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Research Article

ISOLATION OF FISETIN 3-O-GLUCOSIDE FROM *VITEX NEGUNDO* AND ITS HYPOTONICITY INDUCED MEMBRANE STABILIZATION STUDIES

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ABSTRACT

The attractive violet flowers of *Vitex negundo*.-Linn of Verbenaceae family are very popular for its pharmacological properties. Literature survey reveals that *Vitex negundo* was a frequently used traditional plant for its high medicinal properties. The plant can be further explored for the study of traditionally claimed unexplored activities, as well as isolation and identification of active constituents may lead to new findings. In the present study, the flowers of *Vitex negundo* were collected, dried and extracted with various solvents according to solubility index, and finally five extracts were studied. Finally, the isolated compound was identified as fisetin 3-o-glucoside. It was confirmed by thin layer chromatography (TLC), UV and NMR studies and the isolated compound were used to undergo hypotonicity induced membrane stabilization studies.

Keywords: *Vitex negundo*, Verbenaceae, TLC, UV, NMR.

1. INTRODUCTION

The attractive violet flowers of *Vitex negundo*.-Linn of Verbenaceae family are very popular for its pharmacological properties. Alcoholic extract of the seeds of *V. negundo* has been used to prevent liver damage [1]. Decoction of the leaves of *V. negundo* was found to prevent the development of swellings of joints in experimental arthritis in adult albino rats caused by formaldehyde injection. Anti-inflammatory activity has also been studied in *V. negundo* [2]. The flavonoids like fisetin is rare flavonoids found in the Verbenaceae family [3]. The Aerial parts of *Vitex negundo* was found to contain both Kampferol-3-O-rutinoside and 5-hydroxy-3,6,7,3',4'-pentamethoxy flavones [4]. In the absence of any phytochemical work with reference to flavonoids the flowers of *V. negundo* have been chosen for investigation and the results are presented hereunder.

2. MATERIAL AND METHODS

2.1. Plant material

The plant material used in this study was the whole flower of Vitex negundo collected from agricultural area of Nagapattinam district, Tamilnadu, India during January 2019 and was authenticated by the Taxonomist Dr. S. Dharmarajan, Botany Department, Thiru. Vi. Ka. Government Arts College, Thiruvarur, Tamilnadu.

2.2. Extraction and Fractionation

The Whole flowers of *Vitex negundo* were initially rinsed with distilled water and dried on paper towel in laboratory at (34±1°C) for 72 hours and choked in alcohol for a week. It was then extracted with benzene, diethyl ether, ethyl acetate, chloroform and ethanol in a soxhlet extractor. The solvent was completely removed by distillation and dried in vacuum desiccators. The standard extracts obtained were then stored in a refrigerator at 4°C for further use.

2.3. Isolation of Compound

Ethyl acetate fraction was evaluated by UV, thin layer chromatography (TLC). Fraction was eluted with petroleum ether, ethyl acetate (30:70, v/v) and further purified by repeated CC on silica gel using dichloromethane and methanol gradient elution to yield compound (40mg). Finally, the compound was identified by using NMR spectrometry [5].

2.4. Hypotonicity induced membrane stabilization studies

Inflammatory reaction is basic defensive response to a variety of stimuli which may be biological, chemical or physical [7]. The term inflammation originates from lat; 'inflammare' meaning 'to burn'. The clinical sign that

inflammation evoke are heat, redness, swelling and loss of function. Inflammation may be broadly classified under two categories *viz*. acute and chronic inflammation. The acute inflammation is the response of tissues to severe but transient stimuli. The chronic inflammation occurs when a stimulus is persistent [8]. Lysosomal enzymes play an important role in the development of acute and chronic inflammation [9]. Increased enzyme activity has been reported in certain types of experimental inflammation [10]. Anti-inflammatory agent is a drug that inhibits any of inflammation of an experimentally induced nature or as a part of clinic syndrome [11]. Aspirin and Sodium Salicylate have been widely used as remedial drug for inflammation. The hormonal and metabolic side effects of the steroidal drugs have led to the development of non-steroidal anti-inflammatory drugs (NSAIDS) [12].

The mechanism of action of non-steroidal anti-inflammatory drugs lies in their ability to either inhibit the synthesis or back the activity of prostaglandins which mediate the inflammatory response [13]. The inhibitory effects of these drugs on lysosomal enzymes are responsible for their mode of action [14]. Anti-inflammatory agents like phenyl butazone and indomythacin either inhibit the activities of released lysosomal enzymes or stabilize membrane [15].

It has been reported that the structure of red blood corpuscles (RBC) is similar to lysosomal membrane components. Since lysosomal membrane resembles sheep RBC membrane, its stabilization effects have been studied by SRBC. When the RBC is subjected to hypotonic stress, the release of hemoglobin from RBC is prevented by anti-inflammatory agents because of membrane stabilization. So the stabilization of SRBC membrane by dugs against hypotonicity-induced hemolytic serves as useful *in vitro* methods for assessing the anti-inflammatory activity of various compounds [16].

