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FULL LENGTH ARTICLE

Phytochemical screening, TLC, GC-MS/MS analysis, and *in vitro* anti-inflammatory activity of methanolic extract of *Strophanthus wightianus* Wall. ex Wight: a preliminary approach

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Strophanthus wightianus Wall. ex Wight belongs to the family Apocynaceae and is well known for its medicinal properties. The present study was carried out to evaluate the possible bioactive compounds present in the methanolic stem extract of *S. wightianus*. and find out the *in vitro* anti-inflammatory activity of the plant. Preliminary phytochemical analysis of the stem methanolic extract revealed the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, glycosides, coumarins, and saponins. The TLC analysis confirms the presence of phytochemicals in the extract. The Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analysis revealed the presence of 23 compounds in the extract. Some bioactive compounds screened were apiol, methyleugenol, iridomyrmecin, bipyridine, paromomycin, and eicosanoic acid. Compounds were identified by comparing their retention time and peak area with that of available literature and by interpretation of mass spectra. Many of these compounds have various activities like anthelmintic, anti-inflammatory, anaesthetic, insect repellent, abortifacient, antinociceptive, antioxidant, antiallergic, and anti-bacterial. Some of the compounds are used as chelating agents, flavoring agents, making Dyes, and non-ionic surfactants. Anti-inflammatory activity was carried out using the COX assay. COX assay showed 51.42 % inhibition at a concentration of 200 µg/mL with an IC50 value of 171.15 µg/mL, this is an indication that the methanolic stem extract of *S. wightianus* has the potential to be developed as non-steroidal anti-inflammatory drug (NSAID).

Keywords: *Strophanthus wightianus* Wall. Ex Wight, Phytochemicals, GC-MS/MS, COX, Bioactive compounds, Anti-inflammatory activity, TLC.

INTRODUCTION

The plant contains chemicals in the form of primary and secondary metabolites (Vandana and Deora, 2020). It is used to cure a wide range of illnesses, including serious conditions. Numerous chemical compounds with a diverse spectrum of biological activity can be found in plants. Certain illnesses have been shown to respond better to herbal treatments than to conventional types of medication (Sam, 2019). Many physiologically active molecules that humans can employ as medications, meals, additives, flavors, pesticides, colorants, perfumes, and chemicals are produced using these bioactive substances found in plants (Nithya *et al*., 2018).

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Apocynaceae is a family of plants used for centuries in Indian Systems of Medicine (ISM) and is one of the most medicinally varied in the plant kingdom (Islam and Lucky, 2019; Kumar *et al*., 2022). It has been observed that several Apocynaceae species possess anti-inflammatory, antibacterial, anti-hypertensive, antioxidant, anticancer, cytotoxic, and antimalarial properties (Willie *et al*., 2021, Wong *et al*., 2013).

The genus Strophanthus consists of about 39 species and is distributed in the tropics. *Strophanthus wightianus* Wall. ex Wightis endemic to Southern Western Ghat, India, and is a climbing shrub, called kambatti in Malayalam (Singh *et al*., 2020). It is a liana found along the sacred groves of the Western Coast area of Kerala and it is narrowly distributed. It has antibacterial activity against many strains of bacteria (John *et al*., 2012). The plant parts used to treat many diseases like arteriosclerosis, heart problems, and high blood pressure (Warrier and Warrier, 2019).

MATERIALS AND METHODS

Collection of plant materials

The selected plant material like stem of *S. wightianus* was collected from Vandanam Kavu of Alappuzha district, Kerala, India. The collected plant part was washed, shade-dried, and ground into coarse powder using an electric grinder. The powdered plant material was subjected to soxhlet extraction using methanol for 72 hours followed by evaporation of solvent using a rotary evaporator. The extract was subjected to Preliminary phytochemical analysis, TLC, GC-MS/MS analysis, and anti-inflammatory analysis.

Preliminary phytochemical screening

The methanol extract was tested for alkaloids, flavonoids, terpenoids, phenols, coumarins, cardiac glycosides, steroids, tannins, saponins, and phlobatannins.

