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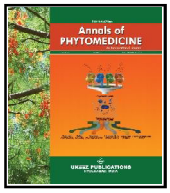

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Ethanopharmacological anti-inflammatory study of phytol in pet ether extract of *Woodfordia floribunda* Salisb.

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Abstract

The *Woodfordia floribunda* Salisb. plant is significant in the domains of medicinal chemistry and pharmacology. The phytoconstitutents such as diterpene alcohol, alkaloids, tannins and phenolics are qualitatively verified by phytochemical analysis. Literature shows that *W. floribunda* possess anti-inflammatory, antioxidant as well as anticancer properties. The primary goal of this research is to prove ability of phytol to reduce inflammation by carrageenan induced paw edema model. This acyclic diterpene alcohol is found in aromatic herbs and essential oils such as *Cleome serrate* and *Lantana radula*. The dried leaves of *W. floribunda* used for the ethanolic extraction by Soxhlet. Phytol is obtained by successive fractionation and which is monitored by thin layer chromatography and GC-MS. The isolated phytol was characterized by IR, ^{1}H-NMR, COSY and C13-NMR spectroscopy. Phytol was administered to Wistar rats at dose size 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. Phytol shows significant anti-inflammatory potency due to the release of prostaglandins (69.21%), histamines (37.54%) and bradykinins (49.76%). Phytol shows significant anti-inflammatory potency due to the release of Serotonin (37.54%) prostaglandins (61.59%) and histamines (70.0%).

1. Introduction

The medicinal plant has great importance in the field of phytochemistry. The *W. floribunda* belongs to the family Lythraceae and commonly it is known as Dhataki. All parts of this plant having medicinal properties. The Dhataki flowers are used extensively in the preparation of asava and aristha containing self-gene rated alcohol (Nautiyal, 2017). Treatment of many diseases has been greatly aided by ayurvedic knowledge. The consumption of ayurveda day-by-day increasing due to its importance. Phytol is an herbal phytochemical phytoconstituents that is extensively spread in nature (Gyawali *et al*., 2020). Phytolisan unsaturated alcohol found in plants as chlorophyll, has a branched chain structure (Felix and Katharina, 2012). It is used as a fragrance component, also the phytol has numerous biological effects so the researcher focuses on its phytochemical applications (Mikailu *et al*., 2022). The silver nanoparticle prepared from the plant, *W. floribunda* shows anti-inflammatory activity (Korde *et al*., 2020). Phytochemicals are active ingredients and these phytochemicals possess therapeutic properties which are very usefully considered as medicine or drug. In ancient times, many countries use the natural source of phytol from fish and barley. The phytol has similar chemical entities known as diterpenoids. The diterpenoids are seen in vitamins E and K and also some amount in tocopherols. The phytol is an acyclic triterpene and is mostly observed in aromatic plants. In the chronic phase of arthritis, the phytol plays an important role. It has a preventive effect on microscopic and macroscopic inflammation. The phytol mostly uses arthritis and ameliorating effect in rats (Tanaka and Kashiwada, 2021). So, efficacy of phytol is evaluated in current research work by carrageenan induced paw edema model as a new method of analysis. Carrageenan used for inflammation in the paws of animals. In addition, phytol inhibits the paw edema caused by prostaglandin E2, histamine, serotonin and bradykinin (Carvalho *et al*., 2020).

Cartilage, synovial membrane and bone in joints are the primary targets of arthritis, making it the most prevalent inflammatory disease. There were important biological activities and modes of action associated with phytol and its derivatives. The recent trend reports the effect of *W. floribunda* on bacteria, arthritis pain and inflammation remedies (Islam *et al*., 2018). The significance of bioactive constituents in ethanopharmacological research (Piwowarski *et al*., 2014) is derived from the fact that traditional medicine is very effective in this field. The *W. floribunda* plant is crucial for treating arthritis. Antiproliferative activity against four different cancer cell lines was observed *in vitro* and the resulting phytol derivatives were found to be cytotoxic (Gliszczyska *et al*., 2021). *W. floribunda* can be used to treat health problems. It has a wide range of secondary metabolites, such as saponins, flavonoids, alkaloids, phenols, glycosides and sterols. The screening process could be helped by finding bioactive principles and making new medicines. By using GC-MS analysis, the crude ethanolic extract was evaluated and getting information about different phytoconstitutents and bioactive compoundspresent in *W. floribunda* (Pawar *et al*., 2021). This investigation attempts to identify

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the phytochemical components present in the plant *W. floribunda*. The active extract was studied using a variety of models (Raghuvanshi and Nuthakki, 2021). The bioactive phytoconstitutents of steroidal saponins, tannins, flavonoids, steroids and terpenoids are found in the crude methanolic extract of *W. floribunda* (Groverand and Patni, 2013). Soxhlet, apparatus was used to carry out extraction. Semi-suspension liquid was obtained followed by filtration. The crude liquid isolated was purified by successive fractional extraction (Abate *et al*., 2021). The fraction No. 5 observed as oily droplets verified by TLC as a single spot. Which is identified as phytol after characterization IR, ^{1}H-NMR and C13-NMR, COSY. The dose dependent (1 mg/kg, 5 mg/kg and 10 mg/kg) anti-inflammatory activity was evaluated by using carrageenan induced paw edema model (Bharathi *et al*., 2021).

2. Materials and Methods

2.1 Plant collection and identification

The plant leaves of *W. floribunda* are obtained from the Sahyadri (Rajur, Tal-Akole) forest in the Ahmednagar district of Maharashtra, India. The herbarium was prepared and it will be submitted to the Botanical Survey of India in Pune, India. It is authenticated by the Indian Botanical Survey of India, made sure that the leaf samples in the herbarium (No. BSI/WRC/Iden.Cer./2021/1905210003955) were from the right plant.

2.2 Plant profile

The given medicinal plant *W. floribunda* is commonly known as fire flame in hindi called Dhaay ke phool and in Sanskrit it is called Gucchapushpa.The flowers are in bunches. The kingdom is Plantae and having class Magnoliopsida, the family of this plant is Lythraceae having Genus- *Woodfordia* and species-*floribunda*. The plant's leaf reached a height of about 3.5 m and it had long, spreading branches with fluted stems. The blossoms are a stunning shade of red (Nautiyal *et al*., 2017).

Figure 1:*W. floribunda* leaves.

2.3 Processing of plant material

The plant leaves are collected, dried and stored in shadow for 4-5 days. The dried leaves are grinded to get the powdered form which was sieved and stored in air tight plastic bags at room temperature (Roshanak *et al*., 2016).

