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GC-MS studies and anti-inflammatory effect of methanolic leaf and stem extracts of Leucas biflora (Vahl) R. Br. on lipopolysaccharide-induced uveitis in swiss albino rats

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***Abstract*---**The bioactive compounds present in Leucas biflora (Vahl) R.Br. leaf and stem methanolic extract was analyzed using GC-MS studies. GC-MS analysis was carried out using GC Clarus 500 perkin Elmer system interfaced to a Mass spectrometer instrument. The study was carried out to investigate anti-inflammatory effect of methanolic leaf and stem extract of Leucas biflora (Vahl) R.Br. on Endotoxin induced uveitis in Swiss Albino rats. Clinical signs including miosis, flares, hyperemia and iris were pursued for and counted in 1.0mg/kg lipopolysaccharide (LPS) induced uveitic mice treated orally with methanolic leaf and stem extract (200mg/kg), Dexamethasone (5mg/kg) or normal saline (0.1ml/kg). The protein concentration as well as levels of tumor necrosis factor – α (TNF- α) in the aqueous humor were also determined after various methods. The Methanolic extract of Leucas biflora(Vahl) R.Br. compounds studied using GC-MS were compared with the database stored in NIST library. The compounds such as Hexadecanoic acid, octadecanoic acid, 2-Ethylacridine were analysed form the plant samples. The methanolic leaf and stem extract of Leucas biflora (Vahl) R. Br. and dexamethasone treatment significantly reduced several signs of uveitis such as hypermia, flare, anterior chamber, pus and miosis. The level of protein and concentration of TNF- α in the aqueous humor were also significantly reduced in a dose dependent manner. The

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methanolic leaf and stem extract of Leucas biflora (Vahl) R. Br exhibits anti-inflammatory effect on LPS - induced uveitis possibly by reducing the production of pro-inflammatory mediators.

***Keywords*---**Hexadecanoic acid, Octadecanoic acid, TNF -α, Dexamethasone, flares, Uveitis, Iris, Hypermia, Leucas biflora (Vahl) R.Br.

Introduction

Ethno-medicine is the oldest method of curing ailments and contagions. Several plants have been used in diverse parts of the world to treat human sicknesses and infections [Caceres A L, et al., 1991; 31:263-276]. Phytochemicals are used as masters for key optimization lineups, which are intended to make benign and active drugs [Balunas M J, et. al., 78: 431-441]. Higher plants may hold a new foundation for natural antimicrobial agents with conceivably innovative mechanisms of action [Ahmad I et. al., 2007; 162(3):264-275]. Still, such plants should be investigated to better understand their properties, safety, and efficiency [S. Arunkumar, et. al., 2009; 572-576].

Intra-ocular inflammatory disease or uveitis is a collection of conditions that were known to the ancients. Today, uveitis is a condition that causes about 10% of the visual handicap in the western world(Robert B. Nussenblatt, 1990; 303-308).The ocular surface presents an unreceptive atmosphere for pathogens looking to bind the epithelial cell surface. Nevertheless, trauma, immunodeficiencies, or wearing contact lens may cause physical devastation of the ocular surface by increasing the prevalence of sight-threatening corneal contamination due to common pathogens, such as Staphylococcus aureus and Pseudomonas aeruginosa [Gudmundsson OG, 1989; 115-121]. While the precise morbific mechanisms underlying uveitis are poorly unstated, cytokines, act as significant intermediaries of immunologic and inflammatory responses, seem to be involved in this condition. Several cytokines, including tumor necrosis factor-a (TNF), interleukin-1 (IL-1), IL-2, IL-6, and interferon-7 (IFN-7), have been detected in ocular material obtained from patients with uveitis [De Vos A F, 1990; 581-597].

Leucas (Lamiacea) genus plants have been used by the traditional healers to cure many sickly conditions which oblique that these plants have massive usage in development of new remedies. The genus Leucas encompasses of approximately 80 species [Hedge I C et. al., 1990; 192]. Leucas biflora (Vahl) R. Br., a perennial herb belonging to Lamiacea family was found to be distributed at river bank and on rocky wet soil. It is distributed widely in India and Sri Lanka. (Sankara Rao, Ket al., 2019)Leucas biflora (Vahl) R. Br. leaf infusion was used in the treatment of Conjunctivitis. The mature leaves of Leucas biflora (Vahl) R. Br. along with leaves of Centella asiatica was used unswervingly to treat nose bleeding (Hdp, Gunatilaka). The plant leaves along with piper betel is prescribed traditionally for women's suffering with white discharge (Majumdar K). The plant is also used in treatment of Skin diseases (Ayyanar M) and headache (Veerabahu RM).

The goal of the present study is to explore the different phytochemical metabolites present in the leaf and stem extracts of Leucas biflora (Vahl) R. Br. The study also emphasizes the GC-MS analysis of methanolic leaf and stem extract to identify the different compounds. Based on the results, the plant extracts were further investigated in the treatment for Lipo-Polysaccharide induced uveitis infection in Swiss albino mice.

Materials and Methods

Plant Material

The aerial parts of Leucas biflora (Vahl)R.Br. were collected in December, 2017 from Maruthamalai Hills, Coimbatore District, Tamil Nadu. After proper washing, Leaf and Stem was dried under shade at a room temperature for seven days and then grinded with a mechanical grinder. The rough powder was separated by sieving using 40 mesh filter and stockpiled in an air tight flask for further use.

