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Stevioside potent anti-inflammatory compound of *Woodfordia floribunda* Salisb.

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Abstract

The anti-inflammatory activity of *Woodfordia floribunda* Salisb leaf ethanolic extract was examined. The literature review gave evidences for treatment of inflammatory diseases. This plant is frequently used in the traditional medicine for the treatment of inflammatory and microbial diseases. Polarity based study of leaf ethanolic extract by column chromatography with different types of solvent on the basis of polarity. Then collect the fraction and take the TLC and make preparative TLC. Finally, the solid form of bioactive compound is stevioside.The isolated compound stevioside is characterized by IR spectroscopy at 1000 cm^{-1}, 1735 cm^{-1}, the broad 3200-3600 cm^{-1}, shows C-O, esteric and alcoholic stretching, respectively. COSY shows correlation of carbon bonding with respective to NIST.1H-NMR and ^{13}C-NMR, confirmed the structure of isolated compound. Finally, stevioside was analysed for anti-inflammatory efficacy in a carrageenan induced paw edema model using diclofenac as the benchmark.The activity was carried in different dose size concentration (1 mg/kg, 5 mg/kg, and 10 mg/kg). The isolated bioactive compound stevioside gives (73.01%) inhibition for inflammation. Diclofenac (5 mg/kg) used as standard. After 1, 2 and 3 h, stevioside shows 41.05% inhibition while 63.96% and 74.76%, respectively.

1. Introduction

The medicinal plant has significant role in the pharmaceutical sciences. The naturally occurring plant in the region of Ratangadh, Kalsubai, Harichandra gadh, Ahmednagar district of Maharashtra has fruitful role in the natural products. The plant *W. floribunda* has predominantly grow in this region and also it has significant natural products. The Ayurveda mentioned that the natural plant is best remedy for curing variety of diseases. The plant possesses the lower surface of the broad, lanceolate leaves, are pale. The name “fire flame bush” comes from the abundance of bright red flowers that are produced by this shrub. Fruits are typically ellipsoid, membranous capsules filled with countless little, brown-shiny seeds. Dementia is a gradual illness marked by memory impairment and loss of intellectual abilities. During last decade’s, 60-70% cases increases due to Alzheimer’s disease. World Health Organization (WHO) reports this diseases 5% in men and 6% in woman above age of sixty years (Fratiglioni *et al.*, 2017). Several investigations have demonstrated that, in addition to its possible sweetening properties, it has antihypertensive, antihyperglycemic, antihuman rotavirus, antioxidant, anti-inflammatory and anticancer properties (Chatsudthipong *et al.*, 2009). Stevioside, the main glycoside derived from the leaves of *W. floribunda*. Stevioside is an antioxidant, anticancer diterpenoid derived from the leaves of *W. floribunda* (Alavala *et al.*, 2019). The *W. floribunda* is a shrub of the Lythraceae family, and from this plant, medicinally bioactive compound was extracted (Gregersen *et al.*, 2003). Both compounds undergo screening to determine how well they bind to various pain and inflammation related targets (Ullah *et al.*, 2016). *In vivo* analgesic and anti-inflammatory potency analysed by animal model of pain and inflammation. The carrageenan and formalin-mediated paw inflammation were used to evaluate the derivatives potential both for acute and longterm anti-inflammatory effects (Boonkaewwan *et al.*, 2006). The plant *W. floribunda* has been contain the active constituents of chemical, mineral and amino acid. In the previous literature, it is mentioned that that the stevioside possesses the anti-inflammatory property (Rao *et al.*, 2011). Corticosteroids, immunosuppressive medicines and nonsteroidal anti-inflammatory medications are currently the most widely given treatment drugs for inflammatory illnesses (Kharashi *et al.*, 2018). The current work studied to isolated the anti-inflammatory compound stevioside from the plant, *W. floribunda* and investigate its anti-inflammatory potency using the carrageenan induced paw model (Raj *et al.*, 2020; Mohideen, 2021).

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2. Materials and Methods

2.1 Collection of plant and identification

The plant leaves of *W. floribunda* Salisb are obtained from the Sahyadri (Rajur, Tal- Akole) forest in the Western ghats of Akole Tahsil (M.H.). The herbarium was prepared and it is 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 Dhaaykephool 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* (Soejarto *et al*., 2015). 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.