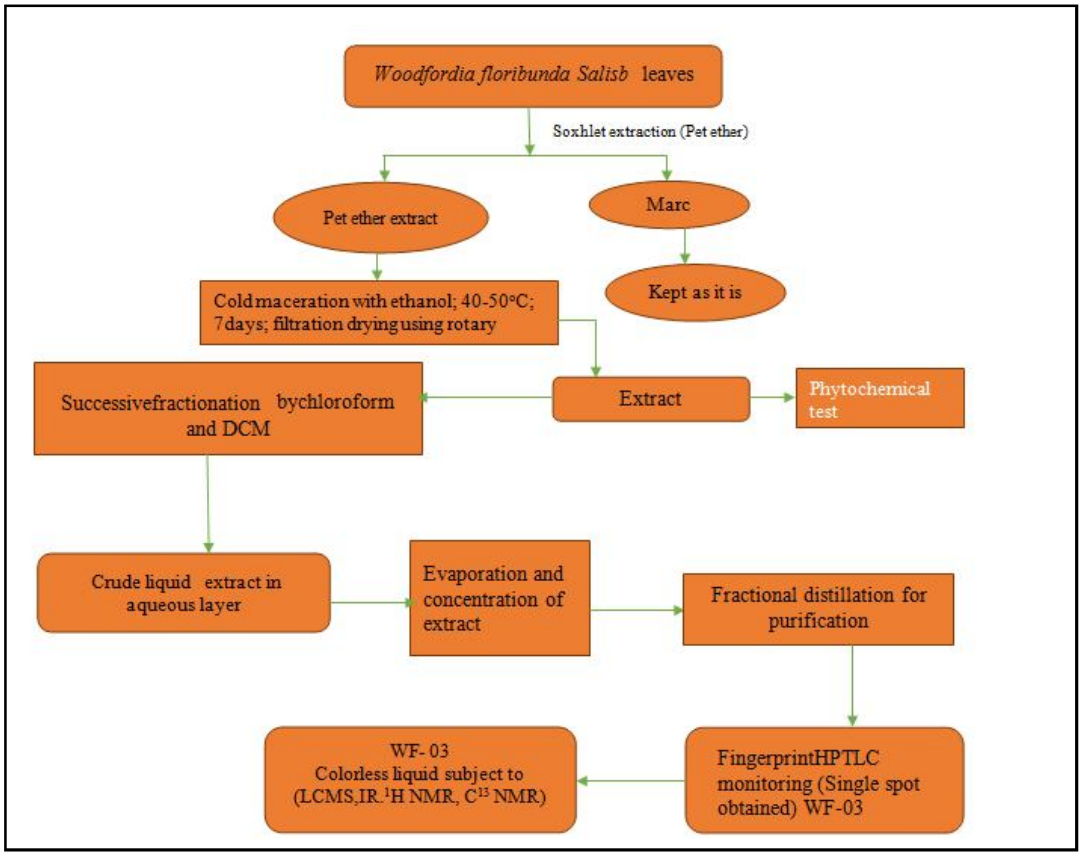

Figure 2: Flow sheet diagram of isolation of phytol (WF-03) from the pet ether extract of *W. floribunda* leaves.

2.4 Preparation of leaves extract

The powdered leaves of *W. floribunda* was hot macerated with ethanol solvent for five days (Redfern *et al*., 2014). The temperature ranging between 60-90^{0}C for the extraction, after fractionation the mobile phase use for the TLC was n-hexane:EtOAc:01drop methanol 3:6:0.5 (Phatangare and Deshmukh *et al*., 2017).

2.5 Identification of the components using GC-MS analysis

The plant of *W. floribunda* pet ether leaf extract GC-MS chromatogram showed 9 main peaks, corresponding to nine compounds. (Figure 3). Table 1 displays the retention times (RTs) and molecular weights of the principle peaks**.** Sophisticated analytical instrument facility (SAIF) provided a library of more than 65,000 patterns to aid in the interpretation of GC-MS mass spectra. The unknown substance's spectrum was compared to the spectra of recognised substances kept in the NIST library. All of the test material's constituents were identified and their molecular weights and structures were calculated.

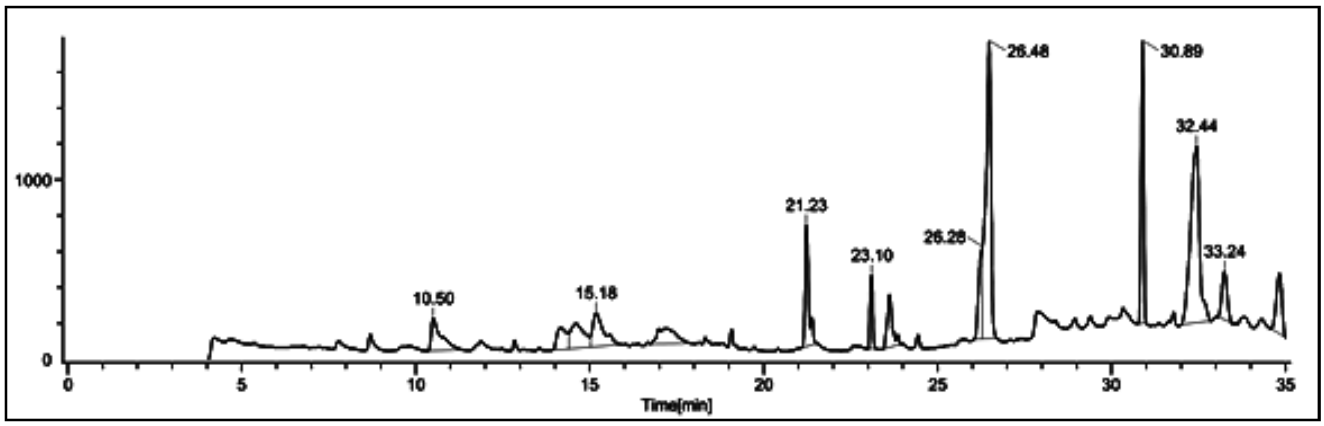

Figure 3: The chromatogram of *W. floribunda* leaves ethanolic extract.