Preparation of Plant Extract

The Powdered material of Leaf and stem extract of Leucas biflora (Vahl) R.Br. was extracted successively with aqueous, methanol, acetone and petroleum ether by maceration cold extraction method (Dharajiya et al., 2014; 2017) for a period of 3 days with occasional shaking and stirring. The mixture was coarse filtrated through Whatman filter paper. The filtrates obtained were evaporated and dried in a rotary vacuum evaporator under reduced pressure and temperature. It concentrated a gluey material of chocolate black color. The concentrate was chosen as crude extract.

Phytochemical screening

The Leucas biflora (Vahl) R. Br. Leaf and Stem extracts (viz., Methanol, Acetone, Petroleum Ether and Aqueous) was analysed for preliminary phytochemical compounds based on Standard method described by Trease and Evans (2002) and Sofowora(1993). The procedure was carried out to detectthe presence of alkaloid, tannin, saponin, carbohydrate, glycoside, flavonoid, steroids and cardiac glycosides.

GC-MS Analysis and identification of compounds

The GC-MS analysis of Leucas biflora(Vahl) R.Br. Leaf and stem methanol extract was performed with a GC Clarus 500 perkin Elmer system including AOC-20i auto sampler interfaced to a mass spectrometer (GC-MS) instrument. The name, molecular weight and the peak value of the components of plant extracts were determined from the database of National Institute of Standard and Technology (NIST), having more than 62,000 patterns. Devet al., (2016).

Invivo studies on lipopolysaccharide induced uveitis

Experimental animals and Husbandary

Swiss albino Mice weighing 20-25g were purchased from Biogen Bangalore, India**.** All animals were housed 5 per cage and kept in the animal house for one week for proper acclimatization before starting the experiment under controlled conditions of illumination (12hr light/12hr darkness) and temperature ranging 20-25°C. Animals were maintained on standard pellet diet and water.

Ethical and Biosafety Considerations

All the studies were conducted in compliance with strategies of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA NO. 971/bc/06/CPCSEA), (IAEC-P.No.13/P. No - 03/PPK/2015; 02/15) Government of India and approved by the Institute of Animal Ethics commission. All the experiments were conducted as per the norms of the committee for the purpose of supervision of experiments on animals.

Drugs and Chemicals used

Lipopolysaccharides from Escherichia coli O111:B4 (SigmaL2630), USA) an endotoxin form was used to induce uveitis. Protein assay reagent kit (Pierce, Rockford, IL. USA) was used to determine total protein in the aqueous humour. Dexamethasone (D1756 Sigma, USA) was the reference anti-inflammatory agent in this study. Mouse TNF-α ELISA Kit (Biotrak ELISA system (RPN2744) were used in assaying for marker of intraocular inflammation. Isofurane was used to euthanize the animals and normal saline solution (Claris life sciences limited, Chacharwadi, India) was the vehicle in which other drugs were dissolved.

Lipopolysaccharide (LPS) induction

Swiss Albino Mice was injected with LPS (Lipopolysaccharide from Escherichia coli 0111: B4) an endotoxin to induce Uveitis. Endotoxin Induced Uveitis was induced by injection of 0.1ml of pyrogen-free saline dissolved Lipopolysaccharide. Dexamethasone (D1756 Sigma, USA) was used as the reference anti-inflammatory agent. Lipopolysaccharide was injected into each hind footpad on the concentration of 1mg/kg. The dosage was selected according to results of a preliminary study, which showed that LPS at 1mg/kg was the optimal dose in inducing moderate inflammation in both eyes without causing obvious lesion in retina.

Effect of Leucas Biflora (Vahl) R. Br leaf and stem methanolic extract on LPS induced Uveitis

Lipopolysaccharide induced uveitic rats were grouped into 5 (n=5), labelled I-V and treated as follows. Briefly, a control group animals received normal saline. Group II animals received Lipopolysaccharide along with normal saline. Group III animals was treated with methanolic leaf extract of Leucas biflora (Vahl) R.Br. (200mg/kg) p.o. Group IV animals was treated with methanolic Stem extract of

Leucas biflora (Vahl) R.Br. (200mg/kg) p.o. Group V animals was treated with Standard Dexamethasone (1mg/kg). All treatments were administered orally using an oral gauge and dosing done once. After 5 days treatment, following parameters were measured.

Clinical Scoring Activity

After 24hrs of Lipopolysaccharide injection both eyes of the rats were examined again for vasodilation and exudation of cells and proteins (Clinical signs of uveitis) using slit lamp(Iba T, Saitoh D.). The aqueous humor from anterior chamber of eye was collected from both eyes of mice using 30-gauge needle and were stored at -20ºC before clinical scoring activity. The scoring system is followed to grade uveitis condition (0=no obvious inflammatory response. 2 – discrete dilation, 3 – moderate dilation, 4- intense hyperemia with intense flare, 4- same signs as 3 with fibroid exudation).