Figure 1: ***W. floribunda*** **leaves.**

2.3 Processing of plant material

The harvested leaves were collected and washed with water and dried under shadow for 3-5 days. The leaves were crushed by hand manually and make it fine powder and was sieved and stored in a plastic bag at room temperature.

2.4 Experimental outline of work

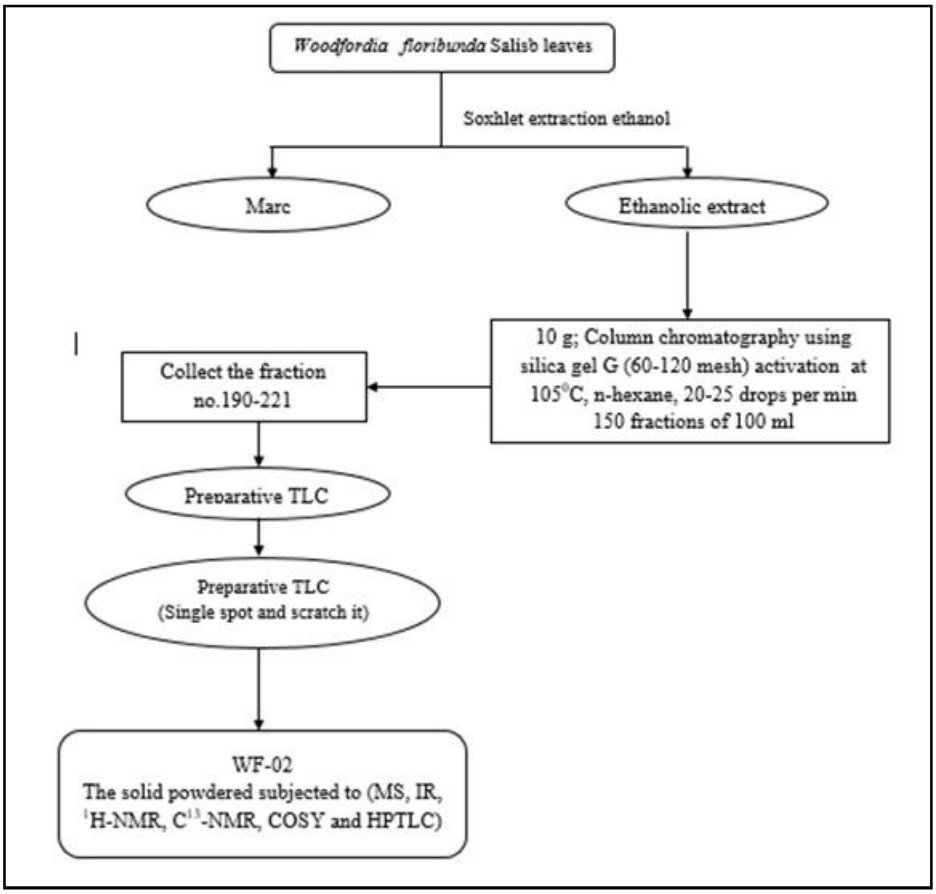

Figure 2: Extraction outline of stevioside (WF- 02) from ***W. floribunda*** **leaves.**

2.5 Preparation of leaves extract

The medicinal plant, *W. floribunda* was extracted and by evaporating it gets dried, powdered leaves in 75% ethanol in a Soxhlet extractor. After 5 days, the Soxhlet extract was taken for further study. Temperatures between 150 and 250°C were maintained (Gardan, *et al*., 2020). After running the crude extract *via* a column chromatography and collecting the fractions, made a preparative TLC and after getting single spot scratch it and subjected to spectroscopic characterization. Lastly, taking the animal activity by using paw edema animal models were used to determine its anti-inflammatory effectiveness (Hossain *et al*., 2017).

2.6 Characterization of stevioside byMass, IR,^{1}H-NMR,^{13}C-NMR, COSY

Infrared spectroscopy is used to identify the functional group and ^{1}H-NMR,^{13}C-NMR, Mass, COSY, are used to clarify the structure (Simatzu 500 MHz). (Figures 4 to 9).

