Available free online at www.medjchem.com

Mediterranean Journal of Chemistry **2023**, 13(1), 35-41

New biologically active allelochemical from the leaves of *Dalbergia Paniculata* Roxb

Alok Kumar Singh*, Raj Nath Yadava, Ritu Yadav and Satyaprakash shrivastava

Department of Chemistry, Dr H.S. Gour Vishwavidyalaya (A Central University), Sagar 470 003, M. P. India

Abstract: A new biologically active Allelochemical 3,5,7,3',4'-pentahydroxyflavone-3-*O*-*β*-*D* xylopyranosyl -(1→4)- *β*-*D*-glucopyranosyl-4`-*O*-*α*-L-arabinoside (**A_1**) whose m.p. is 340–345 °C, m.f. [M^+] 728 (EIMS) was isolated from methanolic leaf extracts of *Dalbergia paniculata* Roxb. It was characterized by several color reactions, spectral analysis FTIR, 1HNMR, MS, and chemical degradations. The results of the antimicrobial activity of **(A_1)** at high concentration (100µg/mL) was 20, 17, and 15 mm zone of inhibition against *E. coli*, *B. cereus*, and *S. aureus aeruginosa*, respectively. The data demonstrated that the antimicrobial activity of CH_3OH soluble fraction **(A_1)** at all concentrations was highest against *E. coli* and lowest *S. aureus. E. coli*, *B. cereus*, and *S. aureus aeruginosa*, respectively 7.3, 2.1, and 0.02 mm, MIC was defined as the minimum concentration of assayed samples that inhibited the visible growth of the tested microorganism. MIC in *E. coli*, *B. cereus*, and *S. aureus aeruginosa* was 7.3, 2.1, and 0.02mm, respectively.

Keywords: Flavone glycoside; *Dalbergia paniculata* Roxb.; Antimicrobial activity; Allelochemicals.

1. Introduction

Dalbergia paniculata Roxb. [1-18] belongs to the Leguminosea family, commonly known as Dhoban, phansi in Hindi. It is a large deciduous tree with smooth bark and yellowish blaze growing up to 30 m in height and widely distributed throughout India. Its bark and root are used for the treatment of diarrhea. Leaf and bark extract showed maximum antimicrobial activity. Many bioactive constituents are isolated from the root, stem, and bark of *Dalbergia paniculata* Roxb. Paniculatin, Paniculatin, Isocavuinin7-*O*-glucosides,7-*O* Rutinosides of biochanin A and formononetin from the bark *Dalbergia paniculata* Roxb. Dalpanitin Dalpetin, from the seeds, minor isoflavonoid glycosides from the stem bark of *Dalbergia paniculata* Roxb. have been isolated by earlier workers. This study focuses on isolation and structure elucidation of new allelochemical (**A_1**) from methanolic leaves extracts of *Dalbergia paniculata* Roxb. which is confirmed by color reactions and analytical techniques such as FTIR, NMR, MS, and chemical degradations, which have shown antimicrobial activity.

2. Materials and Methods

2.1. General Experimental Procedure

A Thiele's tube was used to determine melting points, which are uncorrected. Bruker Alpha II FTIR was used to record the IR spectra on KBr disks. 1HNMR and 13CNMR spectra are recorded in a Bruker DRX at 500 MHz and 125MHz spectrometer, respectively, using $CDCl_3$ as a solvent, EI (70eV) mass spectrometer.

2.2. Collection of *Dalbergia paniculata* Roxb.

Leaves of *Dalbergia paniculata* Roxb. were collected in the Sagar region. The voucher specimen no-Bot/H/03/57/562 has been deposited and taxonomically identified by the department of botany Dr H.S. Gour Vishwavidyalaya, Sagar, M.P, India.

