Advances in Bioresearch
Adv. Biores., Vol 14 (1) January 2023: 247-256
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.1.247256

Advances in Bioresearch

ORIGINAL ARTICLE

Isolation and Characterization of Lupeol from the leaves of *Woodfordia floribunda* Salisb and its Antinflammtory activity

Pankaj H. Naikwadi, Narendra D. Phatangare and Dhananjay V. Mane*
Agasti Arts Commerce and Dadasaheb Rupwate Science College Akole, Department of Chemistry and School of Science and Technology, Yashwantrao Chavan Maharashtra Open University, Nashik- 422222, Maharashtra, India
Department of Chemistry, S.N. Arts, D.J.M. Commerce and B.N.S. Science College, Sangamner- 422605, Maharashtra, India
* School of Science and Technology, Yashwantrao Chavan Maharashtra Open University Nashik,(PG and Research Center, Department of Chemistry, Shri. Chhatrapati Shivaji College, Omerga (MS) 413606)
Corresponding author:Email id: dvmane11@gmail.com

ABSTRACT
The lupeol is pentacyclictriterpenes were isolated in the pet ether extract. The leaves and flower of Woodfordia floribunda Salisbhas been used ethnomedicinal as the remedy. Many researches indicated that lupeol possesses many beneficial pharmacological activities including antioxidant, anti-inflammatory, anti-hyperglycemic, anti-dyslipidemic and anti-mutagenic effects. The lupeol isolation from this species was carried out by column chromatography after successive fractionation and with help of TLC identification was done. The structure was determined by analysis of the isolate by IR, ^{1}H-NMR, ^{13}C-NMR and COSY spectral analysis as well as comparison with reported data.Lastly find out the anti-inflammatory efficacy of lupeolwas administered to Wistar rats at dose sizes 1 mg/kg, 5 mg/kg and 10 mg/kg with paw edema model for its in vivo anti-inflammatory potency. Diclofenac (5 mg/kg) was used as standard. Lupeol shows significant anti-inflammatory potency due to the release of prostaglandins (57.29%), histamines (47.37%), and bradykinins (52.91%).

Keywords: -*Anti-inflammatory, TLC, Carrageenan, vivo, W.floribunda*

Received 02.12.2022 Revised 29. 12.2022 Accepted 25.01.2023

How to cite this article:
P. H. Naikwadi, N. D. Phatangare and D. V. Mane. Isolation and Characterization of Lupeol from the leaves of *Woodfordia floribunda* Salisb and its Antinflammtory activity.. Adv. Biores. Vol 14 [1] January 2023. 247-256

INTRODUCTION

The medicinal plant has a rich source of natural triterpenoids also known as phytosterol due to their variety of biological activities [1]. *Woodfordia floribunda* Salisbisa member of the Lythraceae family. It grows on the mountain and hilly regions, it has a bunch of shrubs with smooth stem [2]. The previous studies indicated that they show anti-inflammatory activity [3]. Literature also reported that leaves and flowers were used to treat a number of diseases including ulcers, hypertension, and weakness of vision, for promoting muscular relaxation before delivery, and antimicrobial, antifungal, and anti-allergic symptoms. Aim of this paper is to report the isolation of lupeol from the pet ether extract of *W. floribunda* .Lupeol has demonstrated several pharmacological activities, including demon tree, and anti-microbial anti-inflammatory activity [4].Day by day growing interested in natural triterpenoids and it is known as a phytosterol. In ancient times natural products are used as remedies for human diseases. Actually, the triterpenoids are called secondary metabolites and their Pharmacology activities are derived from the medicinal plant .Pharmacological importance are a god gift of healthy the lifestyle [5].Triterpenes are widespread a group of natural compounds which having considerable practical significance which are produced by the arrangement of epoxide in a chair-chair-chair arrangement [6].Most of the triterpene contain 28 or 29 carbon and one or two carbon carbon double bond, typically one in the sterol nucleus and sometime second in the alkyl side chain are the natural components of the human diet [7].Lupeol shows a variety of pharmacological activities under in vitro and in Vivo conditions. This shows a beneficial activity against inflammation, arthritis, Diabetes, heart diseases, renal toxicity, and hepatic toxicity [8-16].For the study of inflammation under in vitro and in animal model inflammation, lupeol is

preferentially used. Several studies were carried out to compare the anti-inflammatory efficacy of lupeol with a known anti-inflammatory agent [17-20]. Lupeol and its several derivatives were shown to exhibit higher anti-inflammatory activity than the commonly used non-steroidal anti-inflammatory drug indomethacin in rat and mouse models of inflammation [21-24].For the first time, the lupeol compound is used to reduce inflammation in a mouse model of arthritis, which is an information-associated disease. Another major development in the anti-inflammatory potential of lupeol use for the treatment of information in the mouse model of bronchial asthma [25]. Lupeol is commonly used to reduce the production of mucus and overall inflammation in the lungs. Different studies were carried out to understand the molecular mechanism of lupeol that inhibits the inflammatory processes under in vitro and vivo situations such studies provide a few facts about the mechanistic inflammatory action of lupeol [26-27].

MATERIAL AND METHODS

Plant Collection and identification of plant Material

The plant leaves of *Woodfordia floribunda* Salisb as collected from the Western Ghats of Sahyadri region, akole taluka, state Maharashtra. The herbarium was made as per the BSI pune.The herbarium was send to the Botanical Survey of India.They provide the authentication number after the authentication number (No.BSI/WRC/Iden.Cer./2021/1905210003955.

Plant profile

The *W.floribunda* plant is commonly called as dhaaykephool. The flowers of the plants are in bunches.It belongs to the lythereace family. The plant leaf are evergreen shrubs. It grow height about 4.5 m and having long branches.