Table 1: GC-MS analysis of an ethanolic extract of *W.floribunda* (Source of reference: Dr. Duke's phytochemical and ethnobotanical database)

S. No.	Peak name	Retention time	Molecular formula	Molecular weight	Pharmacological activities
1.	Squalene	10.50	$C_{30}H_{50}$	410	Antioxidant, antitumor
2.	n-Hexadeacanoic acid	15.18	$C_{16}H_{32}O_2$	256	Antioxidant, auto-inflammatory
3.	Lupulon	21.23	$C_{26}H_{38}O_4$	414	Antibacterial
4.	(5β)Pregnane-3,20β-diol,14α,18α-[4-methyl-3-oxo-(1-oxo-(1-oxo-4-azabutane-1,4-diyl)]-diacetate	23.10	$C_{28}H_{43}NO_6$	489	Antibacterial
5.	8,11,14-E icosatrienoic acid (Z,Z,Z)	26.28	$C_{20}H_{34}O_2$	306	Antimicrobial
6.	Phytol	26.48	$C_{20}H_{40}O$	296	Anti-inflammatory, antioxidant, antinociceptive
7.	Vitamin E	30.89	$C_{29}H_{50}O_2$	430	Antioxidant
8.	Vitamin E	32.44	$C_{29}H_{50}O_2$	430	Antioxidant
9.	Lupeol	33.24	$C_{30}H_{50}O$	426	Anti-inflammatory, antimicrobial, antiprotozoal, nephroprotective, antidiabetic

The sophisticated analytical instrumentation facility (SAIF), IIT Bombay, constructed a GC-GC-TOF-MS facility. Purpose: to isolate and identify organic components in a crude plant extract. Used instead is a GC-MS attached to a Gerstel sampler, with attention paid to

specifics like the two types of columns in use. Rxi 5-MS (30 m) and Rxi 17 Sil MS (7 m) are the two columns used (2 m). Front injector liquid injection, head space injection and stir sorptive extraction using the cooled injection system and thermal desorption unit (CIS-TDU). A higher precision can be achieved by separately setting the temperatures of each column. Over a chromatographic peak of 50 MS, the MS may acquire at least 20 full-range spectra. Software capability and increased sensitivity allow for a wider dynamic range to be realised (Koelmel *et al*., 2020).

2.6 Separation of the compound from the extract

Column chromatography is used to separate out the various components of the plant extract. Ethyl acetate and methanol are used as the mobile phase to purify the compound's constituent parts during extraction (Mallavadhani *et al*., 2017). After the column was completely empty, we used thin layer chromatography (Figure 4) to verify that the components had been separated, and observed a single spot, indicating that the compound was indeed pure.

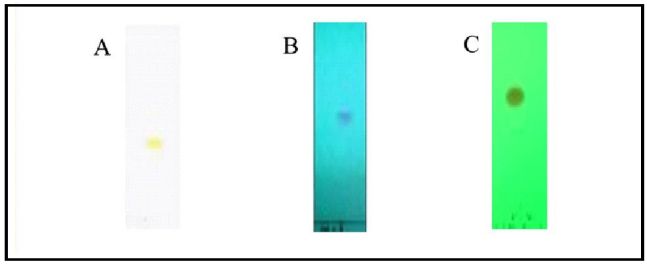

Figure 4: The isolation pattern of isolated compound on thin layer chromatography using iodine (A) UV detection (B) and spraying reagent (C) (H_2SO_4: vanillin) reagent.

After separation of the phytol, properties are checked as follows:

Density: 845 g/cm^{-3}

Boiling point: 206^{0}C-213^{0}C

Odour: Odourless

2.7 Phytochemical analysis

Take 2.5 g of fine powder and individually soaked it in 15 ml of various solvents for phytochemical analysis (Ethanol, chloroform, methanol, benzene, and ethyl acetate) in a weight-to-volume (w/v) ratio of 1.8. These may be left out at room temperature for 24 h (Khoddami *et al*., 2013). For the phytochemical analysis, a crude extract of the plants was prepared, kept in the refrigerator. The following list of distinct bioactive phytoconstituents was evaluated for in these extracts (Jaradat *et al*., 2015).

Table 2: Result of phytochemical analysis

Phytoconstituents	Test	Result of ethanolic extract
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	++
Steroids	Liebermann-burchard reaction	-
Triterpenes	Vanillin-sulphuric acid	++
Tannin and phenolic	5% $FeCl_3$ solution and dilutenitric acid	++
Cardiac glycosides	Keller-Killani	-
Flavonoids	Shinoda	+
Phlobotannins	HCl test	+
Saponins	Frothing test	+
Tannins	$FeCl_3$ test	++

(-): No presence, (+): Less presence, (++): Moderate presence, (+++): High presence

2.8 Characterization of phytol by IR, H^1-NMR, C^{13}-NMR, COSY

Infrared spectroscopy is used to identify functional groups and NMR spectroscopy is used to clarify structure (Simatzu 500 MHz) (Figures 5 to 10).

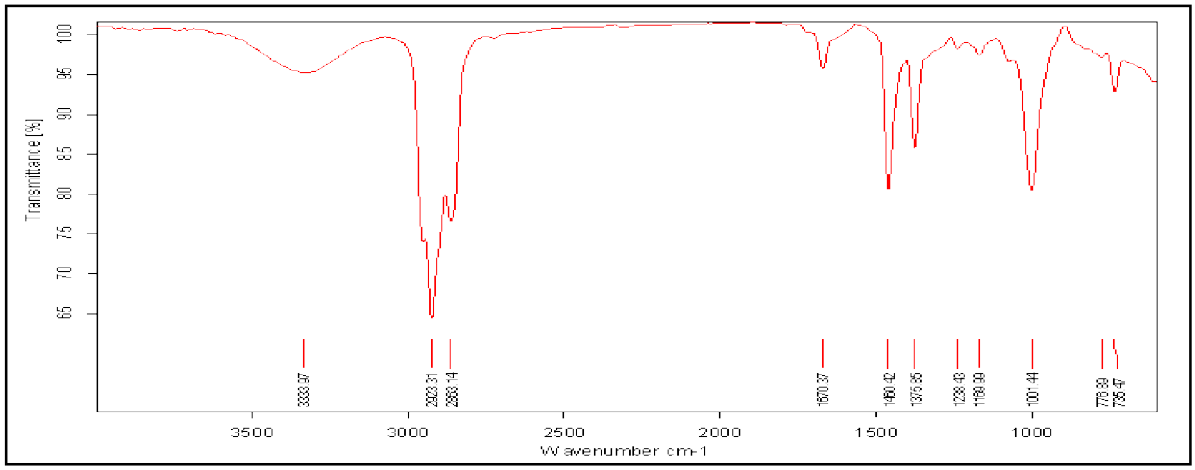

Figure 5: Infrared spectrum of phytol.