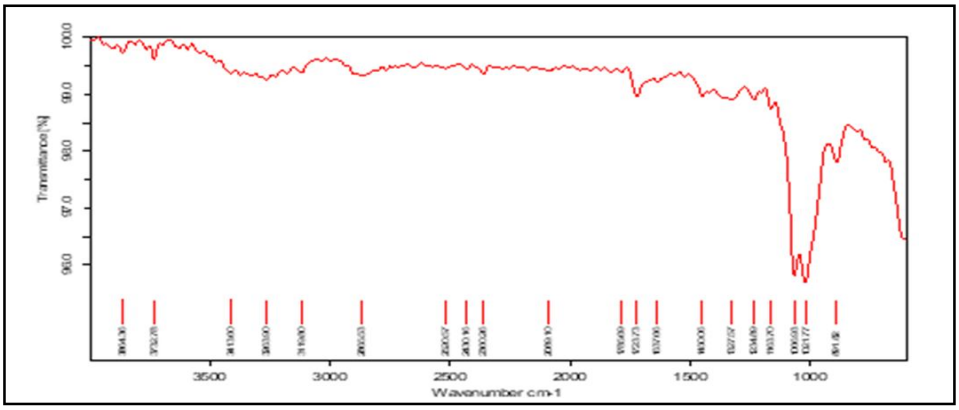

Figure 3: IR spectrum of stevioside.

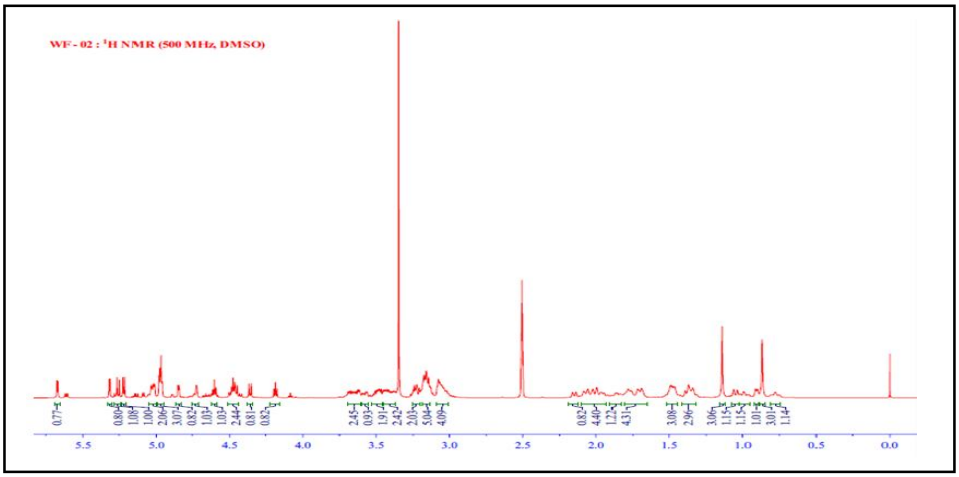

Figure 4: ^{1}H-NMR spectrum of stevioside.

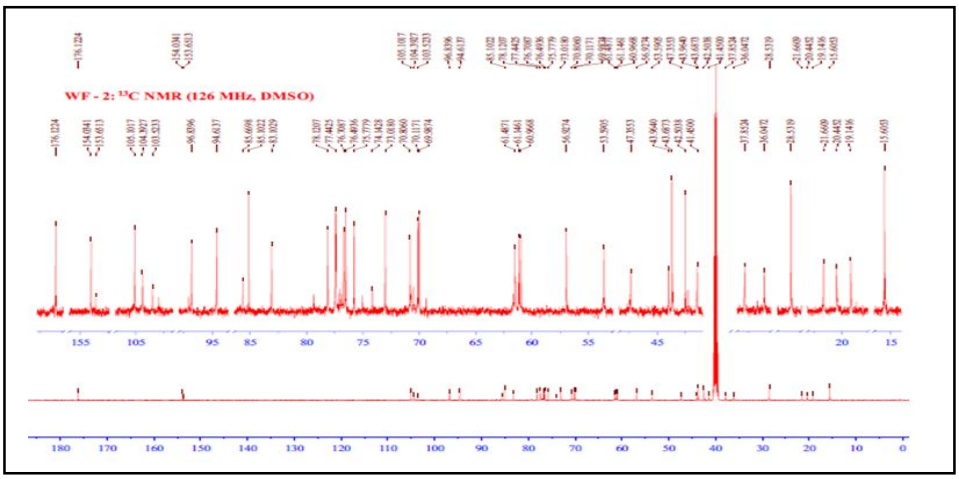

Figure 5: ^{13}C-NMR spectrum of stevioside.