Figure 1. The *W.floribunda* Salisb leaves

Outline of work

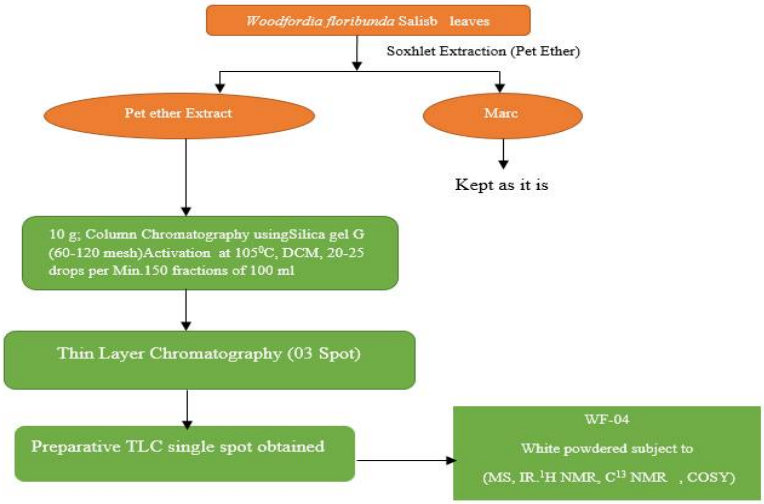

Figure 2. Extraction outline of *W.floribunda* Salisb leaves of pet ether extract

Preparation of leaf extract

The leaves of *W.floribunda* Salisb were subjected to Soxhlet extraction. It requires 4-5 days of completion of the whole cycle.The crude pet ether extract filter through whatmann filter paper no.42 .After that the marc was taken and subjected to column chromatography. Collect the fraction and taking the TLC. Normal phase adsorption chromatography was carried out with gradient elution using different solvent systems of hexane-ethyl acetate (10.0, 9.5:0.5, 9.0:1.0, 8.5:1.5, 8.0:2.0, 7.5:2.5, 7.0:3.0, 6.5:3.5, 6.0:4.0, 5.5:4.5, 5.0:5.0) increasing order of polarity. The solvent system in the ratio 9:1 was used as the basis of the TLC monitoring of the column chromatography. By taking the fraction and subjected to successive fractionation by chloroform.

RESULT

IR

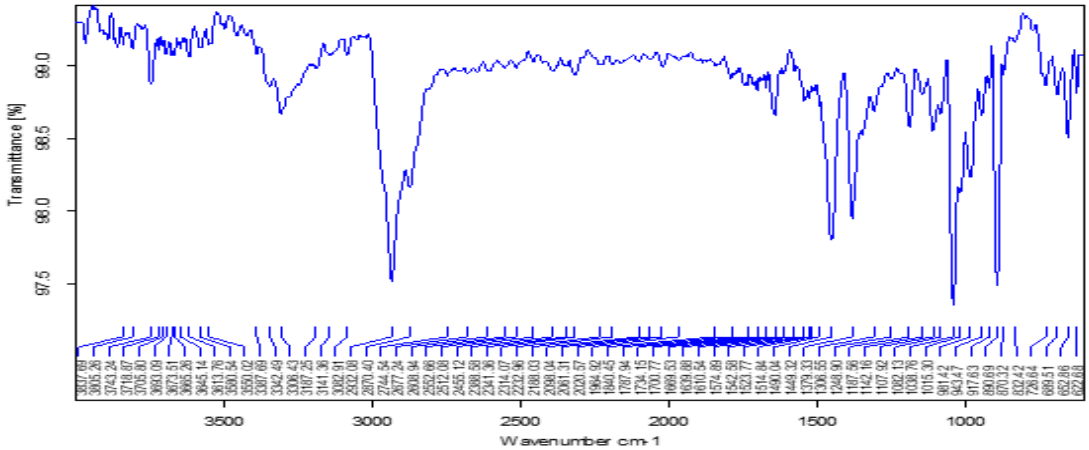

Figure 3.IR spectrum of WF-04

The IR spectrum oflupeol showed a very intensely broad absorption frequency peak at 3387 cm-1 of typical –OH bond stretching frequency of the hydroxyl group.The intense band observed at 2477 cm^{-1} confirmed the presence of aliphatic –C-H bond stretching frequency. The C=C bond stretching frequency was observed at 1490-1248 cm^{-1}.

WF-04-'H NMR (500 MHz, DMSO)

4.68 (d, J 2.4 Hz, 1H), 4.54 (dd, J=2.4, 1.3 Hz, 1H), 4.27 (d, J = 5.2 Hz, 1H), 3.00-2.94 (m IH), 2.37 (td, J-11.0, 5.8 Hz, 1H), 1.92-1.82 (m, 1H), 1.67-1.59 (m, 6H), 1.58-1.53 (m, 1H), 1.47-1.41 (m, 4H), 1.38-1.30 (m, 7H), 1.30-1.08 (m 5H), 1.07-1.01 (m, 1H), 0.99 (s, 3H), 0.95 (dd, J=8.4, 5.0 Hz, IH), 0.91 (s, 3H), 0.87 (s, 3H), 0.77 (d, J-3.8 Hz, 6H), 0.65 (s, 4H).