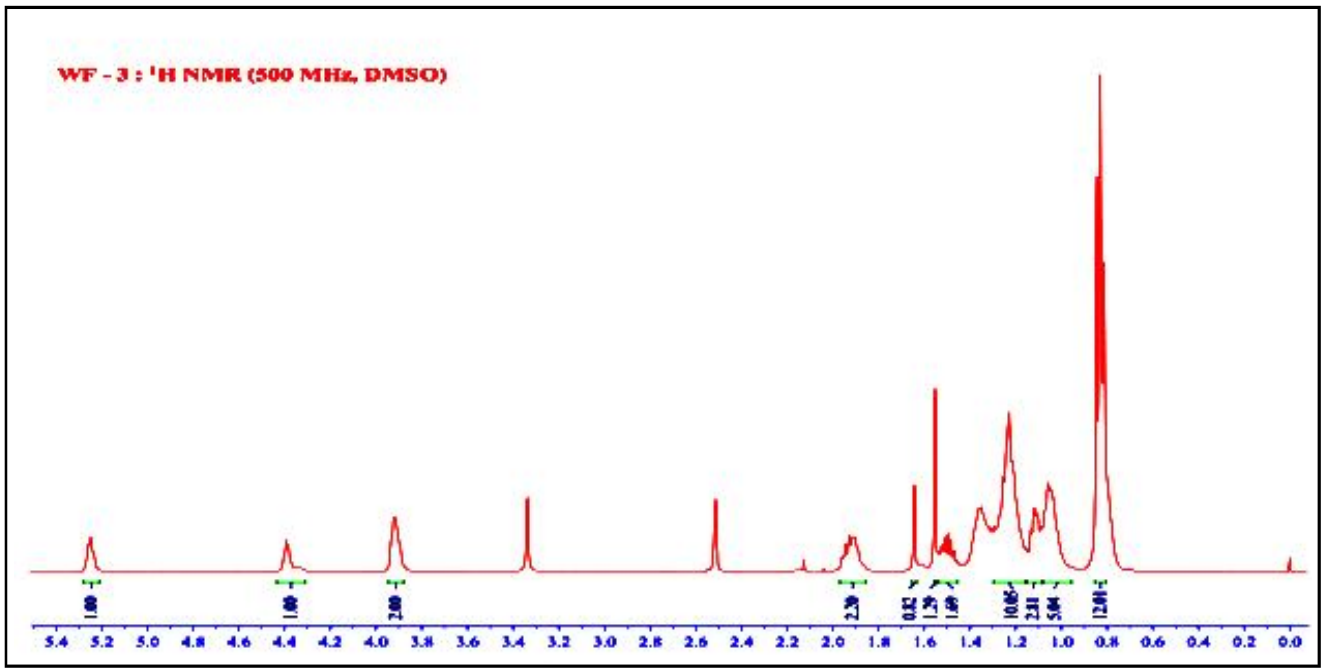

Figure 6: ^{1}H-NMR spectrum of phytol.

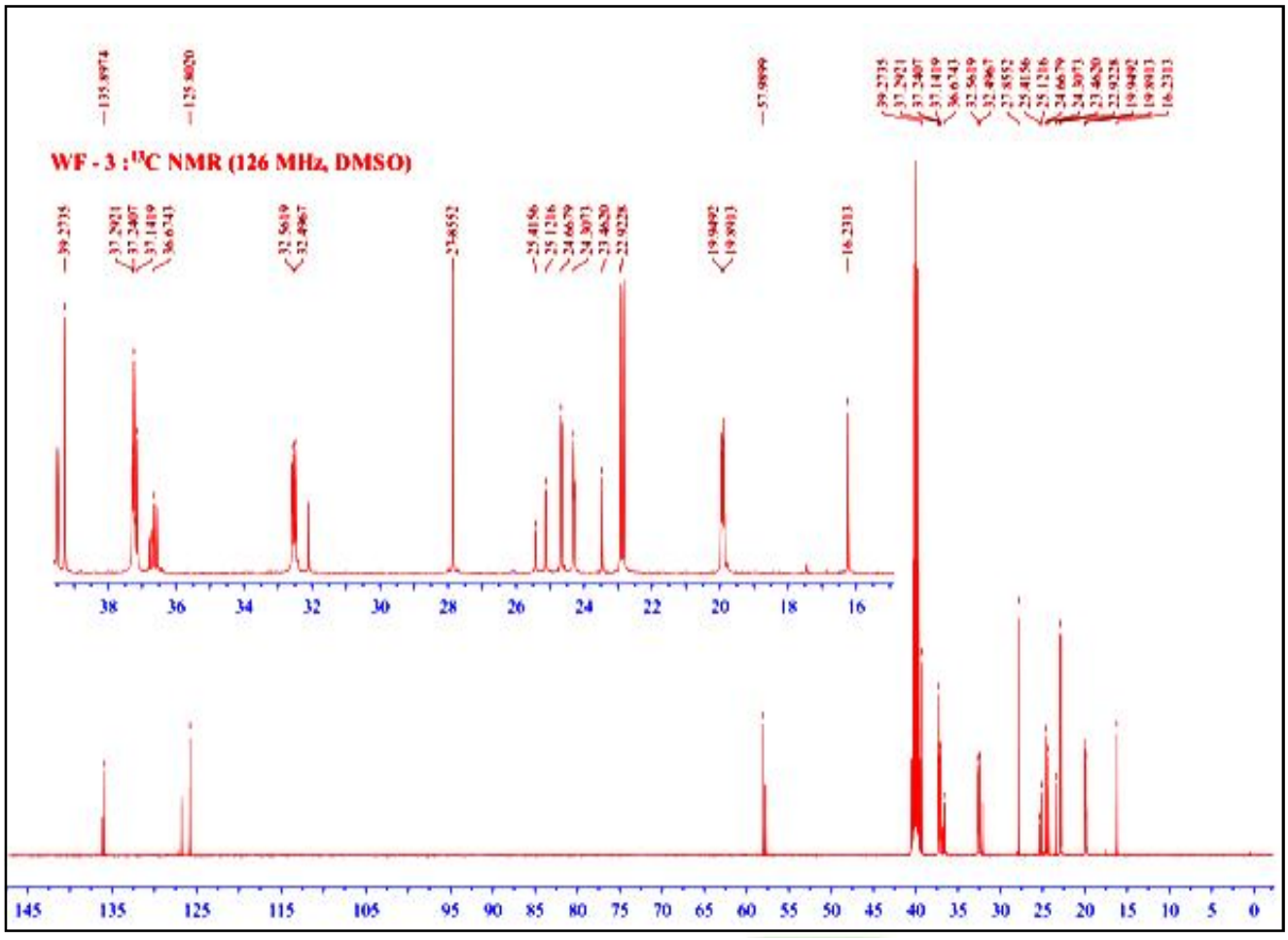

Figure 7: ^{13}C-NMR spectrum of phytol.