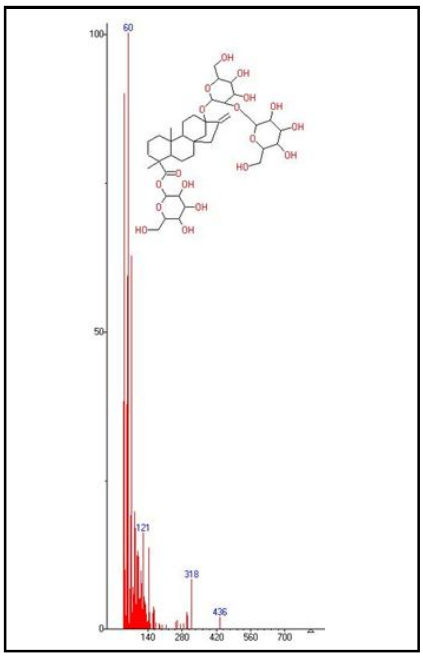

Figure 6: The MS spectrum of stevioside.

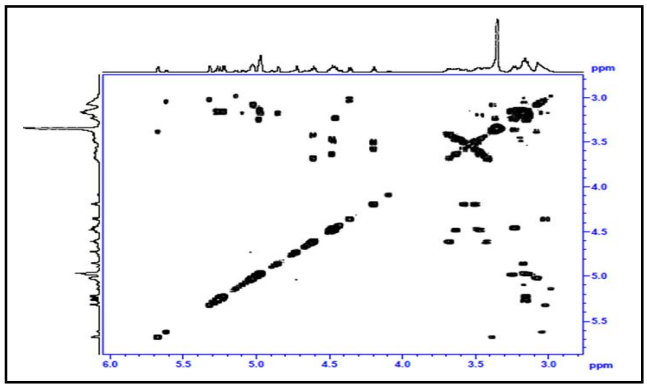

Figure 7: COSY spectrum of stevioside.

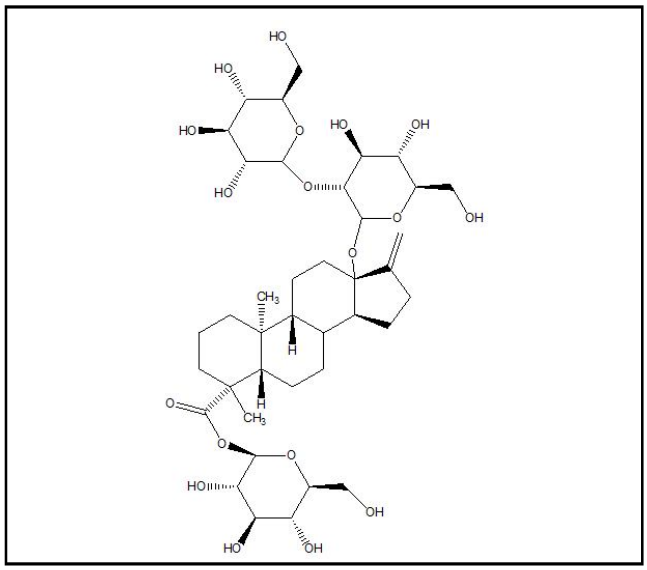

Figure 8: Final structure of stevioside.

2.7 Animal

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 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. Because water can be harmful to living things, different measures are used to ensure that it is suitable for human consumption. *W. floribunda* shows anti-inflammatory activity, has an ethanoic extract dose size depends on the weight of the animal and previous literature and the doses may be easily calculated using the information provided in this study (Ullah *et al.*, 2019).

2.8 Drugs

The diclofenac (Standard) purchased from Pharma Cure Laboratories Garha, Jalandhar and carrageenan soy lecithin from Indore, Madhya Pradesh, India were utilised in the further experimental work.

2.9 Ethical considerations

The anti-inflammatory potency of *W. floribunda* has been investigated by the ethical committee approval (AVCOP/IAEC/2021-22/1153/26/01). During the experimental work, the animals are separated in three group (n = 6), administered ethanolic extract doses of 10, 5 and 1 mg/kg p.o. The right hind paw sub-planter in each rat injected peritoneal with carrageenan (0.1 ml, 1%) using a plethysmometer (Medicaid System) 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 × 100, where Vt and Vc are the volumes used for the comparison between the turkey and the edema control. It shows significance statistical impact as p-values. Among the many impacts of the plant are its anti-inflammatory, antibacterialand antioxidant properties. The ethanolic extract of *W. floribunda* produced noticeably better results $p<0.05$ demonstrating its anti-inflammatory properties.