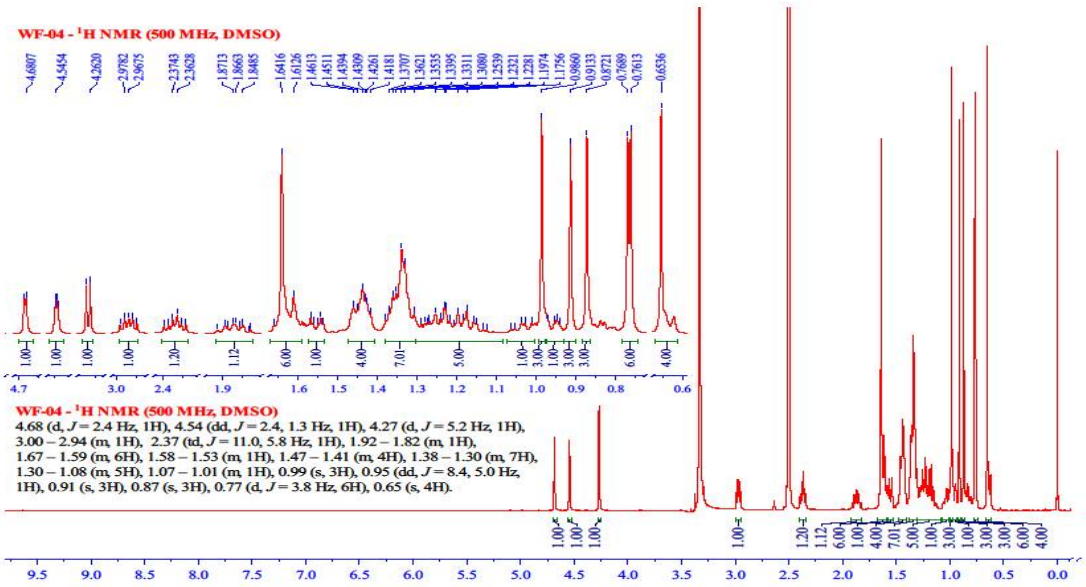

Figure 4.¹H-NMR spectrum of WF-04

^{13}C-NMR

WF-04-C NMR (126 MHz, DMSO) 150.68, 110.15, 77.26, 55.32, 50.30, 48.25, 47.87, 43.02, 42.83, 40.82, 38.97, 38.73, 38.03, 37.15, 35.54, 34.30, 29.66, 28.57, 27.63, 27.46, 25.14, 20.88, 19.43, 18.43, 18.24, 16.40, 16.28, 16.17, 14.80.