Determination of Total Protein Concentration

The total protein concentration was determined in aqueous humor samples with the Biuret method, using Bovine Serum Albumin as a standard. The total protein in the aqueous humour was determined using Protein assay reagent kit. 5µl aqueous humor was diluted with 1µl 0.01 M PBS and liquid part of the sample was centrifuged for 2500rpm for 3 minutes. Then the supernatant was then collected and subjected to protein estimation. 5mg of BSA was dissolved in 1ml distilled water and serially diluted to make the concentration range of 5-0.31 mg/ml.10µl of the sample and 190µl of working Biuret Reagent were added to 96 well plate. Then the plates were incubated for 20 minutes at room temperature. The maximum absorbance was taken at 540nm using a spectrophotometer. (Rao, Narsing A., Mary Ann Fernandez, Alex Sevanian, Jorge L. Romero, Gerd O. Till, and George E. Marak Jr. "Treatment of experimental lens-induced uveitis by dimethyl thiourea." Ophthalmic research 20, no. 2 (1988): 106-111)

Determination of Tumour Necrosis Factor Alpha Levels in Aqueous Humour

The Enzyme Linked Immunosorbent Assay were widely used to estimate the TNF-α in the ocular inflammation. ELISA Kit (Biotrak ELISA system (RPN2744) was used in assaying of intra-ocular inflammation. The experiment was analysed as per the manufacturer's instructions. Optical density of each well was noted using a spectrophotometer set at 450nm.

Statistical Analysis

All the data obtained in the present investigation were subjected to statistical analysis using SPSS 16 version. Parametric test was selected based on the normal distribution of the data by DMRT analysis. One-way and two-way ANOVA (parametric) was carried out for multiple group comparisons of treatments on normally distributed data respectively. The significance level was set at 5% ($p<0.05$) and 1% ($p<0.01$) interval.

Result and Discussion

Phytochemical Analysis

Phytochemical analysis was done in Leucas biflora(valh) R.Br. in different solvents like aqueous, methanol, acetone and petroleum ether. The phytochemical screening of the leaf and stem extract of Leucas biflora(valh) R.Br.Table 1revealed that Alkaloids, carbohydrates, saponins, phenols, tannins and proteins were present in the extract. The results showed that Leucas biflora(valh) R.Br. encompass a number of chemical ingredients, which may be responsible for several pharmacological activity, like anti-bacterial, anti-ulcer, anti-cancer, anti - inflammatory and chemo protective activities. The result emphasized that methanol acts as the best solvent for the extractions of Phytochemicals in the Leucas biflora(valh) R.Br.plant.

GC-MS Analysis and identification of compounds:

The present study was carried out to determine the possible bioactive compounds of methanolic extract of the Leaf and Stem Leucas biflora(valh) R.Br. The National Institute of Standard and Technology (NIST) Library was used for comparing and identifying the compound present in the Methanolic leaf and Stem extracts. The composition and identification of the main components present in the leaf and stem of Leucas biflora (Vahl) R. Br. were shown in Fig.1&2. The active principles with their Compound name, retention time (RT), Molecular formula, molecular weight (MW), and Peak area (%) are presented in Table 2 & 3

Invivo Animal Studies

Effect of plant extract on Endotoxin induced Uveitis:
a) Clinical scoring Activity

The first clinical signs of uveitis were observed 6 hours after footpad injection of LPS in Swiss Albino Mice. The response increased at 18 hours and reached its maximum at 20 to 24 hours, with iris hyperemia, miosis, flare, and cells in the anterior chamber, forming a hypopyon and miosis. There was remarkable infiltration of cells and flares (protein exudates) in the aqueous humour, as well as intense iris hyperemia and vasodilation (clinical score: 4.40 ± 0.40) of uveitic mice treated with normal saline At, 24 hours, the response declined. Group 2 mice induced with Lipopolysaccharide and saline, which signs Hyperemia, moderate dilation in iris and conjunctival vessels with moderate flare in the anterior chamber. The methanolic leaf and stem extract treated mice (Group 3 and Group 4) showed no infiltration, or significantly reduced ($p \leq 0.001$) infiltration of cells and flares, iris hyperemia and vasodilation; reducing clinical scores to between 1.0 to 1.8.

b) Estimation of protein

The protein concentration of aqueous humor from Swiss Albino Mice were determined in 5 groups of mice. The protein concentration first increased at 4 hours, raised at 15 hours, and peaked at 24 hours. Clinical signs of uveitis could

be observed in Swiss Albino Mice in initial stage, after LPS injection, the protein concentration in aqueous humor increased during the period investigated. The concentration at 24 hours was almost peaked. The standard curve was determined by Bovine Serum Albumin Protein (Fig: 3). Standard was taken in different concentration and optical density were taken at 540nm and mean value was calculated (Tab: 5). The changes in infiltering cells and protein content in aqueous humor were also analysed. The level of protein in the groups treated with the Leucas biflora (Valh) R.Br. leaf extract and stem extract was significantly lower than that observed in the Group-2 LPS induced mice group.The changes in infiltrating cells and protein content in aqueous humor were also analyzed. Treatment with methanolic leaf and stem extract of Leucasbiflora (Vahl) R.Br produced significant decrease in infiltration cells ($p \leq 0.05$) and accumulation of protein ($p \leq 0.05$) when compared with LPS group (Fig.1), though these effects were not as potent as dexamethasone ($p \leq 0.01$).

c) Tumour necrosis factor - alpha TNF-α, Biotrak ELISA system (RPN2744)

TNF-α were not detectable in serum and aqueous humor of Swiss Albino Mice before LPS infection. After the injection of LPS, the release of TNF-α were detected and rise in the TNF-α level after few hours. Dose response activity is determined in LPS induced TNF-α estimation. Leaf extract induced mice shows 45.23% of inhibition. Stem extract induced mice showed 49.03% of inhibition and Dexamethasone (standard) induced mice showed 82.51% of inhibition. When compared with Lipopolysaccharide induced mice, TNF-α in the aqueous humor in the Leucas biflora (Valh) R.Br. leaf and stem extract treated mice were significantly decreased in a dose dependent manner. The pro-inflammatory factors TNF-α, were determined in the aqueous humor using ELISA technique. Lipopolysaccharide caused a surge in TNF-α, which were barely detected in normal control group(Fig. 2). The treatment caused a significant reduction of TNF-αfactors when compared with the Lipopolysaccharide group (LPS) ($p \leq 0.05$). Dexamethasone treatment caused a significant reduction in TNF-α.