2.10 Statistical analysis

Values of $p<0.05$ were considered statistically significant calculated using one-way ANOVA.

3. Results

3.1 Mass spectroscopy

The mass spectrum of stevioside shows the significant peak at (M^+436), 318, 121, 60 (figure: 3).

3.2 IR spectroscopy

The IR spectrum of are observed at the C-O stretching frequency at 1000 cm^{-1}, the broad peak of –OH observed at 3500-3600 cm^{-1}and spectrum shows the functional group of ester at 1735-1740 cm^{-1}. Inaddition to this the C=C stretching frequency at 2190-2260 cm^{-1} (Figure 4). The details about the ^{1}H NMR and ^{13}C NMR are as follows (Figures 5, 6)

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

δ: 5.64 (d, *J* = 4.4 Hz, 1H), 5.32 (d, *J* = 3.3 Hz, 1H), 5.26 (d, *J* = 8.2 Hz, 1H), 5.22 (d, *J* = 5.8 Hz, 1H), 5.05 – 5.00 (m, 2H), 4.99 – 4.95 (m, 3H), 4.85 (d, *J* = 5.1 Hz, 1H), 4.73 (d, *J* = 8.3 Hz, 1H), 4.61 (t, *J* = 6.0 Hz, 1H), 4.51 – 4.44 (m, 2H), 4.36 (d, *J* = 7.7 Hz, 1H), 4.19 (t, *J* = 5.7 Hz, 1H), 3.70 – 3.61 (m, 2H), 3.58 (ddd, *J* = 11.1, 4.9, 2.1

Hz, 1H), 3.53 – 3.46 (m, 2H), 3.41 (ddd, *J* = 19.3, 9.2, 3.8 Hz, 2H), 3.26 – 3.20 (m, 2H), 3.19 – 3.13 (m, 5H), 3.09 – 3.01 (m, 4H), 2.15 (d, *J* = 11.4 Hz, 1H), 2.10 – 1.93 (m, 4H), 1.87 (dd, *J* = 17.2, 10.8 Hz, 1H), 1.74 (dd, *J* = 34.8, 11.3 Hz, 4H), 1.52 – 1.43 (m, 3H), 1.37 (t, *J* = 13.0 Hz, 3H), 1.14 (s, 3H), 1.05 (d, *J* = 12.4 Hz, 1H), 0.99 (td, *J* = 13.2, 3.9 Hz, 1H), 0.91 (d, *J* = 7.3 Hz, 1H), 0.87 (s, 3H), 0.82 – 0.73 (m, 1H), 3.34 (H_2O), 2.50 (Solvent Residual)

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

δ 176.12, 154.03, 153.65, 105.10, 104.39, 103.52, 96.84, 94.61, 85.67, 85.10, 83.10, 78.12, 77.44, 76.71, 76.49, 75.78, 74.14, 73.02, 70.81, 70.12, 69.99, 61.49, 61.15, 60.97, 56.93, 53.59, 47.36, 43.96, 43.69, 42.50, 41.45, 37.85, 36.05, 28.53, 21.66, 20.45, 19.14, 15.61,39.9 (Solvent Residual

Table 1: Inhibition of paw edema in percentage of stevioside (n = 06) compared with standard (diclofenac) with respective to time in minutes

dose size	0 min	30 min	60 min	90 min	120 min	180 min	240 min	300 min
1mg/kg	1.94	16.13	33.16	42.13	62.44	70.95	51.35	34.93
5 mg/kg	5.16	18.82	39.47	44.16	62.44	73.33	56.76	41.61
10 mg/kg	10.97	19.35	41.05	48.73	63.96	74.76	57.30	42.69
STD 5 mg/kg	2.58	32.80	48.42	60.91	68.34	80.48	76.14	70.27