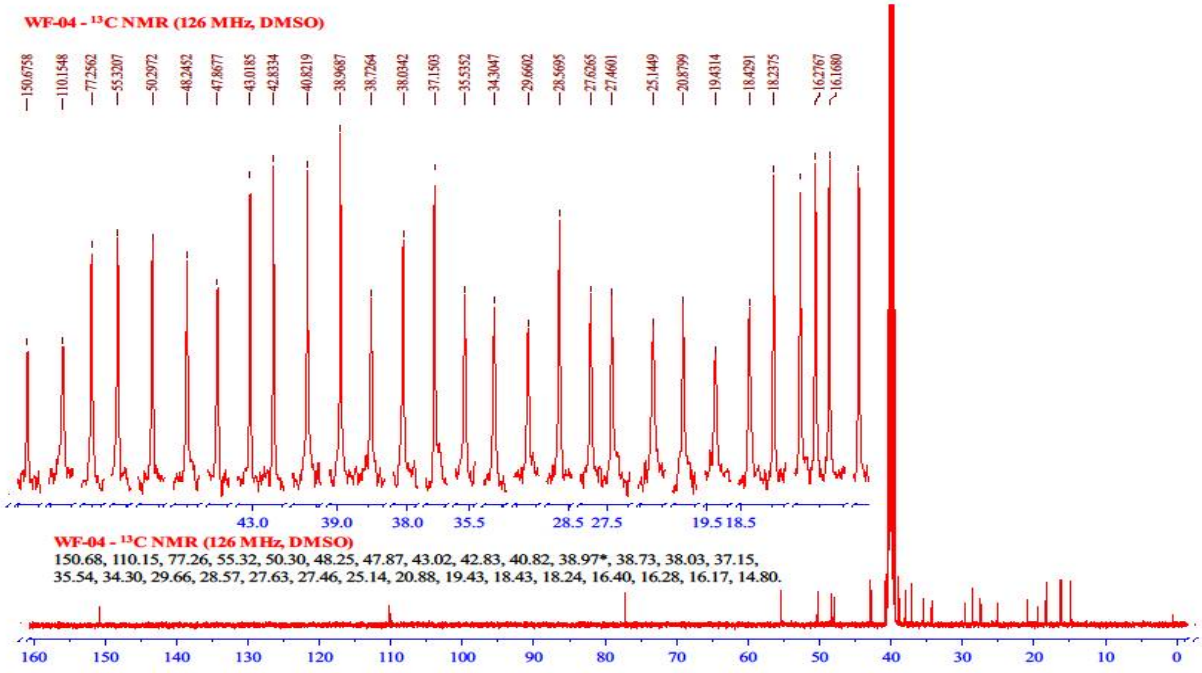

Figure 5. ^{13}C-NMR spectrum of WF-04

COSY

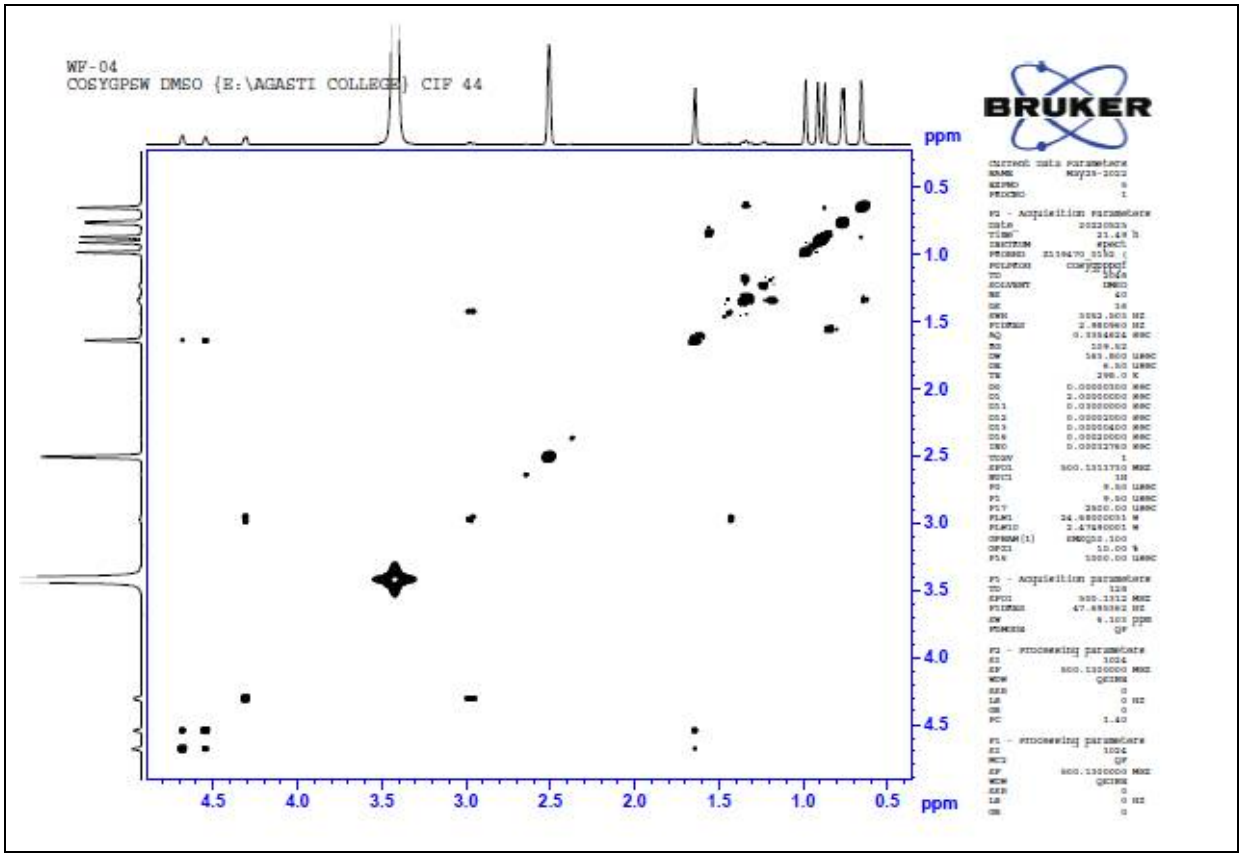

Figure 6. COSY spectrum of WF-04

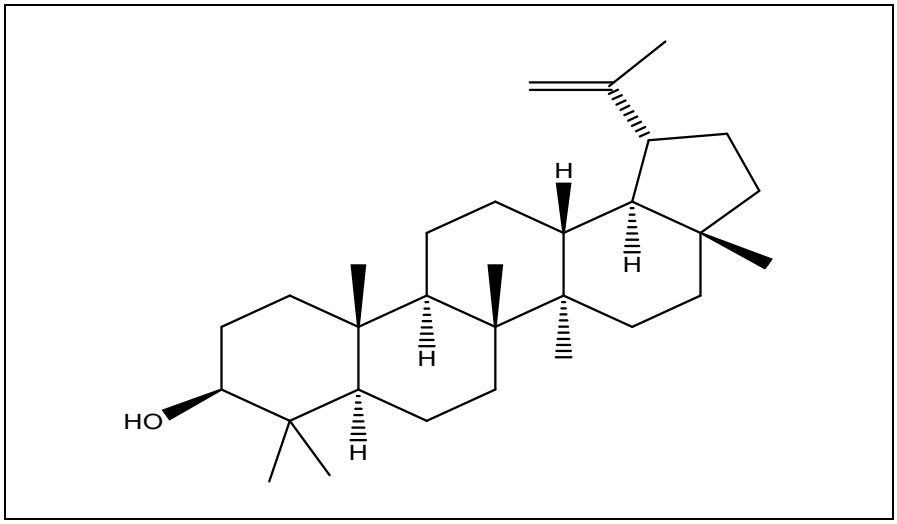

Figure 7. Structure of isolated compound Lupeol WF-04

Antinflammtory activity

The LACSMI BIOFARMS PVT.LTD. Passaydan, Survey No.28/3/21 Samarth colony, Pimple Naka, Pune (Delivery note no.A-106/ 12/03/2022) provided the Rat (Wistar) weighing around (160-200 gm). The housed animal experiences 12-hour light/dark cycles, 40-60% humidity, and 25-34^{0}C temperatures [28]. Polypropylene cages were prepared for the rats and a regular rodent feed and water were provided. The animals fasted for 12 hours before the experiment.And were not given any food or water. Because water can be harmful to living things, different measures are used to ensure that it is suitable for human consumption [29].*W. floribunda*salisb, one of the most important medicinal plants due to its anti-inflammatory activity, has an ethanoic extract dose size depends on the weight of the animal and previous literature[30].The doses may be easily calculated using the information provided in this study .

Drugs

The acquisition of Wistar rats weighing between 160 and 200 g. Purchased from their respective companies, diclofenac (Pharma Cure Laboratories Garha, Jalandhar) and carrageenan soy lecithin (Indore, Madhya Pradesh, India) were utilised in the study.

Ethical considerations

According to the experimental protocol and procedure, the Amruthwahini College of Pharmacy, Sangamner, Dist., A. Nagar, Maharashtra approved the proposal for the animal activity study. Using a model of carrageenan-induced rat paw edema, it confirmed the "Guidelines for care and use of animals in scientific research" (Indian National Science Academy 1998, Revised 2000) (AVCOP/IAEC/2021-22/1153/26/01).All the rats manually divided into three groups (n=6) after receiving doses of 1, 5, and 10 mg/kg p.o. of the pet ether extract as well as distilled water (control). Carrageenan (0.1 ml, 1%) was injected into the right hind paw subplanter in each rat. The injection volume of carrageenan was determined using a plethysmometer (Medicaid System, Mode No. PTH-707, New Delhi, India) at 0, 30, 60, 90, 120, 180, 240, and 300 minutes. After each interval, the following formula is used to compute the percentage inhibition (PI) of edema: PI = 1-Vt/Vc X 100, where Vt and Vc are the volumes used for the comparison between the turkey and the edema control.