Discussion

The present preliminary phytochemical data results were also in correlation with the earlier reports of Geethika and Sunojkumar, 2017 who reported the presence of phytochemical compounds present in various extracts of Leucas family indicating the presence of flavonoid, carbohydratein different extracts such as aqueous extract, methanol extract, ethanol extract and chloroform extract in leaves of leucas species

Earlier studies on phytochemical investigation of methanolic extract of Leucas family by GC-MS analyzed the presence of n-Hexadecanoic acid and 9-Octadacanoic acid, which has anti-inflammatory, anti-oxidant, hypo-cholesterolemic, pesticide and anti-androgenic activity (Shivakumar R., Dhivya A., 2015). Hexadecanoic acid methyl ester, an aliphatic acid ester reported to cause human gastric cancer cell growth inhibition and apoptosis induction [Daniet et al., 2011]. Earlier report on phytochemical investigation of leaf extracts of Jisticiaadhatodaby GC-MS analyzed the presence of 9,12,15-Octadecatrienoic acid and Hexadecanoic acid responsible for traditional biological activity

[Jayapriya G et al., 2015]. The present results were in correlation with GC-MS analysis of Leucas lavandulaefoliaplant extract revealed the existence of major peaks present in methanol were Sym- Tetramethyl dimethoxydisiloxane, Spiro (4,5) decan-7-one, Hexadecanoic acid, 9,12,15-Octadecatrienoic acid, Cyclopropaneoctanoic acid, n- Nonacosane, Hexacosane [Devender R, et. al.,2017; 6(4): 405-406].Anandan A, et. al., (2012) reported the compounds present in essential oil of L. virgata. The results reported to have high content of oxygenated monoterpenes (50.8%). Camphor (20.5%) exo-fenchol (3.4%), fenchon (5.4%), and borneol (3.1%) (Ramzi A. Mothana, et. al.,2013, 14, 23129-23139). The major chemical groups in the methanol fractions of L. aspera leaf extracts revealed the presence of Phytol, 9, 12, 15-Octadecatrienoic acid, methyl ester (*z*, *z*, *z*), n-Hexadecanoic acid, Squalene and 1, 2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester [Anandan A, et. al., 2012, vol 5].

The anti-inflammatory property of methanolic leaf and stem extract of Leucas biflora (Vahl) R.Br. was studied using lipopolysaccharide-induced uveitis in mice. Injection of LPS results in the breakdown of the blood-aqueous barrier a series of mechanisms beginning with the activity of the systemically injected LPS on local macrophages which in turn provoke the production of pro-inflammatory cytokines such as TNF-α are released in response to signals from these pro-inflammatory cytokines where they regulate trafficking of inflammatory cells such as monocytes and neutrophils in to the anterior uvea. The lipidA portion of LPS, a component of a gram-negative bacterial cell wall, is responsible for the explicit uveitogenic tendency in rodents [Lee FF, Foster CS (2010)]. Injection of LPS results in the breakdown of the blood-aqueous barrier a series of mechanisms beginning with the activity of the systemically injected LPS on local macrophages which in turn provoke the production of pro-inflammatory cytokines such as TNF-a are released in response to signals from these pro-inflammatory cytokines where they regulate trafficking of inflammatory cells such as monocytes and neutrophils into the anterior uvea [Iba T, Saitoh D, 2014].Under the cytokines, macrophages and neutrophils secrete prostaglandin E2 and nitric oxide, which actually causes the breakdown of the blood-aqueous barrier leading to the observable infiltration of cells and flare (protein exudation) in the anterior chamber during the clinical stage of the disease process [Lee FF, Foster CS (2010)]. The noticeable miosis especially in rabbit with uveitis can be explained by the release substance P from the peripheral nerve endings in reaction to deleterious stimuli [Chao SC et al., 2015]. Results from this study indicated that the Root and Stem has potent anti-inflammatory effect in endotoxin induced uveitis which gives credence to its traditional use in managing inflammatory eye disorders [Iba T, Saitoh D, 2014].]. TNF-α is one of the earliest cytokines involved in the pathogenesis of Lipopolysaccharide induced uveitis. Other researchers have reported that symptoms of Behcet's disease uveitis could be relieved by anti-TNF-α antibody treatment (Nishida T et al., 2015). It is an established fact that the transcription of TNF- α is under the influence of nuclear factor-κB (NF-κB) and a positive loop exist between amplification of cytokine cascade during inflammation (TNF-α) and activation of NF-κB (Chen et al., 2012). In this study methanolic leaf and stem extract of Leucas biflora (Vahl) R. Br. presumably down-regulated TNF-α in the aqueous humor, by obstructing the generation of the positive loop between TNF-α and NF-κB. The result of the study indicated that Leucas biflora (Vahl) R.Br. leaf methanolic extract and stem methanolic extract suppresses the development of