Thin layer chromatography (TLC) of the extract

The stationary phase- Silica gel G for TLC is mixed with the appropriate amount of water and is applied onto the plate uniformly and then allowed to dry and stabilize. With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots. Then, sample solutions are applied on the spots marked on the plate. The mobile phase is poured into the TLC chamber to a level of a few centimetres above the chamber bottom. Now, the plate prepared with sample spotting is placed in the TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid. The plate is then immersed, such that the sample spots are well above the level of the mobile phase for development. Allow sufficient time for the development of spots. Then remove the plates and allow them to dry. The sample spots can now be seen in a suitable UV light chamber.

GC-MS/MS analysis

The GC-MS/MS analysis of the sample was performed using a Shimadzu Nexis GC- 2030, fitted with a SH-I- 5Sil MS column (30 X0.25 mm, with 0.25 cm film thickness). The oven temperature was programmed from an initial 80ºC and held for 2 minutes. 15ºC/Minutes were increased, at 150ºC will hold for 1minute. After that 10C/minute will be increased. At 220 ºC will hold 5 minutes and a total of 50 minutes taken for a complete run. Data handling was done using GCMS solution software. The identification of compounds was based on a comparison of their mass spectra with those of NIST 20 Libraries.

In vitro anti-inflammatory screening

Cyclooxygenase (COX) assay

RAW 264.7 cells were grown to 70% confluence followed by activation with 1 µL lipopolysaccharide (LPS) (1 µg/mL). LPS-stimulated RAW cells were exposed with different concentrations of test sample solution and the standard provided. The plates were then incubated for 24 hours. After incubation, the anti-inflammatory assays were performed using the cell lysate.The COX enzyme activity was assayed by the method of Walker and Gierse with slight modifications. The cell lysate in Tris-HCl buffer (pH 8) was incubated with glutathione 5 mM/L, and haemoglobin 20 µg/L for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic

acid 200 mM/L and terminated after 20 minutes of incubation at 37°C, by the addition of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 632 nm. (Walker and Gierse, 2010).

RESULTS AND DISCUSSION

The phytochemical analysis is very much important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants (Shaikh and Patil, 2020). The plants in general possess primary and secondary metabolites such as phenolic compounds, flavonoids, alkaloids, tannins, saponins, and steroids (Manasa and Chandrashekar, 2015). The results of the phytochemical analysis are presented in Table 1, The preliminary phytochemical analysis of stem methanolic extract exhibited the presence of alkaloids, flavonoids, phenols, cardiac glycosides, terpenoids, tannins, coumarin, and saponins. According to Jhon *et al*. (2012) Qualitative phytochemical analysis of the ethanolic leaf extract of *S. wightianus* gave positive results for alkaloids, anthraquinones, lipids/fats, glycosides, phenols, carbohydrates, tannins, resins, reducing sugars, saponins, and flavonoids.

Determination of phytochemicals using TLC

TLC profiling of plant extracts in different solvent systems confirms the presence of diverse group of phytochemicals (Talukdar *et al*., 2010). TLC analysis has been carried out in the methanol extract of *S. wightianus* stem revealed the presence of many compounds which are shown in Table 2. The separation under visible and UV light is shown in the Fig.1. The TLC analysis exhibited positive results for phenol, flavonoids, alkaloids, terpenoids, tannins, and saponins. Without chemical treatment, some

Table 1. Preliminary phytochemical analysis of the methanolic extract.

Phytochemicals	Test	Result
Alkaloids	Dragendorf Fs test	+++
	Wanger test	+++
	Mayers test	++
Steroids	Liebermann-Burchardt test	–
Flavonoids	Alkaline reagent test	+++
	Lead acetate test	+++
Phenols	Ferric chloride test	+++
	Lead acetate test	++
	Gelatin test	++
Coumarins	NaOH test	++
Cardiac Glycosides	Keller-Killani test	++
Tannins	Braymer's test	++
	10% NaOH test	++
Terpenoids	Salkowski's test	++
	Copper acetate test	++
Saponins	Froth test	+++
	Form test	++
Phlobatannins	NaHCO3 test	–