Statistical significance was defined as a p-value of 0.05 or less. Among the many impacts of the plant are its anti-inflammatory, antibacterial, and antioxidant properties. The ethanolic extract of *W.floribunda*salisb produced noticeably better results (p 0.05), demonstrating its anti-inflammatory properties [31].

Statistical analysis

Mean and standard error were calculated using one-way ANOVA. Values of $p<0.05$ was considered statistically SIGNIFICANT.

Table 1. Inhibition of paw edema in the percentage of lupeol (n = 06) compared with standard (diclofenac)

dose size	0 Min	30 Min	60 Min	90 Min	120 Min	180 Min	240 Min	300 Min
01 mg/kg	2.58	16.13	33.16	42.13	47.02	66.19	58.68	49.19
5 mg/kg	3.23	19.35	37.89	43.65	48.41	70.00	62.44	55.14
10 mg/kg	5.16	18.28	41.58	47.72	53.87	71.43	63.96	55.14
STD 5mg/kg	0.65	32.80	48.42	60.91	68.34	80.48	76.14	70.27

Animal weight in gm	0 Min	30 Min	60 Min	90 Min	120 Min	180 Min	240 Min	300 Min
200	1.51	1.88	1.87	1.96	1.98	2.09	1.97	1.85
180	1.54	1.87	1.92	1.99	2.02	2.11	1.99	1.87
170	1.61	1.81	1.95	2.01	1.94	2.14	2.01	1.91
180	1.56	1.92	1.88	1.98	2.05	2.08	1.95	1.86
170	1.53	1.86	1.89	1.99	2.00	2.05	1.89	1.53
170	1.55	1.85	1.93	1.95	1.99	2.11	2.03	1.89
SD	0.034	0.036	0.031	0.022	0.037	0.031	0.050	0.146
SEM	0.014	0.015	0.013	0.009	0.015	0.013	0.020	0.058
Mean	1.55	1.87	1.91	1.98	2.00	2.10	1.49	1.39

Table 2. Edema in control (normal saline)

Table 3. The dodoes-dependent anti-inflammatory activity of Lupeol (n = 06).

Sr. No.	Animal weight in gm	Fraction in dose	0 min test	30 min test	60 min test	90 min test	120 min test	180 min test	240 min test	300 min test
I	170	**01 mg/kg**	1.45	1.44	1.13	1.16	0.89	0.83	0.83	1.17
II	175		1.50	1.41	1.20	1.22	0.90	0.89	0.87	1.21
III	165		1.47	1.49	1.21	1.15	0.88	0.74	0.79	1.23
IV	180		1.54	1.51	1.19	1.13	0.91	0.81	0.81	1.19
V	185		1.549	1.47	1.23	1.16	0.83	0.77	0.77	1.25
VI	175		1.45	1.52	1.18	1.11	0.90	0.74	0.80	1.17
Mean			1.49	1.47	1.19	1.15	0.88	0.80	0.81	1.20
SEM			0.018	0.017	0.013	0.015	0.012	0.0239	0.0142	0.0133
SD			0.043	0.042	0.034	0.037	0.029	0.0585	0.035	0.0326
%Inhibition			**3.87**	**20.97**	**37.37**	**41.62**	**52.43**	**52.58**	**45.53**	**25.72**
I	185	**05 mg/kg**	1.45	1.41	1.23	1.15	1.06	0.87	0.84	1.23
II	175		1.51	1.43	1.16	1.13	0.99	0.85	0.91	1.17
III	155		1.44	1.45	1.23	1.03	1.02	0.79	0.78	1.21
IV	165		1.52	1.39	1.21	1.14	1.06	0.74	0.74	1.18
V	168		1.43	1.44	1.24	1.09	1.03	0.81	0.69	1.22
VI	178		1.42	1.50	1.16	1.12	1.06	0.78	0.82	1.16
Mean			1.46	1.44	1.20	1.11	1.03	0.81	0.8	1.2
SEM			0.017	0.015	0.014	0.018	0.011	0.0194	0.0316	0.0117
SD			0.042	0.037	0.036	0.044	0.028	0.0476	0.078	0.0288
%Inhibition			**5.81**	**22.58**	**36.84**	**43.65**	**47.72**	**51.98**	**42.69**	**26.23**
I	175	**10 mg/kg**	1.41	1.42	0.91	1.04	0.79	0.83	0.87	0.98
II	169		1.39	1.41	1.01	0.96	0.76	0.90	0.79	1.01
III	158		1.38	1.39	0.93	1.08	0.62	0.75	0.75	0.95
IV	166		1.35	1.44	1.04	1.07	0.73	0.79	0.85	1.04
V	165		1.41	1.38	1.09	1.02	0.59	0.68	0.71	1.01
VI	180		1.44	1.45	1.02	1.04	0.72	0.81	0.85	1.06
Mean			1.39	1.41	1.00	1.03	0.70	0.79	0.80	1.01
SEM			0.012	0.011	0.027	0.017	0.0324	0.030	0.0261	0.0162
SD			0.03	0.027	0.068	0.042	0.0793	0.074	0.064	0.0397
%Inhibition			**10.32**	**24.19**	**47.37**	**47.72**	**52.91**	**57.29**	**52.18**	**37.76**

Table 4. Effect of a subcutaneous injection of diclofenac as a standard. Values are the mean ± SEM of six animals.