Endotoxin induced Uveitis (EIU). TNF-α is a cytokine produced principally by activated macrophages and monocytes. The result of the present study indicated that Leucas biflora (Vahl) R.Br. leaf and stem methanolic extract decreased the TNF-α concentration invivo. This strongly shows that the methanolic extracts of Leucas biflora (Vahl) R. Br. has anti-ocular inflammatory effect on Endotoxin Induced Uveitis. These findings suggest that Leucas biflora (Vahl) R.Br. leaves and stem may act as a promising agent for the treatment of Ocular inflammation. The present results were in correlation with the Lipopolysaccharide induced uveitis anti- inflammatory result of Leucas cephalotes (Roth.) Spreng. The bioactive compounds such as oleanolic acid, laballenic acid present in Leucas cephalotes (Roth.) Spreng were found to inhibit the inflammatory mediators at half maximal inhibitory concentration of 17.12 to 57.20 µl. Furthermore, LCD at a dose of 50, 100 and 400 mg/Kg was found to reduce Lipopolysaccharide induced tumor necrosis factor (TNF) - α and interleukin (IL)-1β production significantly in female Sprague Dawley (SD) rats. Chitra et al., reported that Leucas biflora leaf extract was used to synthesise silver nanoparticles and they were analysed for anti-cancer activity on the MDA – MB – 231 cell line. The present anti- inflammatory results may lead to a promising pathway for production of novel anti-cancer medications.

All these reports suggested that the anti-inflammatory effects of Leucas biflora (Vahl) R.Br. is moderately mediated through the conquest of pro-inflammatory mediators and hence can be exploited for the development of anti-inflammatory drugs. The Bioactive compounds analysed by GC-MS may be isolated and characterised for further designing of new drugs.

Table 1: Phytochemical Analysis of Leucas biflora (Valh) R. Br. Leaf and Stem extracts

Phytochemicals	Aqueous		Methanol		Acetone		Petroleum Ether	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	+	+	+	+	+	+	+	_
Carbohydrates	+	+	+	+	+	_	_	_
Glycosides	+	_	+	_	_	_	+	_
Steroids	_	_	_	_	_	+	_	+
Flavonoids	_	+	+	+	_	_	_	_
Saponins	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	-
Amino acids	+	+	+	-	+	-	-	-
Proteins	+	+	+	+	+	-	-	-

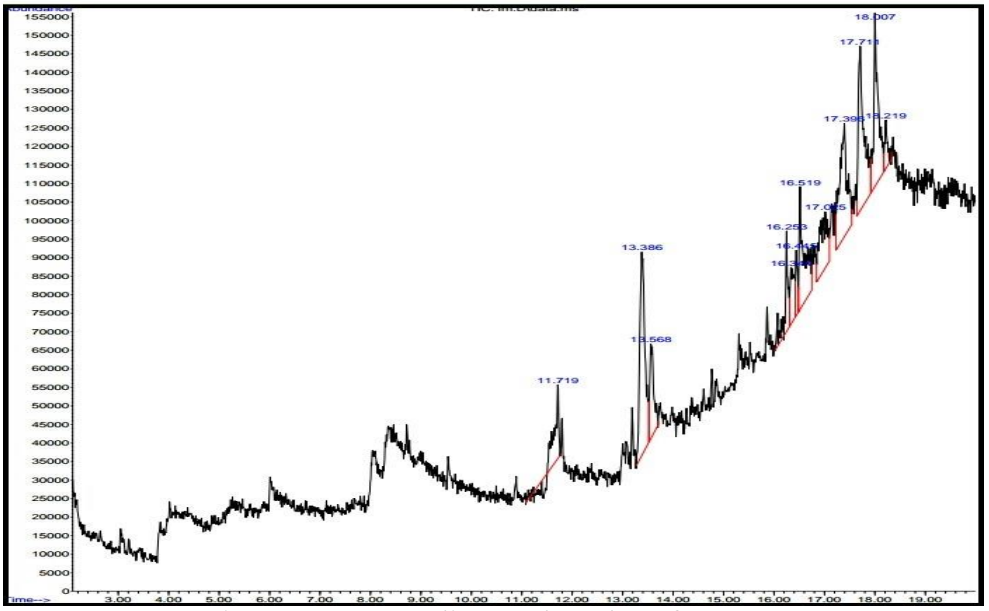
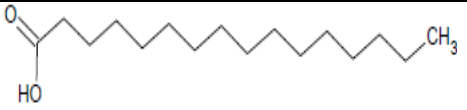
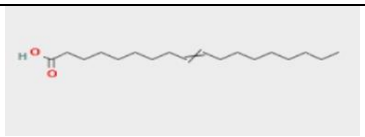
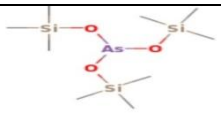
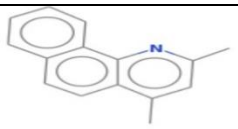
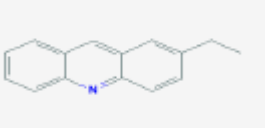


Fig 1: GC MS Studies Methanol Leaf Extract

Table: 2 GC-MS analysis of some Bioactive compounds of Leucas biflora (Vahl) R.Br methanolic leaf extract