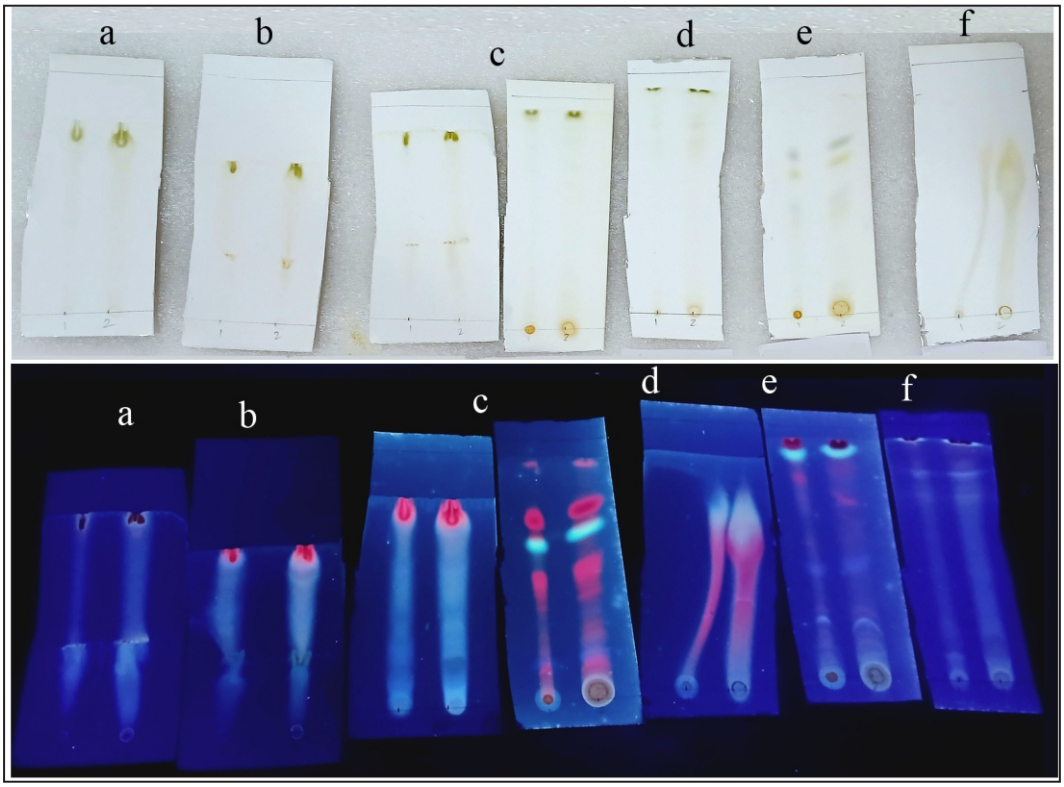
Table 2. TLC analysis of the methanolic extract.

Sl. No.	Phytochemicals	Mobile phase	Proportion of solvent system	Spraying reagent	Observation
1.	Saponin	Butanol:Water Glacial acetic acid	8.4:1.4:1.7	10% H_2SO_4	Straw-coloured, green
2.	Tannin	Butanol: Glacial acetic acid: Water	4:1:5	UV irradiation	Straw-colored and green fluorescence under UV
3.	Flavonoid	Toluene: Ethyl acetate: Formic acid	5:4:0.5	1% Ethanolic ammonium chloride/Liquid ammonia	Bright yellow-brown color
4.	Phenol	Toluene: Acetone: Formic acid	4:5:1	1% Ferric chloride solution	Dark zones/ Bright blue bands
5.	Terpenoid	Toluen: Ethyl acetate	9.3:0.7	Vanillin Reagent	Purple coloured bands
6.	Alkaloid	Methanol: Ammonia	10:0.15	Wagner's Reagent	Stable orange color in visible light

alkaloids, anthraquinones, essential oils, flavonoids, and coumarins, show blue, yellow, green, and red fluorescent zones in the chromatogram.