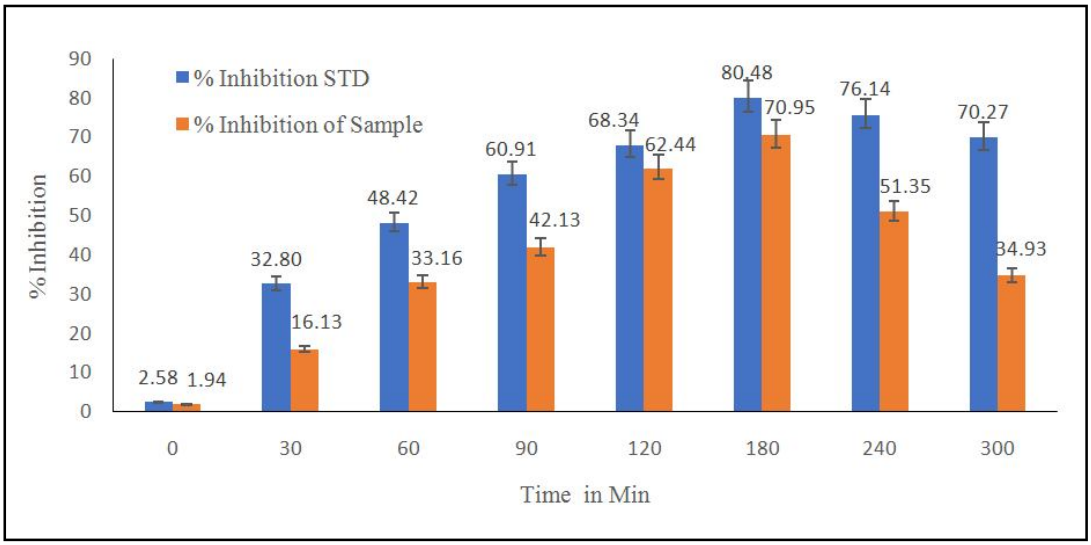

Figure 9: The % inhibition of sample with % inhibition of standard having dose size 1 mg/kg.

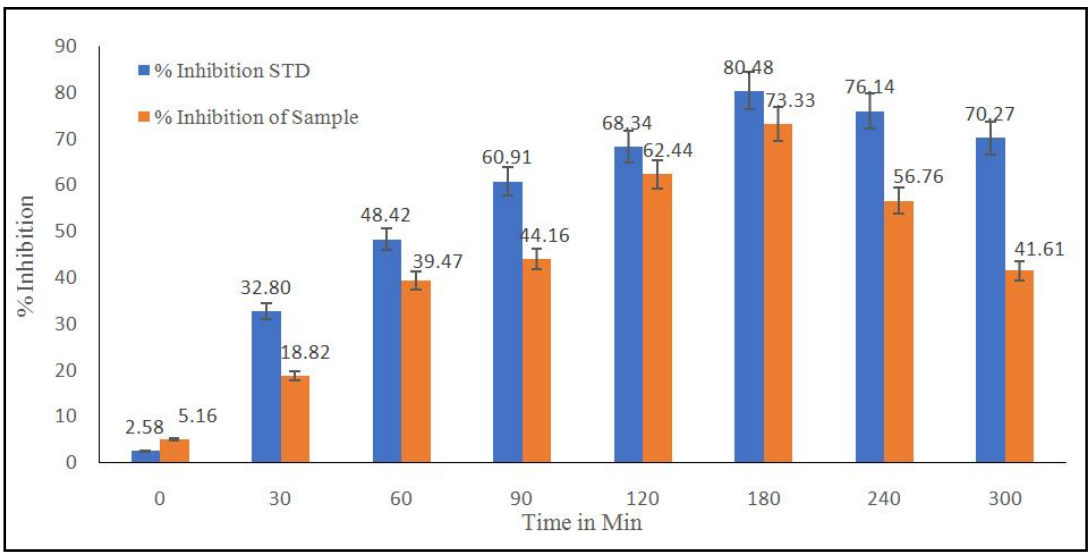

Figure 10: The % inhibition of sample with % inhibition of standard having dose size 5 mg/kg.