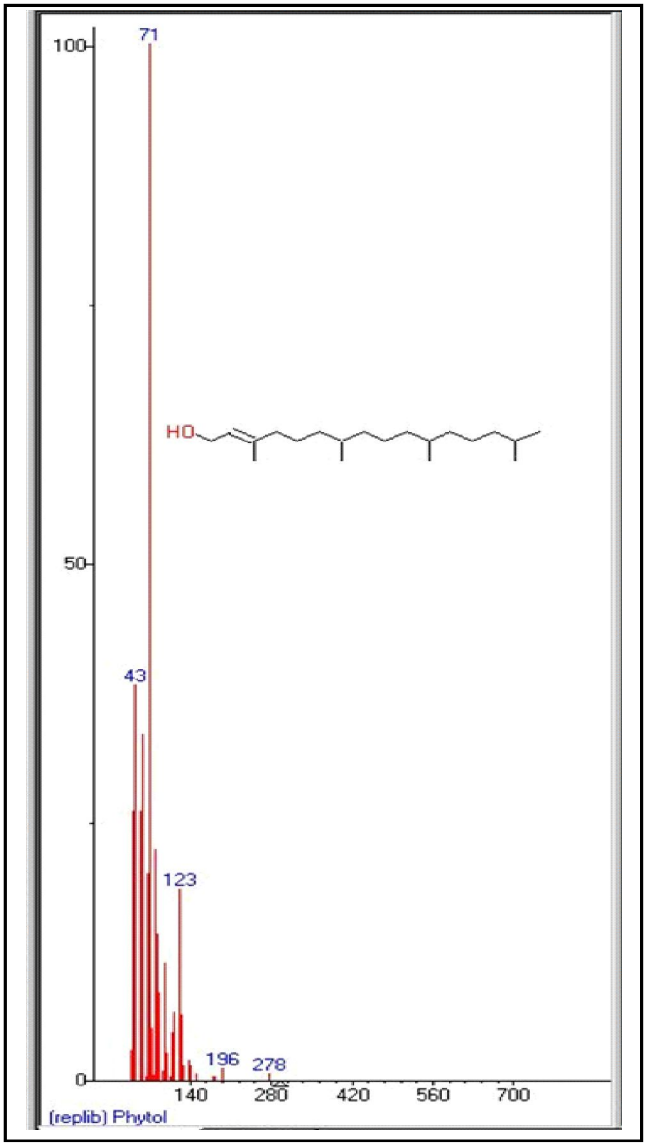

Figure 8: MS spectrum of phytol.

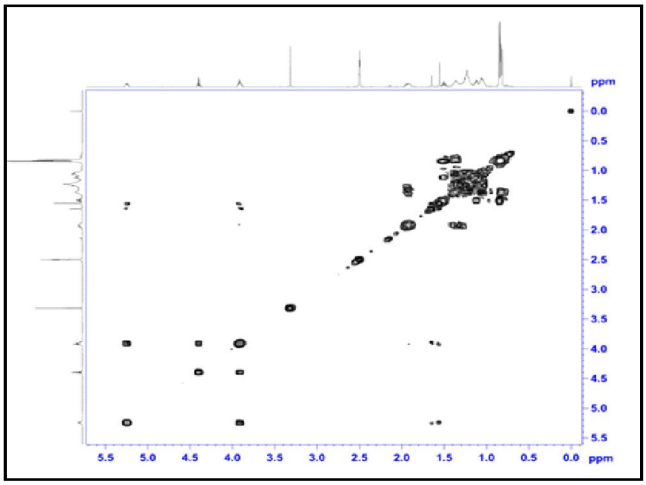

Figure 9: COSY spectrum of phytol.

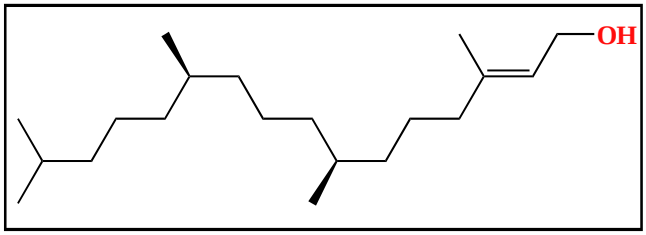

Figure 10: Final structure of phytol (WF-03).

2.9 Anti-inflammatory 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 rats (Wistar) weighing around (160-200 g). The housed animal experiences 12 h light/dark cycles, 40-60% humidity and 25-34^{0}C temperatures. Polypropylene cages were prepared for the rats and a regular rodent feed and water were provided. The animals fasted for 12 h before the experiment and were not given any food or water (Saibaba *et al*., 1996). Because water can be harmful to living things, different measures are used to ensure that it is suitable for human consumption (Mtewa *et al.*, 2021). *W. floribunda* is 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 (Poorniammal and Prabhu, 2022).The doses may be easily calculated using the information provided in this study.

2.10 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.

2.11 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 ethanolic extract as well as distilled water (control). The average weight of Wistar rats was in between 150-200 g which is used to calculate the dose size. Diclofenac (1 mg/kg) was administered as a standard. Carrageenan (0.1 ml, 1%) was injected into the right hind paw sub-planter 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 min. After each interval, the following formula is used to compute the percentage inhibition (PI) of edema: $PI = 1 – Vt/Vc \times 100$, where Vt and Vc are the volumes used for the comparison between the Turkey and the edema control. Among the many impacts of the plant are its anti-inflammatory, antibacterial and antioxidant properties. The ethanolic extract of *W. floribunda* produced noticeably better results ($p<0.05$), demonstrating its anti-inflammatory properties (Najda *et al*., 2021).

2.12 Statistical analysis

Mean and standard error were calculated using one-way ANOVA.Values of $p<0.05$ were considered statistically significant (Kim, 2014). Statistical significance was defined as a *p*-value of 0.05 or less (Gupta and Kaushik, 2022)

3. Results

The concentrated extract was injected into the GC-MS analytical device for analysis. The tool serves the information about the bioactive constituents. GC-MS analysis was performed using the instruments Agilent 7890, FID detector, Headspace injector, combipal autosampler (SAIF, IIT, Bombay), and Jeol, Model Accu TOF GCV, Time of flight analyser mass range 10-200 amu, mass resolution 6000. The results were obtained and identified nine bioactive compounds by measuring their retention time and peak area. The obtained compounds are finger pointed in to the table and w.r.t. data with reported biological activity by NIST. The many chemicals utilised in the inflammatory process lessen the edema caused in the mouse paw (Vasconcelos *et al*., 2011). From this authentication data, we go for separation of compounds by using the Soxhlet and column chromatography and other separation techniques use and isolate this bioactive compound (Jabborova *et al*.; 2020; Ranau *et al*., 2021 ; Goli and Pratap, 2021). Lastly, find out the anti-inflammatory activity by applying different types of dose and it is compared with standard compound (diclofenac).

3.1 Mass spectroscopy

Mass fragment (m/z):- 296(M+), 149, 123, 95, 81, 71, 57, 43, 41, 31, 29 (Figure 8).

3.2 IR spctroscopy

From the IR spectrum, we observed that OH stretching observed at 3250 cm^{-1} to 3500 cm^{-1}, whereas alkyl C-H stretching frequency at 2900 cm^{-1} and the C-O stretching frequency observed that 1005 cm^{-1}. The double bond stretching frequency are at C=C bond due to a-OH (Figure 5).