GC-MS/MS analysis is one of the advanced techniques to identify volatile bioactive molecules like hydrocarbons, alcohols, terpenoids, phenols, esters, etc. The GC-MS/MS analysis of methanolic extract of *S. wightianus* revealed the presence of 23 bioactive compounds. The number of bioactive compounds indicates the medicinal properties of the plant. Compounds are identified by analyzing at their retention time and peak area. The retention time (RT), molecular formula, molecular weight (MW), and peak area in percentage are presented in Table 3.The biological properties of the phytochemicals are presented in Table 4. The GC-MS/MS chromatogram of the compounds perceived is shown in Fig 2. The first compound identified was *Paramomycin*, where the last compound was *Eicosanoic acid*. The bioactive molecules identified through GC-MS/MS exhibit many biological activities that are relevant to the studies.

2-Methoxy-4-vinyphenol possesses antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination properties (Rubab *et al*., 2020; Ashwathanarayana and Naika, 2017). *Methyleugenol* is used as a flavouring added to ice cream, pies, biscuits, puddings, sweets and chewing gum, it also acts as a chemical defence against pathogens and insect herbivores (Raymond *et al*., 2017). *Methyl eugenol* classified as genotoxic carcinogens based on extensive toxicological evidence (Gotz *et al*., 2022). *1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-* or *Myristicin* is an alkenylbenzenes (Gotz *et al*., 2022)



a- Saponin; b- Tannin; c- Flavonoid; d- Phenol; e- Terpenoid; f- Alkaloid

Fig. 1. Chromatogram showing the separation under visible & UV light.

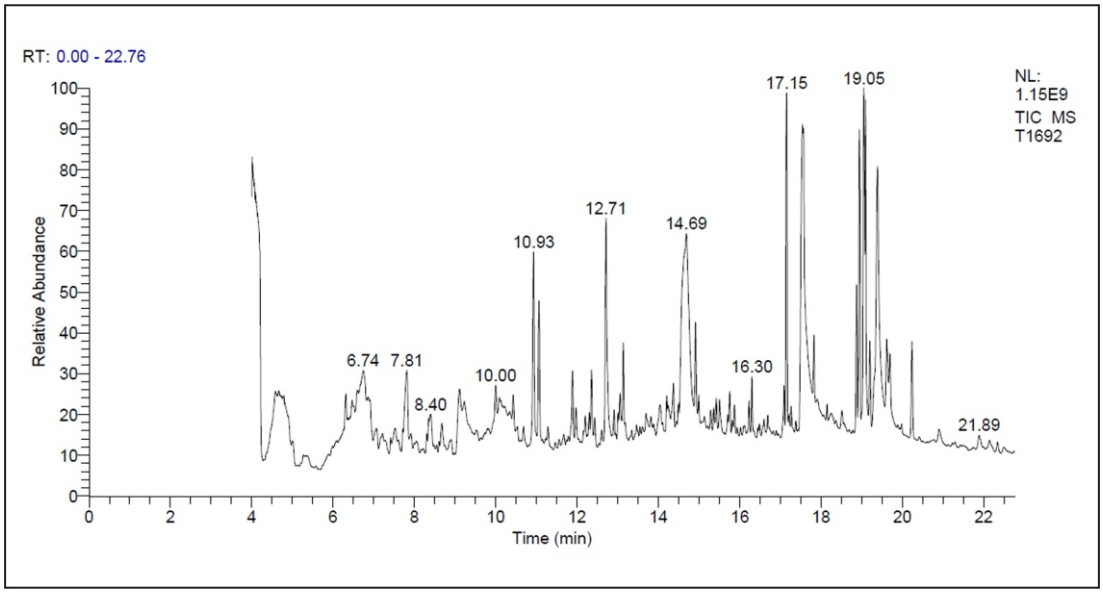


Fig. 2. GC-MS/MS chromatogram of the methanolic stem extract.

Table 3. List of compounds present in GC-MS/MS analysis of methanolic extract.