2.3. Extraction and Isolation

Air-dried and powdered leaves of *Dalbergia Paniculata* Roxb. were extracted by Soxhlet apparatus with different solvents depending on polarity with Pet Ether (40-60^{0}C), $CHCl_3$, $C_2H_5COOC_2H_5$, acetone, CH_3OH. Methanolic extracts were condensed using a rotary evaporator under reduced pressure to yield Chocolate brown colored sticky mass. On TLC examination using n-butyl alcohol: Ethanoic acid: H_2O in (4:1:5) ratio as a solvent and I_2 vapor as visualizing reagent. It showed two spots separated as compounds (**A_1**) and (**B_2**). These compounds (**A_1**) and (**B_2**) were purified and separated by column chromatography over a silica gel using $CHCl_3$: MeOH in a different ratio. Because the compound (**B_2** was found in very small quantities, further characterization has been difficult. 2 g of compound (**A_1**) were crystallized from chloroform needles.

**Corresponding author: Alok Kumar Singh*
Email address: alokbhucham123@gmail.com
DOI: http://dx.doi.org/10.13171/mjc02301311676singh

Received August 22, 2023
Accepted October 2, 2023
Published December 11, 2023

2.4. Study of Allelochemical (A_1)

The molecular formula of Allelochemical (**A_1**) $C_{31}H_{36}O_{20}$, m.p. 340–345 °C, (M^+) 728 (EIMS), Elemental Analysis found (%) C, 51.8 %, H 4.92 %, O 43.90 %, Calculated for m.f $C_{31}H_{36}O_{20}$ found(%), C 51.10 %, H 4.98 %, O 43.92 %, UV absorbance observed at λ_{max} (nm) 268, 371 recorded in methanol. IR spectra showed absorption bands 3448, 2924, 2315, 1644, 1517, 1101 and 885 cm^{-1}.

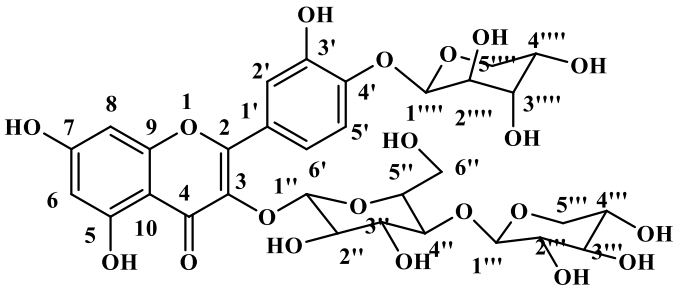
Table 1. 1HNMR (DMSO-d_6 500 MHz)

S.No	δValue	Pattern	J value (Hz)	No.of Proton	Assignment
A	6.30	s		1	H_6
B	6.76	“		“	H_8
C	12.90	“		“	H_5,OH
D	7.50	“		“	$H_{2'}$
E	6.57	d	8.4	“	$H_{5'}$
F	7.60	d	8.4	“	$H_{6'}$
G	5.50	d	7.3	“	$H_{1''}$
H	3.27	m		“	$H_{2''}$
I	3.90	“		“	$H_{3''}$
J	3.36	“		“	$H_{4''}$
K	4.13	“		“	$H_{5''}$
L	3.70	“		2	$H_{6a''}$
M	3.40	“		2	$H_{6b''}$
N	4.24	d	6.4	“	$H_{1'''}$
O	2.97	m		“	$H_{2'''}$
P	3.14	“		“	$H_{3'''}$
Q	3.41	m	9.0	“	$H_{4'''}$
R	3.73	m		2	$H_{5'''}$
S	4.86	d	6.4	“	$H_{1''''}$
T	3.12	m		“	$H_{2''''}$
U	3.31	“		“	$H_{3''''}$
V	3.16	“		“	$H_{4''''}$
W	3.38	“		“	$H_{5''''}$

Table 2. 13CNMR (DMSO-d_6 125 MHz).