3.3 ^{1}H NMR (500 MHz, DMSO)

d 5.28 – 5.21 (m, 1H), 4.43 – 4.31 (m, 1H), 3.92 (d, J = 4.5 Hz, 2H), 1.92 (tt, J = 20.3, 10.1 Hz, 2H), 1.64 (s, 1H), 1.55 (s, 1H), 1.50 (ddd, J = 19.7, 13.1, 6.6 Hz, 2H), 1.31 – 1.16 (m, 10H), 1.16 – 1.08 (m, 3H), 1.08 – 0.96 (m, 5H), 0.85 – 0.80 (m, 12H) (Figure 6).

3.4 ^{13}C NMR (126 MHz, DMSO)

d 135.90(s), 125.80(d), 57.99(t), 39.27, 37.29(t), 37.24(t), 37.14(t), 36.67(t), 32.56(d), 32.50(d), 27.86(d), 25.42(t), 25.12(t), 24.67(t), 24.31(q), 23.46(q), 22.92(t), 19.95 (q),19.89(q), 16.23(q) (Figure 7).

3.5 COSY

From the correlation spectroscopy (COSY), we confirmed that 5.25 d proton-coupled with 3.90 d proton, 3.90 d proton-coupled with 4.40 d proton, 1.90 d coupled with 1.00 d, 1.10 d and 1.20 d proton, also the 5.25 d and 3.90 both coupled with 1.50 d and 1.55 d protons (Figure 9).

Table 3: Inhibition of paw edema in the percentage of phytol (n = 06) compared with standard (diclofenac)

Dose size	0 min	30 min	60 min	90 min	120 min	180 min	240 min	300 min
1 mg/kg	2.58	16.13	33.16	42.13	58.38	68.57	49.19	34.93
5 mg/kg	3.23	19.35	37.89	43.65	62.44	70.00	55.14	41.61
10 mg/kg	5.16	18.28	41.58	47.72	63.96	71.43	55.14	42.69
STD 5 mg/kg	2.58	32.80	48.42	60.91	68.34	80.48	76.14	70.27

Table 4: Edema in control (normal saline)

Animal weight in g	0 min	30 min	60 min	90 min	120 min	180 min	240 min	300 min
200	1.50	1.89	1.86	1.97	1.98	2.09	1.97	1.86
180	1.53	1.86	1.93	1.98	2.02	2.12	1.99	1.88
170	1.62	1.82	1.94	2.01	1.94	2.15	2.01	1.91
180	1.55	1.93	1.87	1.97	2.05	2.09	1.96	1.87
170	1.54	1.85	1.90	1.98	2.00	2.05	1.89	1.54
170	1.56	1.86	1.94	1.96	1.99	2.11	2.04	1.89
SD	0.04	0.037	0.035	0.017	0.037	0.037	0.051	0.14
SEM	0.016	0.015	0.014	0.007	0.015	0.013	0.02	0.057
Mean →	1.55	1.86	1.90	1.97	1.99	2.1	1.97	1.85

Table 5: The dose dependent anti-inflammatory activity of phytol (n = 06)

S. No.	Animal weight in g	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	175	1 mg/kg	1.49	1.54	1.21	1.12	0.86	0.68	0.98	0.92
II	185		1.53	1.59	1.28	1.15	0.88	0.69	0.99	0.95
III	195		1.5	1.66	1.31	1.14	0.77	0.63	0.91	0.89
IV	175		1.52	1.49	1.29	1.05	0.79	0.61	0.92	0.91
V	165		1.51	1.55	1.26	1.21	0.88	0.69	0.94	0.85
VI	185		1.51	1.53	1.25	1.19	0.76	0.68	0.88	0.90
Mean			1.51	1.56	1.27	1.14	0.82	0.66	0.94	0.90
SEM			0.057	0.023	0.014	0.023	0.022	0.023	0.017	0.0126
SD			0.014	0.058	0.035	0.056	0.056	0.056	0.042	0.0310
% Inhibition			**2.58**	**16.13**	**33.16**	**42.13**	**58.38**	**68.57**	**49.19**	**34.93**
I	160	5 mg/kg	1.54	1.46	1.22	1.15	0.75	0.66	0.84	0.87
II	175		1.52	1.42	1.24	1.12	0.81	0.72	0.86	0.79
III	170		1.53	1.41	1.08	1.02	0.71	0.56	0.82	0.83
IV	175		1.48	1.48	1.16	1.15	0.69	0.61	0.79	0.79
V	165		1.45	1.53	1.19	1.19	0.72	0.65	0.84	0.77
VI	155		1.51	1.71	1.22	1.06	0.74	0.59	0.86	0.82
Mean			1.50	1.50	1.18	1.11	0.74	0.63	0.83	0.81
SEM			0.013	0.045	0.023	0.025	0.017	0.023	0.010	0.015
SD			0.033	0.045	0.023	0.025	0.017	0.023	0.010	0.036
% Inhibition			**3.23**	**19.35**	**37.89**	**43.65**	**62.44**	**70.00**	**55.14**	**41.61**
I	160	10 mg/kg	1.41	1.52	1.04	1.06	0.75	0.71	0.87	0.84
II	170		1.46	1.49	1.07	1.02	0.78	0.64	0.88	0.91
III	180		1.45	1.52	1.14	0.98	0.79	0.69	0.91	0.78
IV	160		1.42	1.55	1.15	1.08	0.72	0.55	0.83	0.74
V	170		1.52	1.54	1.17	1.07	0.61	0.49	0.73	0.69
VI	180		1.54	1.53	1.13	0.99	0.63	0.54	0.74	0.82
Mean			1.47	1.52	1.11	1.03	0.71	0.6	0.83	0.80
SEM			0.021	0.008	0.020	0.017	0.031	0.036	0.030	0.032
SD			0.052	0.020	0.050	0.042	0.076	0.089	0.075	0.077
% Inhibition			**5.16**	**18.28**	**41.58**	**47.72**	**63.96**	**71.43**	**55.14**	**42.69**

Table 6: 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	1.86 ± 0.015	1.91 ± 0.013	1.97 ± 0.007	1.99 ± 0.015	2.10 ± 0.013
Standard (5 mg/kg)	1.54 ± 0.055	1.25 ± 0.023	0.98 ± 0.016	0.77 ± 0.021	0.63 ± 0.09	0.41 ± 0.09
1 mg/kg	1.51 ± 0.057	1.56 ± 0.023***	1.27 ± 0.014	1.14 ± 0.023	0.82 ± 0.022***	0.66 ± 0.023***
5 mg/kg	1.50 ± 0.013	1.50 ± 0.045**	1.18 ± 0.023	1.11 ± 0.025	0.74 ± 0.017***	0.63 ± 0.023***
10 mg/kg	1.47 ± 0.021	1.52 ± 0.08**	1.11 ± 0.020	1.03 ± 0.017	0.78 ± 0.031***	0.60 ± 0.036**