Sl. No.	Retention Time	Peak area %	Name of the compound	Molecular formula	Molecular weight
1.	5.84	0.19	Paromomycin	$C_{23}H_{45}N_5O_{14}$	615
2.	7.82	2.22	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
3.	9.24	3.70	Niacin	$C_6H_5NO_2$	123
4.	9.99	1.89	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
5.	10.93	2.50	N-(1-Cyclopenten-1-yl)-morpholine	$C_9H_{15}NO$	153
6.	11.05	3.52	Methyleugenol	$C_{11}H_{14}O_2$	178
7.	11.98	0.38	Iridomyrmecin	$C_{10}H_{16}O_2$	168
8.	12.31	3.14	Benzene, 1,2-dimethoxy-4-(1-propenyl)-	$C_{12}H_{16}O_3$	178
9.	12.44	2.28	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{17}H_{30}OSi$	206
10.	12.70	3.78	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)	$C_{11}H_{12}O_3$	192
11.	12.91	2.27	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{15}NO_5$	208
12.	13.02	0.39	Dodecanoic acid	$C_{12}H_{24}O_2$	200
13.	13.07	0.37	2,4′-Bipyridine	$C_{10}H_8N_2$	156
14.	13.90	0.43	Apiol	$C_{12}H_{14}O_4$	222
15.	14.70	1.62	3-O-Methyl-d-glcose	$C_7H_{14}O_6$	194
16.	16.45	0.2	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
17.	17.07	0.33	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276
18.	17.15	0.82	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
19.	17.27	2.08	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292
20.	17.51	6.34	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
21.	19.05	1.33	Phytol	$C_{20}H_{40}O$	296
22.	19.62	2.21	Octadecanoid acid	$C_{18}H_{36}O_2$	284
23.	22.47	0.34	Eicosanoid acid	$C_{20}H_{40}O_2$	312

Table 4. Biological activity of the compounds present in the extract.

Sl. No.	Name of the compound	Biological activity	Reference
1.	Paromomycin	Anti-parasitic, anti-diarrheal, antiprotozoal activities	Ababutain and Alghamdi, 2021
2.	Niacin	DNA repair, cell death, cell cycle progression, and gene expression, headaches, psychiatric disorders, ischemia, and traumatic traumas, as well as in neurodegenerative illnesses	Gasperi *et al*., 2019
3.	2-Methoxy-4-vinylphenol	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination properties	Rubab *et al*., 2020; Ashwathanarayana and Naika, 2017
4.	Methyleugenol	Chemical defense against pathogens, insect herbivores, toxicological activity	Raymond *et al*., 2017; Gotz *et al.,* 2022
5.	Iridomyrmecin	Neuroprotective, anti-inflammatory, immunomodulator, hepatoprotective, and cardioprotective effects	Tundis *et al*., 2008
6.	Benzene,1,2-dimethoxy-4-)1-propenyl)-	Calming properties, treatment of mood disorders, anxiolytic- and antidepressant properties	Buchbauer and Wallner, 2016
7.	Phenol,2,4-bis (1,1-dimethylethyl)-	Antibacterial and anti-inflammatory activities	Mujeeb *et al*., 2014; Phillips *et al., 2015*
8.	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)	Anticarcinogenic, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, analgesic, hepatoprotective, neurotoxic and anti-cholinergic effect	Seneme *et al.*, 2021; Gotz *et al*., 2022; Al-Qahtani *et al*., 2022; Alarcon *et al*., 2023
9.	Benzene, 1,2,3-trimethoxy-5-)2-propenyl)-	Antifungal, and anti-allergic activities	Al-Qahtani *et al*., 2022; Minh *et al*., 2023
10.	Dodecanoic acid	Anti-Mycobacterium tuberculosis, antibacterial, antiviral and antifungal properties	Sharma *et al*., 2018
11.	Apiol	Used in abortion	Narayanamoorthi *et al*., 2015
12.	Pentadecanoic acid	Antimicrobial activity	Fatema *et al*., 2019; To *et al*., 2020
13.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Antimicrobial and anti-diabetic activity	El-fayoumy *et al*., 2021; Ahmad *et al*., 2023
14.	Hexadecanoic acid, methyl ester	Antifungal, antioxidative, hypocholesterolemic, antiandrogenic, nematicide, 5-α-redictase inhibitor and antibacterial activities	Al Abboud *et al*., 2023; Pant and Rao, 2018
15.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	Antigungal and antioxidant activities	Chirumamilla *et al*., 2022
16.	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, Antiandrogenic, and antimicrobial activity	Al Abboud *et al*., 2023; Shawer *et al*., 2022; Omoighe and Agoreyo, 2022
17.	Phytol	Anxiolytic cytotoxic, metabolism modulating, autophagy, apoptosis-inducing, immune modulating, antioxidant, antinociceptive, antimicrobial, and anti-inflammatory activities	Islam *et al*., 2018; Siswadhi and Saragih, 2021