S.No	δValue	Assignment
A	147.9	C_2
B	134.9	C_3
C	179.2	C_4
D	160.5	C_5
E	99.2	C_6
F	166.2	C_7
G	93.7	C_8
H	156.8	C_9
I	106.0	C_{10}
J	120.5	$C_{1'}$

K	116.7	C_{2}'
L	146.6	C_{3},
M	147.5	C_{4},
N	115.4	C_{5},
O	120.9	C_{6},
P	100.6	C_{1},,
Q	73.8	C_{2},,
R	77.0	C_{3},,
S	79.40	C_{4},,
T	75.50	C_{5},,
U	60.40	C_{6},,
V	103.2	C_{1},,,
W	73.60	C_{2},,,
X	76.50	C_{3},,,
Y	70.10	C_{4},,,
Z	65.80	C_{5},,,
A'	105.10	C_{1},,,,
B'	73.45	C_{2},,,,
C'	75.90	C_{3},,,,
D'	71.24	C_{4},,,,
E'	73.80	C_{5},,,,



Structure of (A_1)

2.4.1. Acid Hydrolysis of (A_1)

400 mg of compound **(A_1)** were dissolved in methanol (30 ml) and hydrolyzed in 7.5% of H_2SO_4 (20 ml) by refluxing for 8 h in the water bath**.** We concentrated the reaction mixture, cooled it, and separated the residue using Et_2O. The ether layer was washed with water, and the residue was chromatographed over silica gel using $CHCl_3$: CH_3OH (6:4) as a solvent to give aglycone **(A_2)**. The aqueous hydrolysates were neutralized with Barium carbonate and Barium sulfate and were filtered off. Paper chromatography was performed after the filtrate was concentrated analysis using n-butyl alcohol: Ethanoic acid: H_2O in (4:1:5) ratio used as mobile phase and Ninhydrine as visualizing reagent showed the presence of L-arabinose (R_f 0.21), D-xylose (R_f 0.27), D-glucose (R_f 0. 18)(Co-Pc) Aglycone **(A_2)** was identified as 3,5,7,3', 4'-penta hydroxyflavone.

2.4.2. A_2 (Aglycon)

Molecular formula $C_{15}H_{10}O_7$ was analyzed by m.p. 315–318°C,

(M^+) 302 (EIMS)

Elemental analysis found (%) C, 59.51 %, H 3.23 %, O 37.04 %, Calculated m.f $C_{15}H_{10}O_7$ found(%), 59.61 %, H 3.33 %, O 37.06 %,

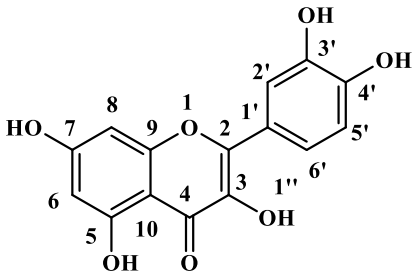
UV (MeOH) λ_{max} (nm) 260, 300, IR (KBr) spectra showed absorption bands 3448-3200, 1664, 1560, 1515.

Table 3. 1HNMR (DMSO-d_6 500MHz) of A2

S.No	δValue	Pattern	J value (Hz)	No.of Proton	Assignment
A	6.20	s		1	H_6
B	6.76	s		"	H_8
C	12.40	s		"	H_5,OH
D	7.70	s		"	H_2,
5	6.92	d	8.4	"	H_5'
E	9.40	s	8.4	"	H_3,OH
F	7.57	d		"	H_6'

Table 4. 13CNMR (DMSO-d_6 125 MHz) of A2

S.No	δValue	Assignment
A	154.7	C_2
B	136.0	C_3
C	176.2	C_4
D	160.9	C_5
E	98.4	C_6
F	164.9	C_7
G	98.7	C_8
H	156.8	C_9
I	104,0	C_{10}
J	122.8	C_1,
K	116.7	C_2,
L	146.6	C_3,
M	148.5	C_4,
N	115.4	C_5,
O	120.9	C_6,



Structure of (A_2)

2.4.3. Permethylation of (A_1)

(**A_1**) [19] 45mg was dissolved in C_3H_7NO (20 ml), refluxed with methyl iodide (5 ml) and silver di-oxide (40mg) for 48 hours, filtered with C_3H_7NO, and washed. The filtrate was dried in a vacuum and hydrolyzed with 10% ethanolic H_2SO_4 for 6 h, methylated aglycone identified as **A_2** (3,4'-dihydroxy 5,7,3'-trimethoxyflavone). The aqueous hydrolysates were neutralized with Barium carbonate and Barium sulfate and were filtered off. Paper chromatography was performed after filtrate was concentrated using n-butenol, acetic acid, and water with a (4:1:5) ratio as developer and Ninhydrine as visualizing reagent and methylated sugars, which were identified as **A_4** {2,3 4-tri-*O*-methyl-D xylose (R_G 0.93)}, **A_5** {2,3,4,6-tetra –*O*-methyl-D-galactose (R_G 0.68)}, **A_6** {2,3 4-tri-*O*-methyl-L-arabinose(R_G)}.