Treatment (mg/kg)	0 Min	30 Min	60 Min	90 Min	120 Min	180 Min
Control	1.55±0.014	1.87±0.015	1.91±0.013	1.98±0.009	2.00±0.015	2.10±0.013
Standard (05mg/kg)	1.54 ± 0.023	1.25 ± 0.023	0.98 ±0.016	0.77 ±0.021	0.63 ± 0.022	0.41 ± 0.011
01mg/kg	1.49 ± 0.018	1.47 ± 0.016***	1.19 ±0.013	1.15 ±0.015	0.88±0.012***	0.80±0.023***
05mg/kg	146 ± 0.017	1.44± 0.015**	1.20 ±0.014	1.11 ±0.018	1.03± .011***	0.81±0.019***
10mg/kg	1.39 ± 0.012	1.41 ± 0.011**	1.0= ± .027	1.03 ±0.017	0.70±0.024***	0.79 ± 0.030**

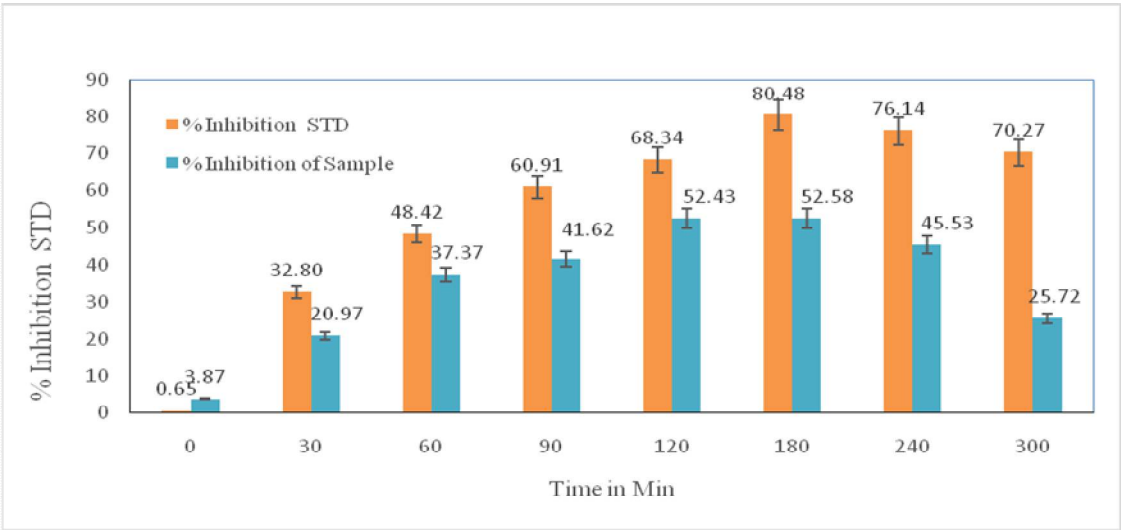

Figure 8. The % inhibition of sample dose size 1mg/kg

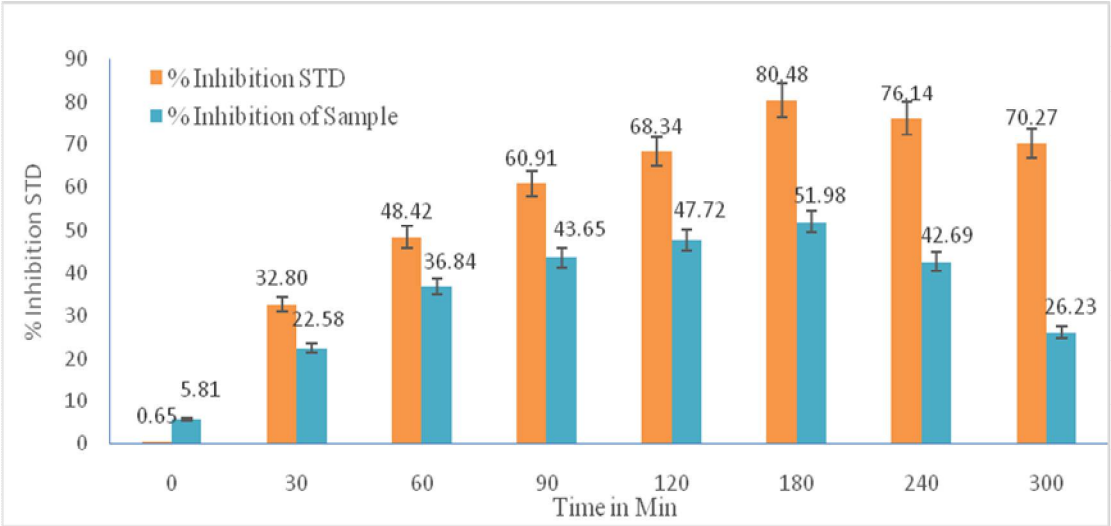

Figure 9. The % inhibition of sample dose size 5mg/kg

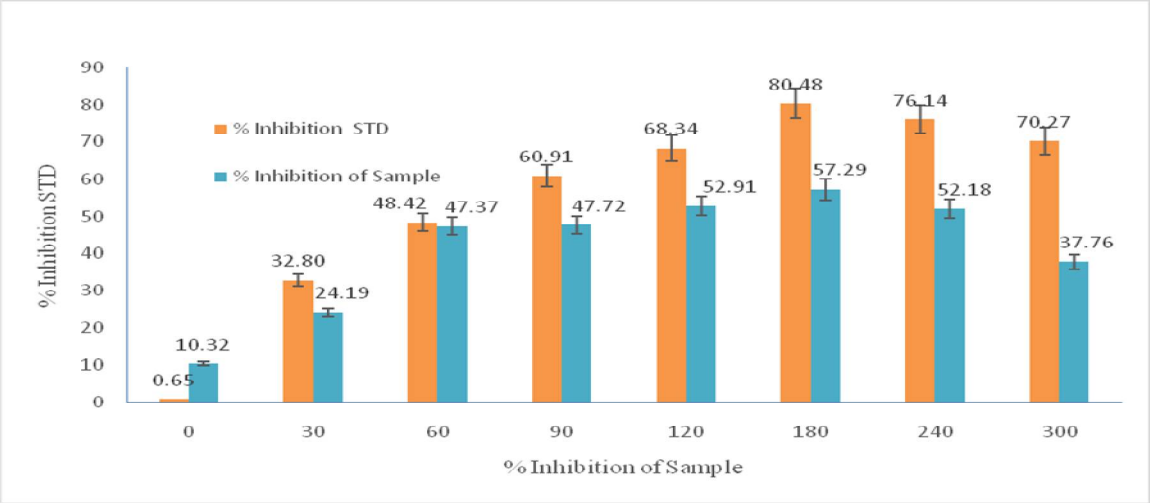

Figure 10. The % inhibition of sample dose size 10mg/kg

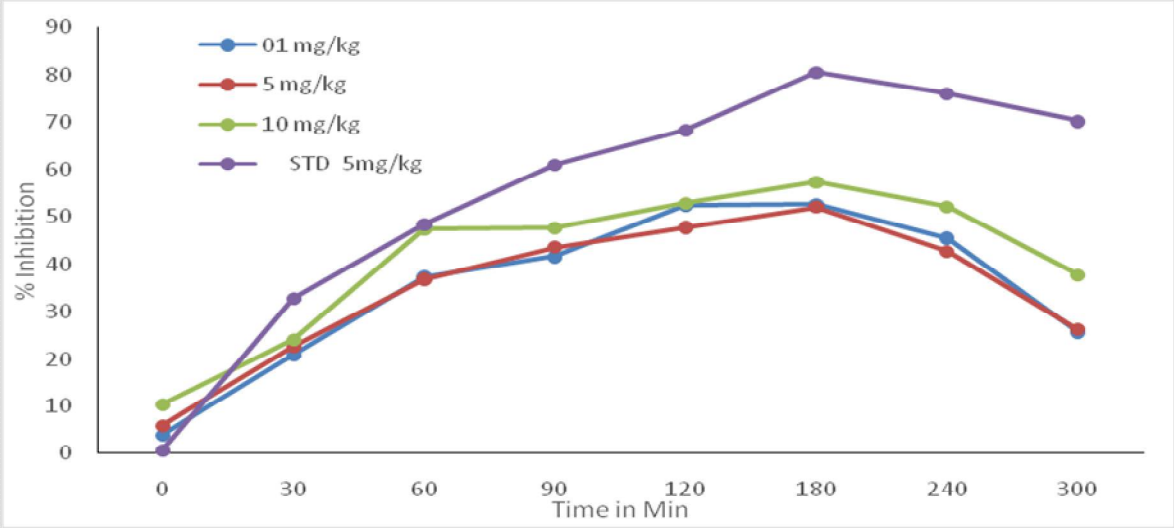

Figure 11. Release in histamine (1 hr) serotonin and bradykinin (2hr) and prostandinin (3 hr)

DISCUSSION

The plant *W.floribunda* Salisb has consider as medicinal plant due to their pharmaceutical properties and also the leaves and flower are used to cure the preliminary diseases. Thedrieddried leaves was subjected to soxhlet extraction apparatus. The crude pet ether extract examined by using GC-MS and column chromatography .With the help of successive fractionation and TLC the compound was isolated and subjected to Characterization such as IR,^{1}H-NMR,^{13}C-NMR,COSY confirmed the structure of lupeol (WF-05).Lastly by taking the anti-inflammatory activity of isolated compound and comparing with standard parameter as per the protocol.