which possesses anticarcinogenic, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, analgesic, and hepatoprotective activities (Seneme *et al*., 2021; Alarcon *et al*., 2023). It is colourless and volatile which is insoluble in water. It is mainly used as an insect and pest repellent exhibiting neurotoxic and anti-cholinergic effect (Al-Qahtani *et al*., 2022). *Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-* or *Elemicin* is an alkenylbenzene (Wang *et al*., 2019), it exhibits better antifungal activity (Al-Qahtani *et al*., 2022), it possesses anti-allergic activity by the inhibitory effect on enzyme 5-lipoxygenase (Minh *et al*., 2023). *Hexadecanoic acid, methyl ester or Methyl palmitate*, exhibits antimicrobial activity (Al Abboud *et al*., 2023), used in Perfumes and Cosmetics (Ashwathanarayana and Naika, 2017), it has antifungal, antioxidative, hypocholesterolemic, antiandrogenic, nematicide, 5-α- reductase inhibitor and antibacterial activities, it is also used as a flavouring agent (Pant and Rao, 2018). *Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester* or *Benzenepropanoic acid* is a chemical compound that has been shown to be an inhibitor of the receptor binding of integrin. It use in biological treatment and as a diagnostic aid for bowel disease. shows strong antifungal and antioxidant activities (Chirumamilla *et al*., 2022).

According to (Al Abboud *et al*., 2023) *n-Hexadecanoic acid* or *Palmitic acid* shows anti-microbial properties, it is a saturated long-chain fatty acid with antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, antimicrobial activity, hemolytic, 5- alpha reductase inhibitor and potent mosquito larvicide. (Shawer *et al*., 2022; Omoighe and Agoreyo, 2022). *Phytol* (PYT) is a diterpene member of the long-chain unsaturated acyclic alcohols. Phytol has been found to display a wide range of biological properties, including anxiolytic, cytotoxic, metabolism modulating, autophagy and apoptosis-inducing, immune modulating, antioxidant, antinociceptive, antimicrobial, and anti-inflammatory activities (Islam *et al*., 2018; Siswadhi and Saragih, 2021). *Paromomycin* is an antimicrobial used to treat a number of parasitic infections, it is used to treat diarrhoea and protozoa infections (Ababutain and Alghamdi, 2021). *Niacin*, sometimes referred to as vitamin B3 or vitamin PP, regulates many biological processes, such as DNA repair, cell death, cell cycle progression, and gene expression. It is involved in other neuropathological situations like headaches, psychiatric disorders, ischemia, and traumatic traumas, as well as in neurodegenerative illnesses (Alzheimer's, Parkinson's, and Huntington's diseases) (Gasperi *et al*., 2019). *Iridomyrmecin* is a defensive chemical, classified as an iridoids exhibits a wide range of bioactivity, such as neuroprotective, anti-inflammatory, immunomodulator, hepatoprotective and cardioprotective effects(Tundis *et al*., 2008). *Benzene, 1,2-dimethoxy-4-(1-propenyl)-* or *Veratrole* or *methyl isoeugenol* (MIE), is a dimethoxybenzene with the methoxy groups at ortho-positions. It is often used as food flavor. This essential oil has calming properties. This additive appears attractive for the treatment of mood disorders. Anxiolytic- and antidepressant-like activities are also present. (Buchbauer and Wallner, 2016). *Phenol, 2, 4-bis (1, 1-dimethylethyl)* - derivative is present in various plants and is known for its antibacterial and anti-inflammatory activities (Mujeeb *et al*., 2014 ; Phillips *et al*., 2015). *Dodecanoic acid* or *Lauric acid*exhibits anti-Mycobacterium tuberculosis, antibacterial, antiviral and antifungal properties(Sharma *et al*, 2018). *Apiol* is an organic compound Used in abortion (Narayanamoorthi *et al*, 2015). *3-O-Methyl-d-glucose* or *3-methylglucose* is often used to study blood-brain barrier transport and the distribution spaces of hexoses in brain (Jay *et al*., 1990). *Pentadecanoic acid* exerts selective cytotoxic effects, it can serve as a novel JAK2/STAT3 signalling inhibitor in breast cancer cells (To *et al*., 2020), it shows antifungal and antimicrobial activity (Fatema *et al*., 2019). *7,9-di-tert-butyl-1-oxaspiro [4.5]deca-6,9-diene-2,8-dione* exhibits antimicrobial activity (El-fayoumy *et al*., 2021), it inhibits the activities of both α-amylase and AChE (Ahmad *et al*., 2023). *Eicosanoic acid* or *Arachidonic acid* is an integral constituent of biological cell membrane, it is necessary for the function of ion channels, several receptors and enzymes, via activation as well as inhibition, critically important for brain reward signalling, motivational processes, emotion, stress responses, pain, and energy balance. Free ARA and metabolites promote and modulate type 2 immune responses, which are critically important in resistance to parasites and