2.4.4. Enzymatic Hydrolysis of (A_1)

The (25 mg) of **(A_1)** was dissolved in 20 ml MeOH and hydrolyzed with an equal volume of Takadiastase enzyme at R.T resulting in the liberation of L-arabinose (Rf 0.21) showing α linkage between D-galactose and L-rhamnose.

Again hydrolyzed with an equal volume of almond emulsion showing β linkage between aglycone and sugars were identified as D-xylose (0.27), D-glucose (R_f 0.18) (Co-Pc).

2.4.5. Antimicrobial activity of (A_1)

The disc diffusion method performed the Antimicrobial activity of the compound (A1). Compound A was dissolved in methanol and diluted to achieve the desired concentrations (25, 50, 75, and 100 µg/mL). To test the microbial activity, two gram-positive (*E. coli* and *B. cereus*) and one gram-negative (*S. aureus*) bacteria strain were used, with ciprofloxacin (10 g/disc) serving as a positive control. The bacterial strains were revived on Muller-Hinton broth by aerobically incubated at 37℃ for 24 hours on a shaking incubator at 180 rpm. After that turbidity of the bacterial suspension was adjusted to the McFarland standard (0.5). Muller-Hinton Agar (MHA) media plates were used to streak the strains using a sterile cotton tip swap. Each test sample (40 µg/mL) was placed on a 6 mm disc (Hi-Media) and was impregnated on the seeded agar plates, and left to stand for 1 hour (h) to allow the extract to pre-diffusion. After the pre-diffusion of extract, the plates were incubated at 37°C for 24 h. The study was conducted using the zone of inhibition method, and inhibitory zone diameters (mm) were used to calculate the antimicrobial activity for each of the four described bacterial strains. Experiments were carried out in triplicate. The results of the antimicrobial activity of **(A_1)** at high concentration (100µg/mL) was 20, 17, and 15 mm zone of inhibition against *E. coli*, *B. cereus*, and *S. aureus aeruginosa*, respectively. The data demonstrated that the antimicrobial activity of CH_3OH soluble fraction **(A_1)** at all concentrations was highest against *E. coli* and lowest *S. aureus.* The results are reported in Table 5 [20-29].

Table 5. Antimicrobial activity of **(A_1)** against bacterial strains.

	Concentration of (A_1)				**Ciprofloxacin (stand. drug)**
	25	50	75	100	(10)
Microorganisms	Zone of inhibition (mm)				
E. coli	11.4 ± 0.02	13.2 ± 0.12	15.2 ± 0.04	20.8 ± 0.45	21.8 ± 0.16
Bacillus cereus	10.54 ± 0.07	14.83 ± 0.15	15.14 ± 0.03	17.1 ± 0.32	23.2 ± 0.12
Staphylococcus aureus	7.30 ±0.05	9.62 ± 0.05	11.3 ± 0.12	15.8 ± 0.24	26.3 ± 0.91
Concentration in (µg/ mL)					

2.4.6. Minimum inhibitory concentration (MIC) Assay

The broth microdilution method was used to determine the MIC_s of **(A_1)**. This method evaluates multiple **(A_1)** dilutions twice in a disposable 90 mm plastic petri dish. Briefly, desired bacterial cultures were activated by transferring a loopful of strains from stock cultures into tubes, inoculating them with Nutrient-broth (NB) medium, and incubating them for 24 hours at 37°C. Fresh NB medium was used to dilute the bacterial cultures to a concentration of 100µg/ml, which was then serially diluted to 50, 25, 12, 5, 6, 2, 1, 5, and 0.78 µg/ mL, respectively.