S.NO	RT	COMOUND NAME	USES	STRUCTURE
1	11.722	n-Hexadecanoic acid	Anti-oxidant, Lubricant, 5-Alpha reductase inhibitor, antipsychotic.	
2	11.722	Dodeconoic acid	Anti-cancerous	
3	13.386	9-Octadecenoic acid, (E)-	Anti-inflammatory, Anti-androgenic Cancer preventive, 5-Alpha reductase inhibitor,	
4	13.386	6-Octadecenoic acid	Anti-cancerous	
5	16.251	Arsenous acid, tris(trimethylsilyl) ester	Anti-oxidant activity	

6	16.449	Benzo [h] quinoline, 2,4-dimethyl-	Anti-cancer	
7	16.516	2-Ethylacridine	Anti-microbial and Anti-tumor	

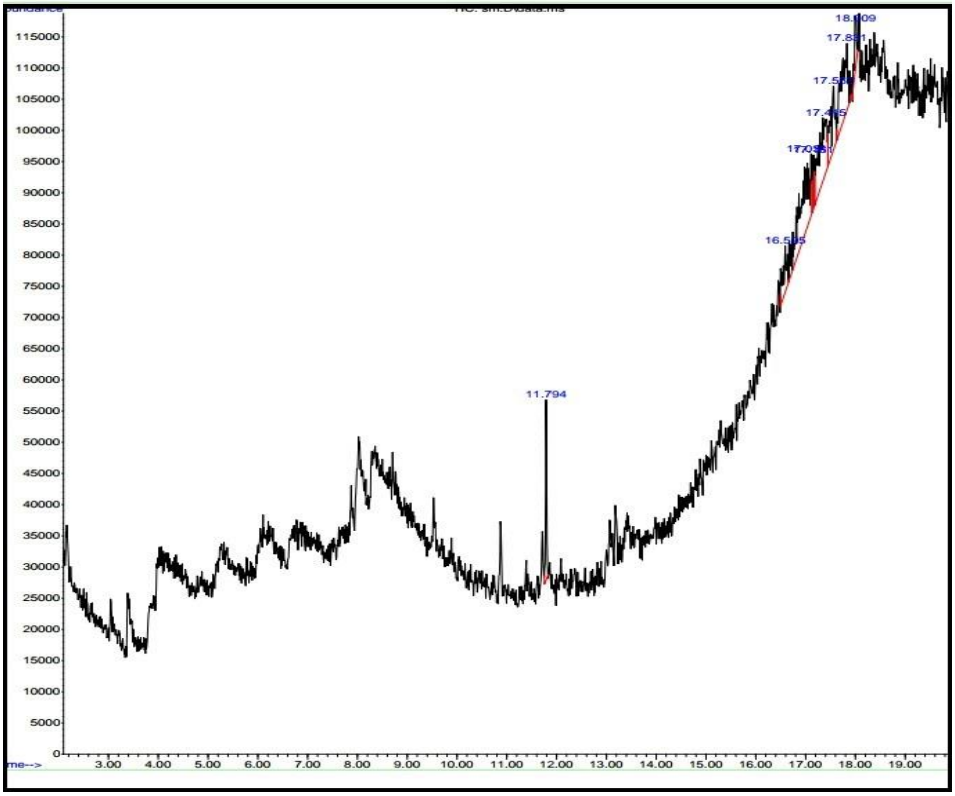
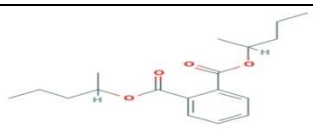


Fig 2: GC MS Studies Methanol Stem Extract

Table: 3 GC-MS analysis of some Bioactive compounds of Leucas biflora (Vahl) R.Br methanolic stem extract

S.No	RT TIME	NAME OF THE COMPOUND	ACTIVITY	STRUCTURE
1	11.797	Phthalic acid, 1-tert-butoxyprop-2-yl pentyl ester	Anti-microbial	

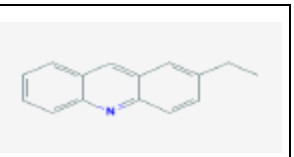
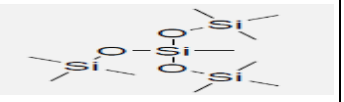
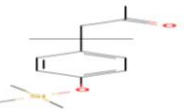
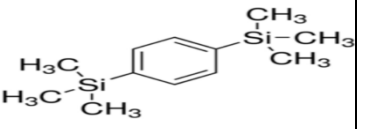
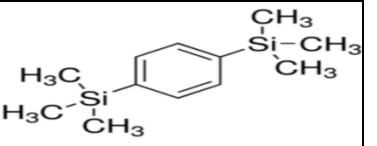
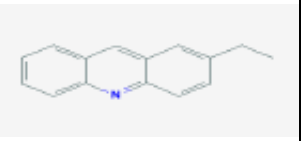
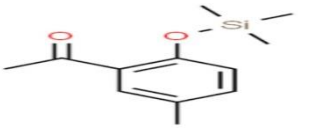
2	16.591	2-Ethylacridine	Anti-microbial and Antitumor	
3	17.036	Methyltris (trimethylsiloxy)silane	Anti-acne agent, Anti-bacterial Agents	
4	17.159	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	Anti - oxidant, anti-bacterial and anti-inflammatory	
5	17.414	1,4-Bis(trimethylsilyl)benzene	Anti-oxidant activity	
6	17.565	1,4-Bis(trimethylsilyl)benzene	anti -oxidant	
7	17.83	2-Ethylacridine	Anti-microbial and Anti-tumor	
8	18.01	5-Methyl-2-trimethylsilyloxy-acetophenone	Anti-oxidant and anti-microbial	