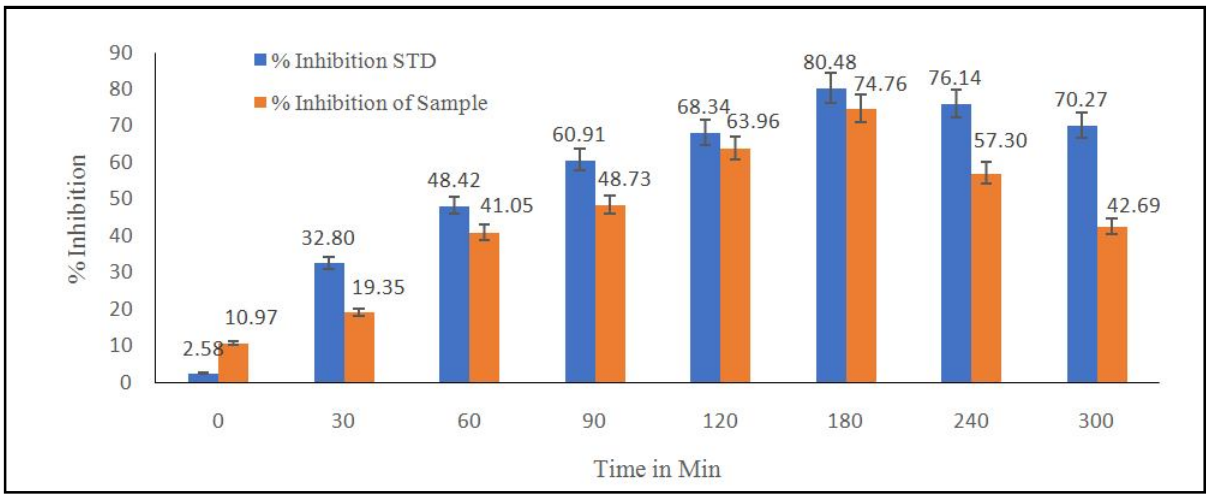

Figure 11: The % inhibition of sample with % inhibition of standard having dose size 10 mg/kg.

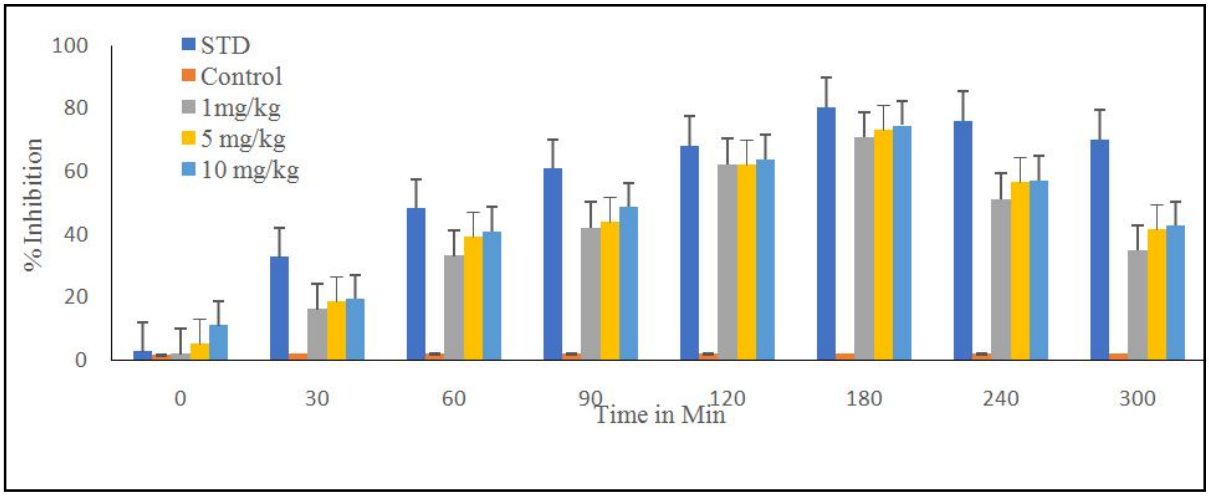

Figure 12: Comparison of % inhibition w.r.t. dose size and time.