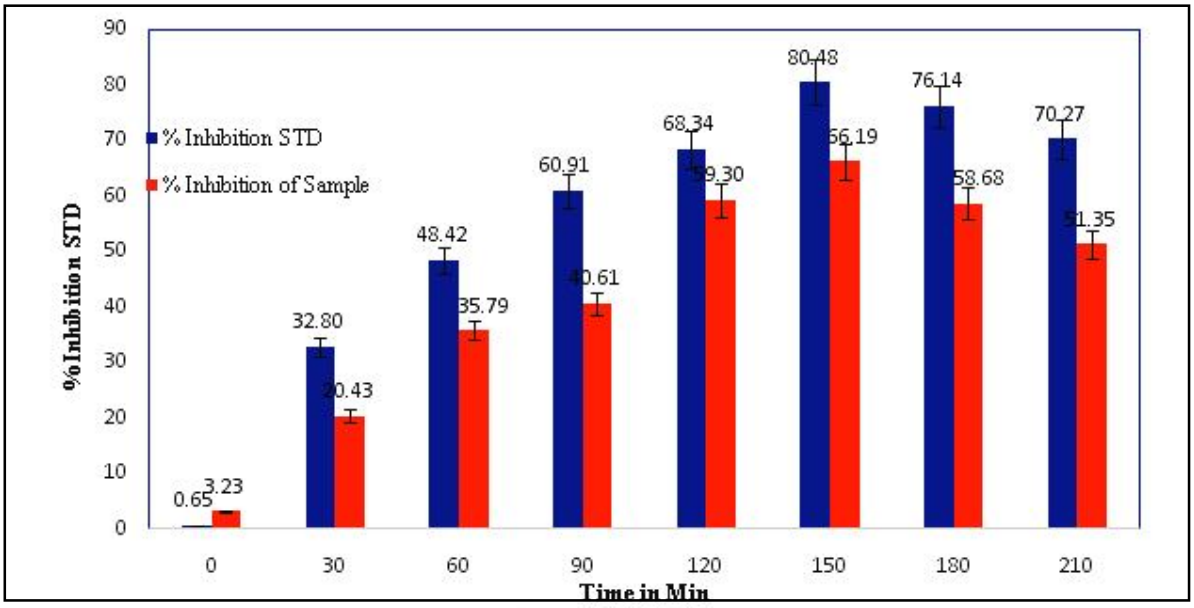

Figure 11: The % inhibition of sample dose size (1 mg/kg).

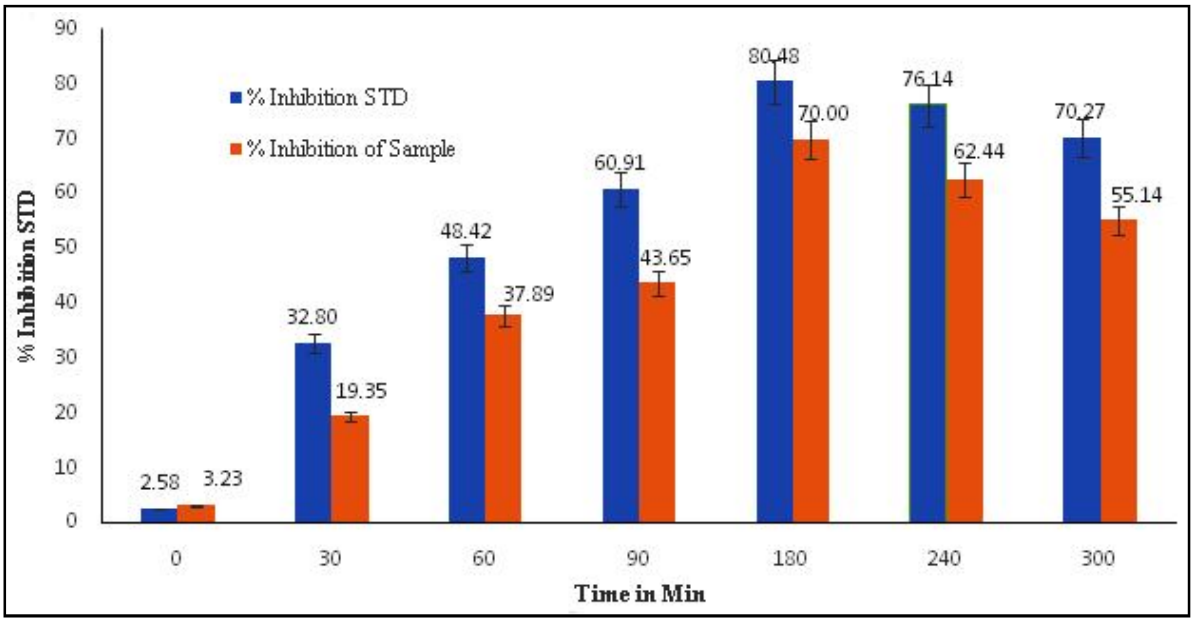

Figure 12: The % inhibition of sample dose size (5 mg/kg).