CONCLUSION

The isolated compound lupeol from the pet ether extract *W.floribunda* shows significantly anti-inflammatory activity. Lupeol (WF-05) is pentacyclic triterpenoids widely distributed in the plant kingdom. The isolated compound shows low cytotoxicity on healthy cells and synergistically used in combine therapies. Lastly the anti-inflammatory efficacy of lupeol was administered to Wistar rats at dose sizes 1 mg/kg, 5 mg/kg and 10 mg/kg with paw edema model for its *in vivo* anti-inflammatory potency. Diclofenac (5 mg/kg) was used as standard. Lupeol shows significant anti-inflammatory potency due to the release of prostaglandins (57.29%), histamines (47.37%), and bradykinins (52.91%).

ACKNOWLEDGMENT

This research was fully supported by Yashwantrao Chavan Maharashtra Open University Nashik, we also thank our colleagues from the Agasti Arts Commerce and Dadasaheb Rupwate Science College Akole, who

provided all the facilities for my research. We thank Head department of Chemistry improving the quality of the research paper. The technical support and instrumentation facility provide by the Savitribai Phule Pune University, Pune and SAIF, IIT Bombay and Amrutwahini College of Pharmacy. Finally, I am thankful to my guide Yashwantrao Chavan Maharashtra Open University provide all the information and outline for my research work every time.

FINANCIAL SUPPORT

The Minor Research Project (MRP) scheme was offered by the Yashwantrao Chavan Maharashtra Open University, Nashik.Letter no. (YCMOU/RO-47/2022)