Table 4: Clinical Scoring Activity

Group	Hyperemia/ Redness (0-2)	Flare (0-1),	Anterior chamber (0-2),	Hypopyon / Pus (0-2)	Miosis (0-1).
Normal	0	0	0	0	0
Untreated	2	1	1	1	0
Stem (10 %)	1	0	1	1	0
Root (10 %)	1	1	0	0	0
Dexamethasone	0	0	0	0	1

The grading is assigned as:0= no obvious inflammatory response, 1 = discrete dilation of iris and conjunctival vessels, 2 = moderate dilation and iris and conjunctival vessels with moderate flare in the anterior chamber, 3 = intense iridalhyperemia with intense flare in the anterior chamber, 4 = same clinical signs as 3 with presence of fibrinoid exudation and miosis.

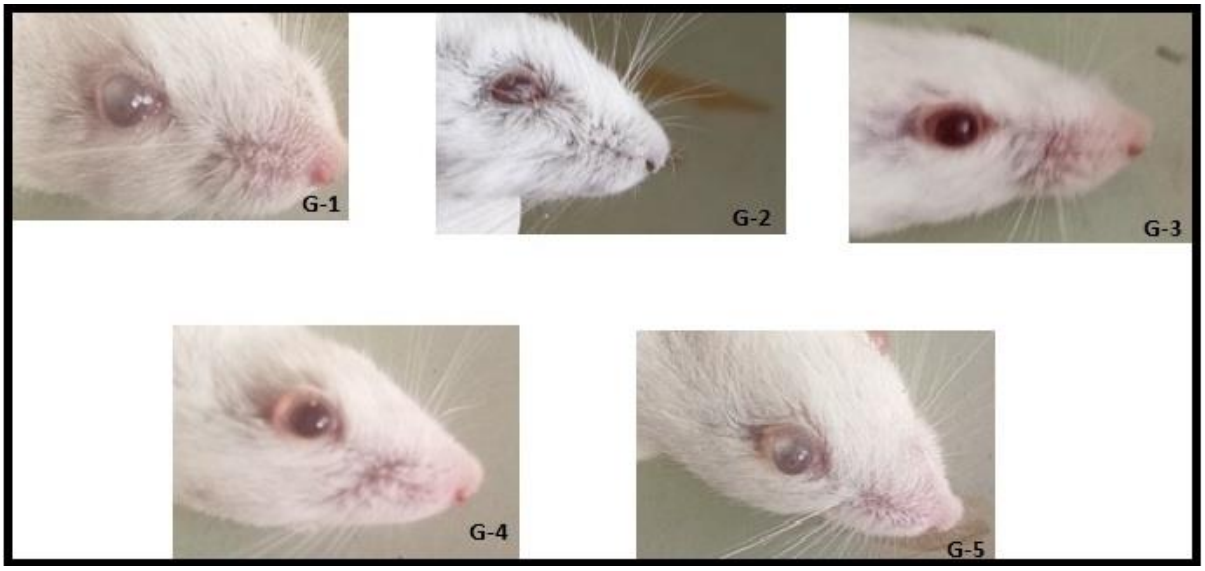


Fig 3: Clinical manifestations after 24 hours of LPS induction

Table 5 - Protein standard curve

Standard concentration (mg/ml)	OD @ 540nm		Mean
5	0.780	0.782	0.781
2.5	0.443	0.448	0.446
1.25	0.254	0.256	0.255
0.62	0.130	0.132	0.131
0.31	0.058	0.059	0.059
Blank	0.063	0.065	0.064