After that, the tubes were inoculated with 20µL of microbial suspension and heated to 37℃ for 24 hours. **(A_1)** MIC was defined as the minimum concentration of assayed samples that inhibited the visible growth of the tested microorganism. The findings are shown in Table 6.

Table 6. Minimum inhibitory concentration of (A_1).

Tested bacterial strains	**MIC (µg/mL) of compound A**	**MIC (µg/mL)Ciprofloxacin (Standard drug)**
1. *E. coli* **2.** *Bacillus cereus* **3.** *Staphylococcus aureus*	• 7.3 • 2.1 • 0.02	• 0.9 • 0.14 • 4.2

3. Results and Discussion

Allelochemical **(A_1)** has m.p. 340–345 °C, molecular formula $C_{31}H_{36}O_2$, (M^+) 728 (EIMS). It gave Molisch and Shinoda test conforming its flavonoids glycosidic nature [30]. Elemental analysis found (%) C 51.8 %, H 4.92 %, O 43.90 %, Calculated m.f $C_{31}H_{36}O_{20}$ found (%), 51.10 %, H 4.98 %, O 43.92 %, UV absorbance observed at λ_{max} (nm) 268, 371 recorded in methanol.IR spectra showed absorption

bands 3448, 2924,2315,1644, 1517, 1101 cm^{-}, and 885 cm^{-1}.

The 1HNMR Showed singlet at 12.9 ppm, which indicates OH at C_5 positions. Two singlets at 6.30, 6.76 ppm for H-6, H-8, and one singlet, one doublet, one double doublet at 7.5, 6.57, 7.60 ppm was assigned for H-2', H-5', H-6' respectively. The anomeric protons of the sugars showed doublets at δ 5.50, δ 4.24, and δ 4.86, which were assigned to H-1", H-1"', H-1"" of D-glucose, D-xylose, and L-arabinose The coupling constant showed β configuration between D-glucose D-xylose and α configuration of L-arabinose with Pro-aglycon. The characteristic ion peak of the **(A_1)** mass spectrum was observed at m/z [M^{+}] 728 and 596,464,302, which is formed by subsequent losses of [M^{+}-arabinose], [M^{+}-D- xylose] [M^{+}-D-glucose and **A_2**], indicating L-arabinose linked with C'$_4$ and D-xylose attached with D-glucose with aglycone at the C_3 position.

(A_1) was acid hydrolyzed with 7.5% H_2SO_4 to produce aglycone **A_2** molecular formula $C_{15}H_{10}O_7$ was analyzed, m.p. 315–318°C, (M^{+}) 302 (EIMS). The aqueous hydrolysates were neutralized with Barium carbonate and Barium sulfate and were filtered off. Paper chromatography was performed after filtrate was concentrated using n-butenol, acetic acid, and water with a (4:1:5) ratio as developer and Ninhydrine as visualizing reagent showed the presence of L-arabinose (R_f 0.21), D-xylose (R_f 0.27), D-glucose (R_f 0.18) Co-Pc). The presence of all sugars in an equimolar ratio1:1:1 was indicated by quantitative sugar estimation [32]. All sugars were sent in pyranose form, confirmed by Periodate oxidation [32] of **(A_1)**. The position of sugars moieties in **(A_1)** was determined by Permethylation followed by acid hydrolysis methylated aglycone identified **A_2** glycosidation was involved at C_3 and C_3' position of aglycone.

Methylated sugars were identified as **A_4**, **A_5**, and **A_6**. Therefore, it was conducted (C-1'''')-OH attached with OH group at C_4' position of aglycone, C-1' –OH of D-glucose attached with OH group at the C_3 position of aglycone, C-4'-OH of D-glucose attached with C-1'''-OH of xylose. The inter glycosidic linkage (1-4) was found between D-glucose and D-xylose.