allergens insult, directly via action on eosinophils, basophils, and mast cell (Tallima and El Ridi, 2018).

Inhibition of cyclooxygenase enzyme activity

Inflammation is pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmic fluid and blood cells (Rajeswari *et al*., 2013). Inflammation is a complicated physiological process that results in a variety of disorders. Several inflammatory mediators are produced during this process, which is responsible for long-term inflammatory conditions like osteoarthritis, rheumatoid arthritis, asthma, cancer, and neurological disorders (Mukhopadhyay *et al*., 2023). COX is a bifunctional enzyme exhibiting both cyclooxygenase and peroxidase activities. The standard used in this evaluation of the cyclooxygenase (COX) inhibitory activity were diclofenac, an anti-inflammatory medication with very high COX inhibition. The alcoholic and aqueous extract of S*trophanthus hispidus* exhibited anti-inflammatory activity. The extract inhibits inflammatory mediators histamine, serotonin, and prostaglandin, using the highest dose (Isholaet *al*., 2013; Agbaje and Fageyinbo, 2012). The crude extracts of the leaf, root, bark, and stem of *Strophanthus sarmentosus* obtained using n-hexane, dichloromethane, and methanol possesses significant anti-inflammatory activity at different concentrations, thus justifying the use of the plant in the treatment of various inflammatory diseases (Muhammad *et al*., 2015). The ethanolic extract of the leaves of *Strophanthus gratus* (Baah and Borquaye, 2019) and the ethyl acetate fraction of ethanol stem extract of *Strophanthus divaricatus* (Miao *et al.,* 2022) showed significant anti-inflammatory activity. Hence this study analyses the *in-vitro* anti-inflammatory activity of the methanolic stem extract of *S. wightianus.*

The percentage of inhibition and IC50 values of standard and the extract are shown in the tables 5 and 6 and its graphical representation are in the Figs. 3 and 4. In the COX analysis, the Diclofenac exhibited 63.37 percentage of inhibition at a concentration of 100µg/mL with an IC50 value of 82.98. According to (Hecken *et al*., 2000) Diclofenac is a highly potent inhibitor of the Cox enzyme. The methanolic extractexhibited 51.42 percentage of inhibition at a concentration of 200µg/mL and the IC50 value was obtained as 171.15 µg/mL. This is an indication that the test sample has the potential to be developed as non-steroidal anti-inflammatory drugs (NSAIDs).