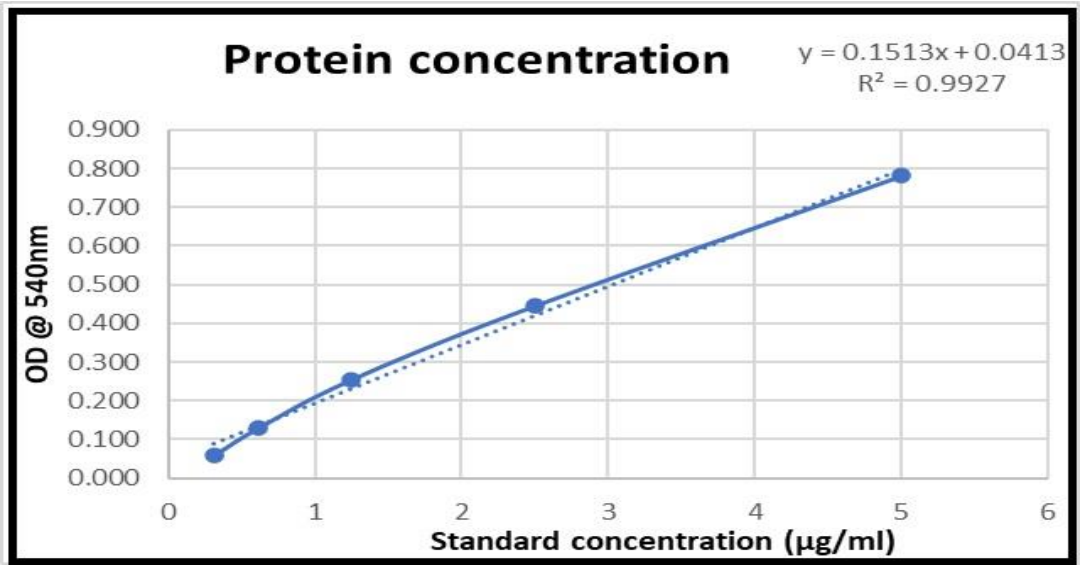


Fig 4: A Bovine Serum albumin Protein – Standard curve

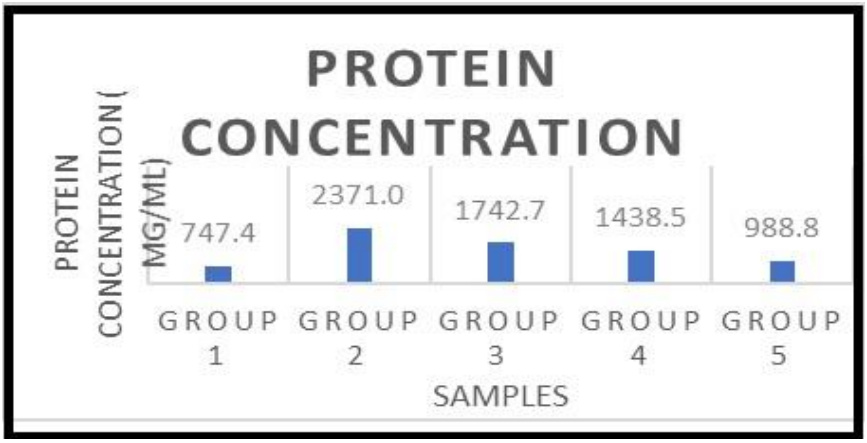


Fig 5: The effect of methanolic leaf and stem extract of Leucas Biflora (Vahl) R. Br. on protein concentration in aqueous humor in mice with LPS induced uveitis. All values are expressed as the mean ± SD. n=5**.** In this figure- 1 Group 1 indicates the Normal Saline, Group 2 LPS+ saline, Group 3 LPS+ Leaf extract, Group 4 – LPS+ STEM extract, Group 5 LPS+ Dexamethasone

Table: 6 Dose response activity of Leucas biflora (Vahl) R. Br. in LPS induced TNF –α estimation

Dose	TNF-α (pg/ml)	
	Levels(pg/ml)	% Inhibition
Group 1	850	-
Group 2	9572	-
Group 3	5243	45.23
Group 4	4879	49.03
Group 5	1674	82.51

%inhibition = (Normal activity-Inhibited activity) X100
(Normal activity)

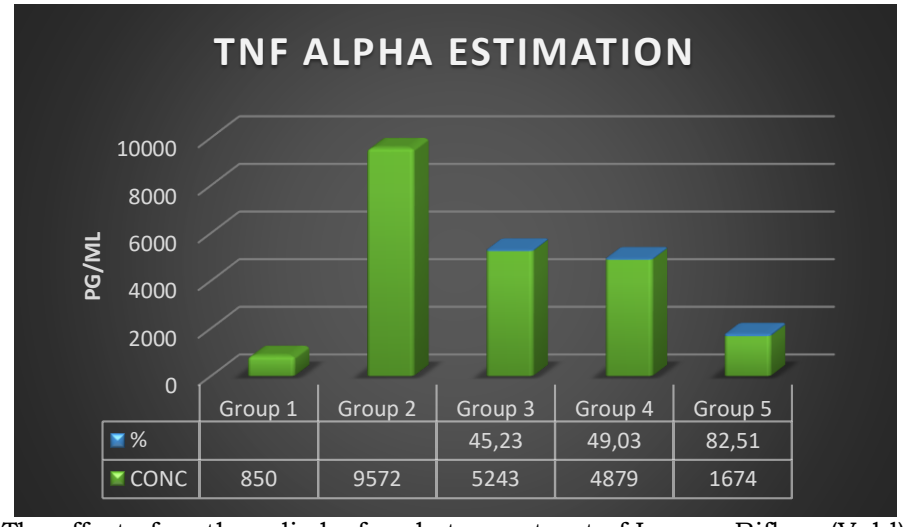


Fig 6: The effect of methanolic leaf and stem extract of Leucas Biflora (Vahl) R. Br. on TNF-α in aqueous humour in mice with LPS induced uveitis. All values are expressed as the mean ± SD. n=5

Conclusion

In the phytochemical analysis it concludes that methanol and aqueous are the best solvents for the extractions of phytochemicals. Preliminary phytochemical screening revealed the presence of Alkaloids, carbohydrates, saponins, phenols, tannins and proteins in Leaf and Stem extracts of Leucas biflora (Vahl) R. Br. GC-MS results showed that Leucas biflora (Vahl) R.Br. contain a number of chemical ingredients, which may be responsible for the pharmacological activity. Thus, this study may give information on nature of active principles present in medicinal plants. These identified phytoconstituents presumed to be responsible for selecting the traditional activity of this plant Leucas biflora (Vahl) R. Br. The isolated compounds from the methanol extract of plant Leucas biflora (Vahl) R. Br. may prove the medicinal importance in future.

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