In the above conclusion, **(A_1)** was present in the methanolic leaf extracts of *Dalbergia paniculata* Roxb. Showed antimicrobial activity. The results of the antimicrobial activity of **Allelochemical (A_1)** at high concentration (100µg/ml) was 20, 17, and 15 mm zone of inhibition against *E. coli*, *B.cereus*, and *S. aureus aeruginosa*, respectively. The data demonstrated that the antimicrobial activity of CH_3OH soluble fraction allelochemical **(A_1)** at all concentrations was highest against *E. coli* and lowest *S. aureus.*

4. Conclusion

Based on the above evidence, the structure of (A1) 5,7,3',4'-pentahydroxy flavone-3-O-β- xylopyranosyl (1→4)- β-D-glucopyranosyl-4'-O-α-L-arabinoside was determined from the CH3OH extract of Dalbergia paniculata Roxb. leaf showed good antimicrobial agent.

Acknowledgment

I want to thank the Director of the Central Drug Research Institute Lucknow for MS analysis, SIC, and the Department of Microbiology, Dr. H.S. Gour University, Sagar (India), for spectral analysis and antimicrobial activity. Thanks to our supervisor Prof. RajNath Yadava of this university, for fruitful suggestions and guidance.

References

1- D. M. Verma, N. P. Balakrishnan, R. D. Dixit, flora of Madhya Pradesh: Pteridophytes and Angiosperms (Ranunculaceae to Plumbaginaceae), Botanical Survey of India, **1993**, 1.
2- R. N. Chopra, S. L. Nayar, I. C. Chopra, L. V. Asholakar, K. K. Kakkar, O. J. Chakra, B. S. Varma, Glossary of Indian medicinal plants with active principles, New Delhi, **1992**, 1, 187.
3- S. N. Chaitra, N. K. Chaitra, P. Shalini, R. Sindhu, K. M. Saru Raj, Phytochemical Analysis and antibacterial activity of *Dalbergia paniculata* Roxb., International Journal of pharmaceutical science and research, **2015**, 6, 712-716.
4- P. M. Dewick, Isoflavonoids, In the flavonoids advance in research since1986, Harbone J. B., Chapman and Hall, NY, **1994**, 117-238.
5- D. M. X. Donnelly, G. Boland Neoflavonoids, Harbone J. B., (Ed) Chapman and Hall., In the flavonoids advance in research science, NY, **1994**, 239-258.
6- I. Shaik. Khalivulla, A. K. B. Reddy, D. Gunasekar, M. M. Murthy, Tadikimalli p, Alain Blond and Bernard bodo, A new C-geranylated Isoflavone from *Dalbergia paniculata* Roxb., **2007**, 11, 1109-1111.
7- V. Narayanan, T. R. Seshadri, Paniculatin, A new isoflavone-di-C- glucoside of *Dalbergia paniculata* Roxb. bark. Indian Journal of Chemistry, **1997**, 9, 14-16.
8- D. Adinaravana D, J. R. Rao, Isoflavonoid glycosides of *Dalbergia paniculata* Roxb., The constitution of Dalpenitin and Dalpatin, Tetrahedron, **1972**, 28, 5377-5384.
9- M. R. Parthasarathy, T. R. Seshadri, R. S. Verma, Minor isoflavonoid glycosides of stem bark of *Dalbergia paniculata* Roxb. isolation of a new C-glycoside, current science, **1974**, 43, 74-75.

