI:10.7324/RJC.2017.1011544
17. H. Salomies and J. P. Salo, *Chromatographia*, **36(1)**, 79(1993), DOI: 10.1007/bf02263842
18. V. Sathiyarayanan, H. Venkatasubramanian and D. Easwaramoorthy, *Rasayan Journal of Chemistry*, **12(4)**, 2141(2019), DOI:10.31788/RJC.2019.1245419
19. Tita Juwitaningsih, Iis Siti Jahro, Ida Dumariris, *Rasayan Journal of Chemistry*, **13(2)**, 1096(2020), DOI:10.31788/RJC.2020.1325614
20. I. A. Holder and S. T. Boyce, *Burns*, **20(5)**, 426(1994), DOI: 10.1016/0305-4179(94)90035-3
21. A. L. Niles, R. A. Moravec and T. L. Riss, *Expert Opinion on Drug Discovery*, **3(6)**, 655(2008), DOI: 10.1517/17460441.3.6.655
22. E. E. Aung, A. N. Kristanti, N. S. Aminah, Y. Takaya, R. Ramadhan and H. T. Aung, *Rasayan Journal of Chemistry*, **14(1)**, 312(2021), DOI:10.31788/ RJC.2021.1416106
23. Ren Bo An, Gil Saeng Jeong, Jin-Seon Beom, Dong Hwan Sohn and Youn Chul Kim, *Archives of Pharmacal Research*, **32(10)**, 1393(2009), DOI: 10.1007/s12272-009-2008-1
24. Si-Yuan Ma, Ling-Gao Shi, Zheng-Bing Gu, Yu-Lan Wu, Liu-Bin Wei, Qi-Qiu Wei, Xing-Ling Gao and Na Liao, *Chemistry & Biodiversity*, **15(9)**, e1800253(2018), DOI:10.1002/cbdv.201800253
25. H. P. C. A. Cane, N. Saidi, M. Mustanir, D. Darusman, R. Idroes and M. Musman, *Rasayan Journal of Chemistry*, **13(4)**, 2215(2020), DOI:10.31788/ RJC.2020.1345818
26. Eun-Mi Noh, Mi Suk Yi, Hyun Jo Youn, Byoung Kil Lee, Young-Rae Lee, Ji-Hey Han, Hong-Nu Yu, Jong-Suk Kim and Sung Hoo Jung, *Journal of Breast Cancer*, **14(1)**, 8(2011), DOI:10.4048/jbc.2011.14.1.8

[RJC-6131/2020]