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

REFERENCES

1. Ovesná Z, Vachálková A, Horváthová K, Tóthová D. (2004).Pentacyclictriterpenoic acids: new chemoprotective compounds. Minireview Neoplasma., 51(1):327–333.
2. Rao SD, Rao BN, Devi PU, Rao A.K. (2017). Isolation of Lupeol, Design and Synthesis of Lupeol Derivatives and their Biological Activity. Oriental Journal of Chemistry., 33(1):173-80.
3. Emaikwu, V; Ndukwe, IG; Iyun, ORA; Anyan, J.V. (2019). Preliminary Phytochemical and Antimicrobial Activity Screening of Crude Extracts of Bird lime (*Tapinanthusglobiferus*). *J.Appl. Sci. Environ. Manage*., 23(2):1-14.
4. Patocka J.(2003).Biologically active pentacyclic triterpenes and their current medicine signification. Journal of Applied Biomedicine., 1(1):7-12
5. Variya, Bhavesh C, Bakrania Anita, K Patel, Snehal S. (2016). Emblica officinalis (Amla): A review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. Pharmacological research., 111:180-200.
6. Moreau RA, Whitaker BD, Hicks K.B. (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res., 41(1):457–500.
7. Beveridge TH, Li TS, Drover J.C. (2002). Phytosterol content in American ginseng seed oil. J Agric Food Chem., 50(1):744–750.
8. Alander J, Andersson A. (2005).The shea butter family – the complete emollient range for skin care Formulations. Cos Toil Man Worldwide., 2(1):28–32.
9. Prasad S, Kalra N, Singh M, Shukla Y. (2008).Protective effects of lupeol and mango extract against androgen induced oxidative stress in Swiss albino mice. Asian J Androl.,10(1):313–318.
10. Zhang L, Zhang Y, Zhang L, Yang X, Lv Z. (2009). Lupeol, a dietary triterpene, inhibited growth, and induced apoptosis through down-regulation of DR3 in SMMC7721 cells. Cancer Invest.,27(1):163–170.
11. Vidya L, Lenin M, Varalakshmi P.(2002). Evaluation of the effect of triterpenes on urinary risk factors ofstone formation in pyridoxine deficient hyperoxaluric rats. Phytother Res., 16(1):514–518.
12. Sudharsan PT, Mythili Y, Selvakumar E, Varalakshmi P. (2006). Lupeol and its ester exhibit protective roleagainst cyclophosphamide-induced cardiac mitochondrial toxicity. J Cardiovasc Pharmacol., 47(1):205–210.
13. Sudhahar V, Kumar SA, Sudharsan PT, Varalakshmi P. (2007).Protective effect of Lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia. Vascul Pharmacol., 46(1):412–418.
14. Lee TK, Poon RT, Wo JY, Ma S, Guan XY, Myers JN, et al. (2007). Lupeol suppresses cisplatin-induced nuclear factor-kappaB activation in head and neck squamous cell carcinoma and inhibits local invasion and nodal metastasis in an orthotopic nude mouse model. Cancer Res., 67(1):8800–8809.
15. Saleem M, Kweon MH, Yun JM, Adhami VM, Khan N, Syed D.N.(2005). A novel dietary triterpeneL upeol induces fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model. Cancer Res., 65(1):11203–11213.
16. Saleem M, Murtaza I, Tarapore R, Suh Y, Adhami VM, Johnson J.J.(2009).Lupeol Inhibits Proliferation of Human Prostate Cancer Cells by Targeting {beta}-catenin Signaling. Carcinogenesis. Online published ahead., 30(5):808-17
17. Fernández MA, de las Heras B, García MD, Sáenz MT, Villar A.(2001). New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. J Pharm Pharmacol., 53(1):1533–1539.
18. Fernández A, Alvarez A, García MD, Sáenz M.T. (2001).Anti-inflammatory effect of Pimentaracemosa var.ozua and isolation of the triterpene lupeol. Farmaco.,56(1):335–338.
19. Sudhahar V, Ashok Kumar S, Varalakshmi P, Sujatha V. (2008).Protective effect of lupeol and lupeolinoleate in hypercholesterolemia associated renal damage. Mol Cell Biochem.,88(7):11–20.
20. Lima LM, Perazzo FF, Tavares Carvalho JC, Bastos J.K. (2007). Anti-inflammatory and analgesic activities of the ethanolic extracts from Zanthoxylumriedelianum (Rutaceae) leaves and stem bark. J Pharm Pharmacol., 59(1):1151–1158.
21. Akihisa T, Yasukawa K, Oinuma H, Kasahara Y, Yamanouchi S, Takido M.(1996). Triterpene alcoholsfrom the flowers of compositae and their anti-inflammatory effects. Phytochemistry.,43(1):1255–1260.
22. Davis RH, DiDonato JJ, Johnson RW, Stewart C.B. (1994).Aloe vera, ydrocortisone and sterol influence onwound tensile strength and anti-inflammation. J Am Podiatr Med Assoc., 84(1):614–621.

23. Ramirez Apan AA, Pérez-Castorena AL, de Vivar A.R. (2004).Anti-inflammatory constituents of Mortoniagreggii Gray. Z Naturforsch., 59(1):237–243.
24. Geetha T, Varalakshmi P. (1999). Anticomplement activity of triterpenes from Crataevanurvala stem bark in adjuvant arthritis in rats. Gen Pharmacol.,32(1):495–497.
25. Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Gupta B.D.(2006). Suppression of T lymphocyte activityby lupeol isolated from Crataevareligiosa. Phytother Res.,20:279–287.
26. Latha RM, Lenin M, Rasool M, Varalakshmi P. (2001).A novel derivative pentacyclic triterpene and omega3 fatty acid. Prostaglandins Leukot Essent Fatty Acids.,64:81–85.
27. Vasconcelos JF, Teixeira MM, Barbosa-Filho JM, Lúcio AS, Almeida JR, de Queiroz L.P.(2008).Thetriterpenoid lupeol attenuates allergic airway inflammation in a murine model. IntImmunopharmacol., 8(1):1216–1221.
28. Saibaba, P.; Sales, G. and Stodulski, G. (1996). Behaviour of rats in their home cages: daytime variations and effects of routine husband dry procedures analysed by time sampling techniques. Laboratory Animals., 30(1):13-21.
29. Mtewa, T., Yapuwa, H. and Mulwafu, W. (2021). Water testing for potential phytochemical contamination and poisoning, in phytochemistry, the Military and Health. Elsevier.,6(4):427-442.
30. Poorniammal, R. and Prabhu, S. (2022). Antimicrobial and wound healing potential of fungal pigments from *Thermomyces*sp. and *Penicillium purpurogenum*in wistar rats. Ann. Phytomed., 11(1):376-382.
31. Najda, A.; Bains, A.; Chawla, P.; Kumar, A. and Balant, S.(2021). Assessment of anti-inflammatory and antimicrobial potential of ethanolic extractof *W. fruticosa* flowers: GC-MS analysis. Molecules., 26(23):71-93.