10- M. R. Parthasarathy, T. R. Seshadri, R. S. Verma,7-O-Rutinosides of biochanin A and formononetin, Two new rhamnoglucosides from the bark of *Dalbergia paniculata* Roxb., India journal of chemistry, **1974**, 12, 518-519.
11- D. Adinarayana, J. R. Rao, Dalpanin, A C-glycosylisoflavanone from *Dalbergia paniculata* Roxb. Proceedings of Indian academy of science, **1975**, 81A, 23-31.
12- D. Adinarayana, J. R. Rao, Isoflavonoids of Dalbergia paniculata, Indian Journal of Chemistry, **1975**, 13, 425-426.
13- M. R. Parthasarathy, T. R. Seshadri, R. S. Verma, New Isoflavonoidglycosides from *Dalbergia paniculata* Roxb., Phytochemistry, **1976**, 15, 1025-1027.
14- M. Radhakrishniah, Chemical constituents of *Dalbergia paniculata* Roxb. root, Journal of Indian chemical society, **1979**, 56, 81-86.
15- M. R. Parthasarathy, P. Sharma, S. B. Kalidhar, Isocavuinin-7-Oglucoside, from bark of Dalbergia paniculata Roxb., Indian Journal of Chemistry, **1980**, 19B, 429-430.
16- J. R. Rao, R. S. Rao, Dalpalatin, A new isoflavone from *Dalbergia paniculata* Roxb seeds Indian Journal of Chemistry, **1990**, 29B, 78-79.
17- J. R. Rao, R. S. Rao, Dalpaniculin, A C-glycosyl isoflavone from *Dalbergia paniculata* Roxb. seeds, Phytochemistry, **1991**, 30, 715-716.
18- N. P. Leena, N. A. Aleykutty, Isolation and spectral identification of quercetin from the alcoholic root extract of Clerodendrum paniculatum Linn., International Journal of pharma science and research, **2016**, 7, 47-50.
19- G. Venugopal, C. S. Reddy, B. N. Rao, V. B. Rao, T.V. Rao, A. P. Rao, pharmacognostic and preliminary phytochemical evaluation of Ocimum basilicum L.var.pilosum(Willd.) benth. and Otenuiflorum var. CIM-AYU, International Journal of Pharmacognosy and Phytochemical Research, **2015**, 7, 519-526.
20- E. J. Park, Y. Kim, Journal of Natural Products, 63, **2000**, 34-36.
21- S. Chaitra, N. N. Kumar, P. Shalini, R. Sindhu, K. M. S. Raj, Phytochemical Analysis and Antimicrobial activity of *Dalbergia paniculata* Roxb., International Journal of pharmaceutical science and research, **2015**, 6, 712-716.
22- E. Leder, M. Lederer, Chromatography, Elsevier publishing company, New York, 1247, 1957.
23- L. V. Nho, P. D. Thang, V. V. Sy, N. H. Hoang, P. T. Huong, Antimicrobial activity and chemical investigation of Pongamia pinnata L. Leaf Journal of Pharmacognosy and phytochemistry, **2022**, 11(4), 30-32.
24- F. S. Mohammed, E. Kına, İ. Uysal, K.Mencik, M. Dogan, M. Pehlivan, M. Sevindik, Antioxidant and Antimicrobial Activities of Ethanol Extract of Lepidium spinosum Turkish Journal of Agriculture - Food Science and Technology, **2022**, 10(6), 1116-1119.
25- R. Gurung, S. Adhikari, K. Parajuli, Evaluation of the Antibacterial and antioxidant activity of Mimosa rubicaulis and Reinwardtia indica Evidence-Based, Complementary and Alternative Medicine, **2020**, 6, 3862642.
26- D. Sunil, S. Shinde, S. Kanshette, D. M. Jadhav, HPTLC profiling and antibacterial studies of *Cassia tora* L. against some pus forming bacteria, International Journal of Botany Studies, **2022**, 7, 527-532.
27- K. Ananda Lakshmi, Review on biosynthesis of silver nanoparticles and its characterization plant archives, **2021**, 21, 2393-2400.
28- *S*. B. Mishra, V. K. M. Rao, S. K. Bose, Quantitative estimation of carbohydrates by paper partition chromatography, Journal of Scientific and Industrial Research, **1960**, 173-176.
29- E.L. Hirst, L. Hough, J. K. N. Jones, Composition of the gum of Sterculia setigera: occurrence of D-tagatose in nature, Journal of chemical society, **1949**, 177-177.
30- J.Shinoda, *J Pharm Soc Japan*, **1928**, 48, 214.
31- K.S. Szablewska, T. Szablewski , A. P. Balcerek , L. Szwajkowska, .M. M. .Krzyzaniak, D. .Swierk, R. C..Radziejewska and Z. Krejpcio, Antimicrobial Activities Evaluation and Phytochemical Screening of Some Selected Plant Materials Used in Traditional Medicine, MDPI, **2023**, 28, 244.
32- S. Abubakar, O.A. Adoum, Antimicrobial Activity and Brine Shrimp Lethality Test of cassia Sieberiana D.C. Leaves Extracts, *Chemistry Research Journal*, **2021**, 6(6), 148-15.