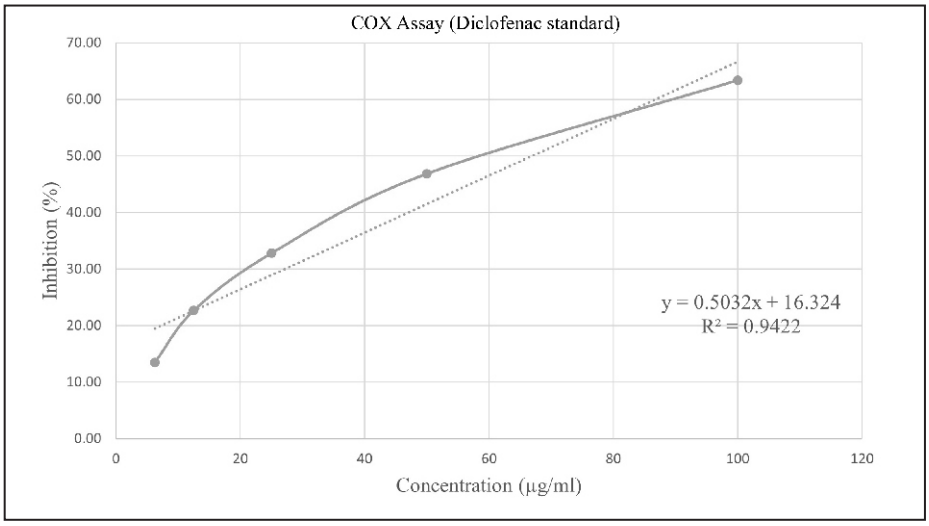


Fig. 3. Percentage inhibition of COX enzyme activity of Diclofenac.

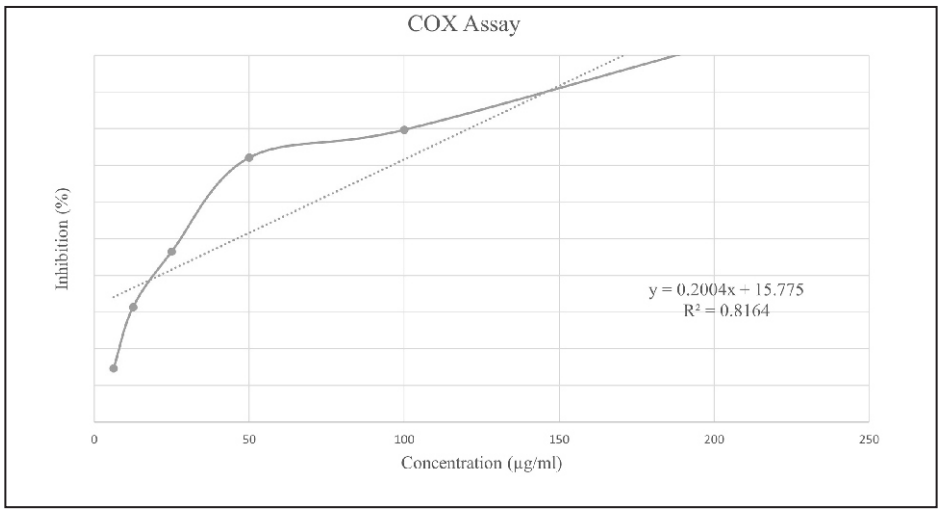


Fig. 4. Percentage inhibition of COX enzyme activity by the extract.

Table 5. Percentage inhibition of COX enzyme activity by Diclofenac standard.

Standard	Concentration (µg/ml)	Percentage of Inhibition
Diclofenac	6.25	13.48
	12.5	22.65
	25	32.77
	50	46.85
	100	63.37
IC 50	**82.98**	

Table 6. Percentage inhibition of COX enzyme activity by the methanolic extract.

Concentration (µg/ml)	Percentage of Inhibition
6.25	7.33
12.5	15.65
25	23.25
50	36.05
100	39.85
200	51.42
IC 50	**171.15**

CONCLUSION

The bioactive compounds present in plants are miraculous and have the potential to cure many diseases. The present study reveals that the stem of *Strophanthus wightianus* Wall. Ex Wight has very high medicinal properties in it. The preliminary phytochemical and TLC analysis showed that the plant contains several phyto-constituents such as alkaloids, phenols, flavonoids, terpenoids, glycosides, tannins, coumarins, and saponins which indicates the significance of the plant as a medicinal herb. GC-MS/MS spectrum analysis of the stem extract reveals the presence of numerous bioactive compounds with pharmacological significance that can be used in pharmaceutical preparations. In the *in-vitro* anti-inflammatory assay, the plant extract has shown a goodIC50 value of 171.15 µg/mL, indicating the plant extract's potential to be developed as a non-steroidal inflammatory drug.

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DISCLAIMER

The author(s) declare no conflict of interest in the work.

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