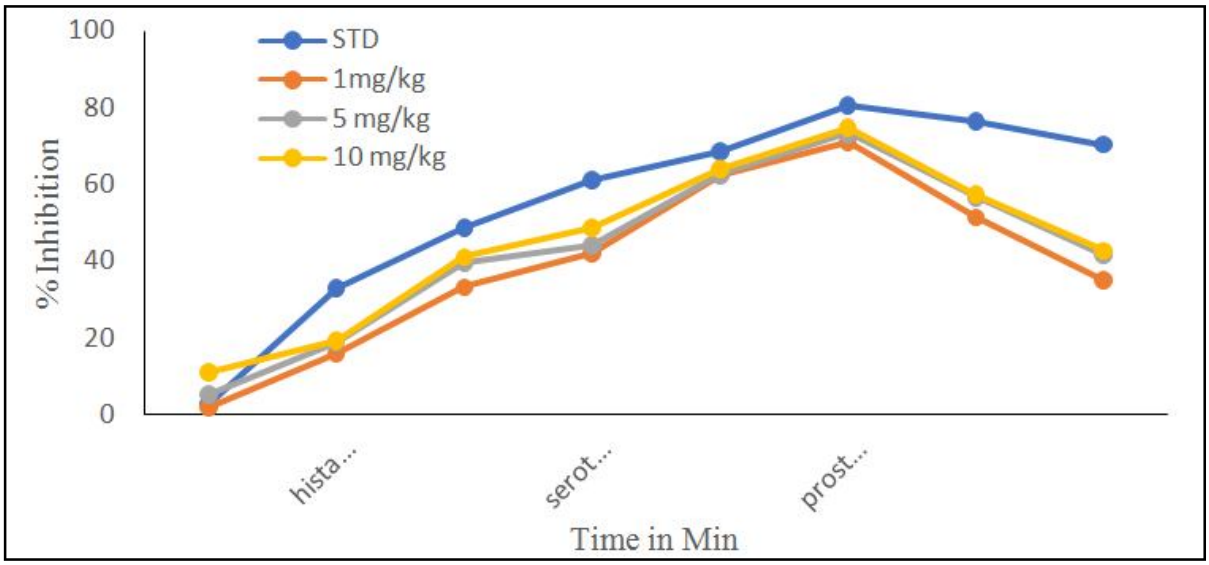

Figure 13: Release of histamine 1h,serotonin 2 h and prostaglandin 3 h.

4. Discussion

The validated in *vivo* study of the carrageenan induced paw edema model was used for *W. floribunda* on the basis of remedies prescribed by traditional ethanophramacological practitioner (Meckes *et al*., 2004). The ethanolic extract *W. floribunda* was effectively used to reduce inflammation in paw of wistar rats (120-200 g in weight). The active stevioside injected peritoneal (Winter *et al*.,1962). Carrageenan is frequently used for artificial inflammation in animal (Humbal *et al*., 2019; Falguni *et al*., 2019 ; Dilfuza *et al*., 2020)

When this drug is inhaled or ingested or regionally into the rat paw, it causes a significant chronic inflammation (Yadav *et al*., 2012). Carrageenan induced inflammation corresponds to events in the acute phase of inflammation (Borgi *et al*., 2007). The collected plant leaves of *W. floribunda* has crucial role in the medicinal chemistry. The compound present in the plant also containing bioactive constituents. In the literature of Indian medicinal plants, Basu and Kirtikar indicated that the *W. floribunda* shows anti-inflammatoryactivity. The biological active compound, stevioside separated by Soxhlet, column chromatography and preparative TLC technique. The isolated compound was subjected to the spectroscopy technique such as Mass, IR.^{1}H- NMR , ^{13}C-NMR and COSY. From the spectroscopic data and literature, the identification was so easy. Lastly, the anti-inflammatory potency of isolated stevioside is confirmed by using Carrageenan induced paw edema model (Bhatt *et al*., 2019; *Shrilakshmi et al*., 2022 ; Winter *et al*., 1962).

5. Conclusion

The ethanolic leaf extracts of *W. floribunda* have rich source of stevioside. It shows traditional medicine for the treatment of inflammatory and microbial diseases. Polarity dependent separation of components like stevioside was carried out. The process was monitored by TLC and preparative thin layer chromatography. The isolated compound, steviosides is characterized by IR spectroscopy at 1000 cm^{-1}, 1735 cm^{-1} broad 3200-3600 cm^{-1}, shows C-O, esteric and alcoholic stretching, respectively. COSY shows correlation of carbon bonding with respective to NIST.^{1}H-NMR,^{13}C-NMR, confirmed the structure of isolated compound. Finally, the isolated solid compound is characterized by IR,^{1}H-NMR,^{13}C-NMR and Mass and confirmed that it is stevioside (WF-02). The anti-inflammatory potency of isolated compound verified by carrageenan induced paw edema model. Stevioside shows significant anti-inflammatory activity (73.01%) with respect to standard (Diclofenac 5 mg/kg). Histamine release (41.05%) after 1 h serotonin and bradykinin release (63.96%) and prostaglandin release (74.76 %), indicate remarkable percentage inhibition.

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Conflict of interest

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

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