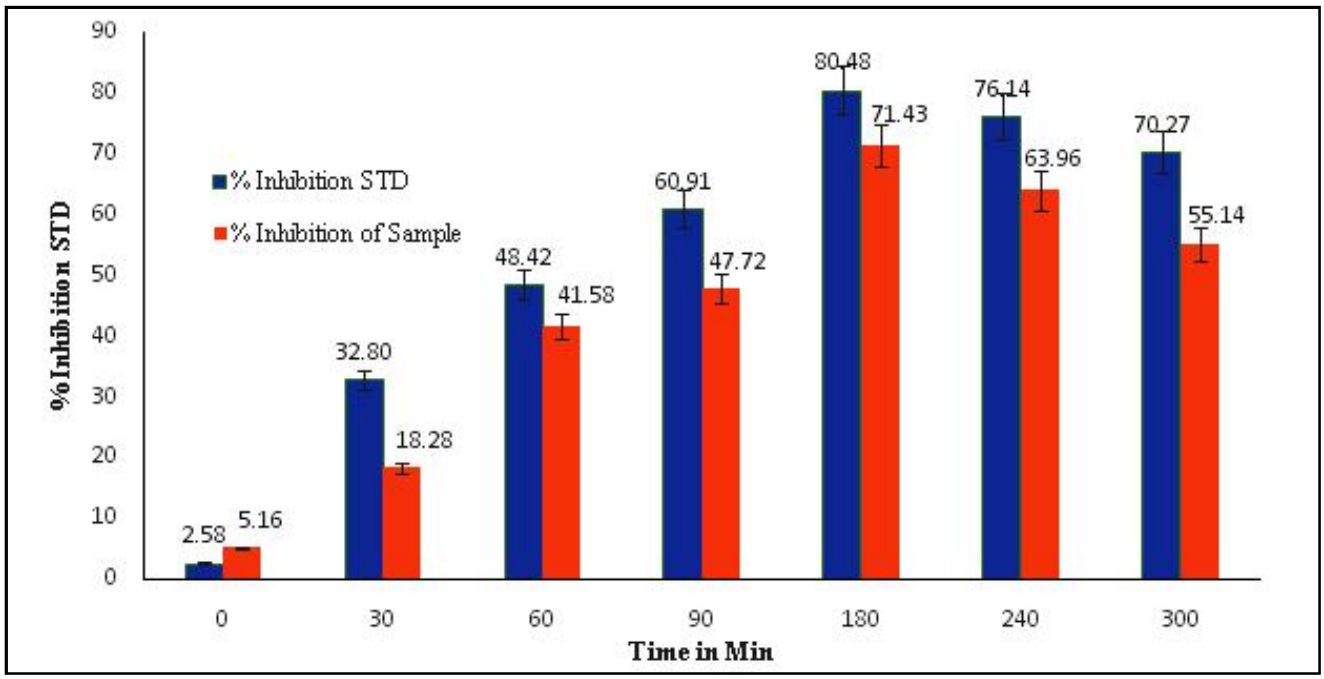

Figure 13: The % inhibition of sample dose size (10 mg/kg).

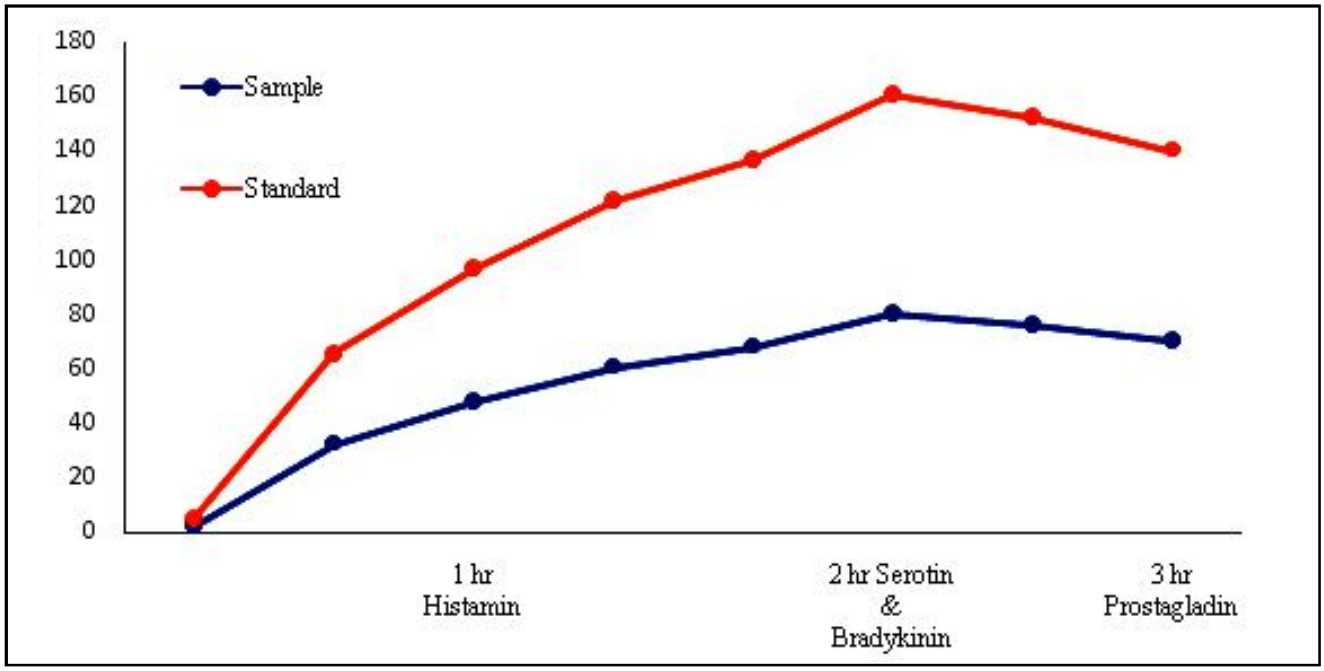

Figure 14: Release in histamine (1 h) serotonin and bradykinin (2 h) and prostandinin (3 h).

4. Discussion

W. floribunda has been documented to have anti-inflammatory properties (Verma *et al*., 2012). (Kirtikar and Basu 1933) published and exhaustive report in the field of Indian medicinal and aromatic plant research. Separation methods are followed in sequence of such as Soxhlet, column chromatography and preparative TLC are used to isolate the bioactive component phytol (Manousi *et al*., 2019). In GC-MS, the different finger prints are observed on the basis of retention time such as squalene, n-hexadecanoic acid, lupulon, 5-methyl-3-oxo-(1-oxo-4-azabutane-1,4-diyl), and 14-, 18-[4-methyl-3-oxo-(1-oxo-4-azabutane-1,4-diyl)] are components of the ethanolic extract obtained from *W. floribunda* by -diacetate, phytol, vitamin E and 8,11,14-E icosatrienoic acid (Z,Z,Z). The phytochemical analysis shows a significance of alkaloids, triterpenes, tannins, phenolic and flavonoids. Phytol is diterpene (Mathew and Ramavarma *et al*., 2019). Spectroscopy methods like Mass, IR.^{1}H NMR and C^{13}NMR, COSY were used to characterize the purified molecule. Raj *et al*., (2020) reported that phytol have anti-inflammatory potency by carrageenan induced paw edema model (Khan and Kuntal, 2019 ; Mohiuddin and Shareef, 2021).

5. Conclusion

By separating the funnel, the semi-liquid pet ether extract was prepared for the separation of a component with various organic solvents based on polarity and non-miscibility. TLC single spot is used to isolate and validate a single liquid ingredient. The isolated liquid compound is identified as phytol (WF-03). The IUPAC name is (7R, 11R) -3, 7, 11, 15-tetramethylhexadec-2-en-1-ol. By using IR, ^{1}H-NMR, C^{13} NMR, COSY and mass spectrometry characterization carried out. By using a model of paw edema caused by carrageenan, the isolated compound anti-inflammatory effectiveness was confirmed. Phytol significantly reduces inflammation (70.0%) compared to the reference (Diclofenac 5 mg/kg). Serotonin, bradykinin and prostaglandin release (37.54%), histamine release (61.59%) after an hour and prostaglandin release (70.0%) show notable percentage inhibition.

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Conflict of interest

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

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