Flavonoids like quercetin, rutin, hyperoside, naringenin and naringin have been reported to exert in vitro stabilizing action on the HRBC membrane against hypotonicity-induced haemolysis [17]. The leaf extract of *Vitex negundo* has been reported to show anti-inflammatory activity [18]. Anti-microbial activity of methanolic extract of the roots of *V. negundo* containing alkaloids, saponin and flavonoids has been reported [19]. *V. negundo* plants reportedly contain vitexicarpin as active principle; which has dlavonoid analogous structure with cytotoxic effect [20]. Apart from well documented antioxidant role, flavonoids behave as pro-oxidants [21]. In the following pages the procedure that had been adopted for stabilizing the HRBC membranes by *Vitex negundo* EtOAc soluble are furnished.

3. RESULTS AND DISCUSSION

3.1. UV spectrum analysis

The UV spectrum of the glycoside exhibited major peaks at 362 (Band -I) and 248 nm (Band II) is indicative of a flavonol skeleton. Decomposition was absorbed on the addition of NaOMe with aglycon. Since the flavonols which have free OH - groups at the 3, 3^1 and 4^1 position are unstable in NaOMe and the absorption peaks in NaOMe spectrum degenerate in a few minutes, it was inferred that there was free OH group at C-3, C-3^1, and C-4^1 in the compound. A shift of +58 nm on the addition of $AlCl_3$ - HCl showed the presence of a free 5-OH in the ring.

Comparing the $AlCl_3$ spectra +30 nm shifts was absorbed in the case of $AlCl_3$ without acid, which also revealed a B - ring o-dihydroxyl group. The presence of free OH at C-7 was ascertained by shift of +20 nm (Band -II) on the addition of NaOAc the catechol type of di-hydroxyl group in B-ring was further evidenced by the bathochromic shift 16nm on the addition of H_3BO_3.

3.2. Identification of glycoside

The yellow aglycone obtained above on crystallization from MeOH afforded yellow needles. The filtrate from the aqueous hydrolysate was neutralized with Ba_2CO_3 and filtered. The concentrated filtrated on paper c gave R_f values corresponding to that of glucose the running properties of the glycoside on pc pointed to the presence of glucose. Thus from these observations in table 1 it could be concluded that the glycoside must be glucose.

3.3. NMR spectrum analysis

In the ^{1}H NMR spectrum of the glycoside recorded with a (100 MHz, DMSO-d_6 and TMS). The spectrum shows a signal at 10.24 ppm corresponding to a -OH at C-7. The 5-OH proton resonates as a distinct singlet at 12.62 ppm. The sharp singlet at 10.779 ppm can be traced to the OH at C-7 [6]. The C-5^1proton appears as a doublet at 6.916 ppm (J-8Hz). The signals at 6.213 ppm (d, J=2Hz) and 6.417 ppm (d, J-1.8 Hz) can be traced to the protons at C-6 and C-8. The C-6^1 proton resonate s at 7.638 ppm (d, J=2Hz). The C-2^1 proton shows up at 8.049 ppm. The signal at 4.60 ppm conform the presence of the H-1 of the glucose.

Supporting evidence for the structure of the aglycone is also provided by the analysis of ^{13}C NMR data (100 MHz, DMSO-d_6 and TMS). The ^{13}C NMR spectral data for fisetin as collected from the literature are also listed for easy comparison. The compound isolated from diethyl ether fraction was processed NMR study gave following table II. On this basis the isolated compound was conformed as fisetin 3-o-glucoside.

Table 1: R_f (X 100) values of sugar from the glycoside of V. negundo (Whatmann No: 1/ Ascending 30± 2°C)

Sugar	Developing solvents			
	F	g	h	i
Sugar from glycoside	77	9	39	90
Glucose (Authentic)	77	9	39	90

Table 2: ^{13}C- NMR spectral data of the constituents of the flowers of *Vitex negundo* and assignment of signals to various carbons

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C -8	C-9	C - 10
Aglycon isloated from Et_2O fraction	147.5	135.8	175.6	160.8	98.3	163.9	93.4	156.3	103
Authentic	147.6	135.6	175.7	160.7	98.2	163.9	93.4	156.2	103
Compound	$C1^1$	$C-2^1$	$C-3^1$	$C-4^1$	$C-5^1$	$C-6^1$			
Aglycon isloated from Et_2O fraction	122.1	115.4	145.2	146.7	115.7	120.1			
Authentic	122	115.3	145	146.9	115.6	120			

3.4. Significance for membrane stabilization study of *V. negundo*

The hypotonicity induced hemolytic property of the EtOAc soluble has been investigated for their screening anti inflammatory studies. For that a fresh blood was collected and mixed with equal volume of sterilized alsever solution (2% dextrose, 8.87% Sodium citrate, 0.05% citric acid and 0.42% NaCl) [22]. It was used within 5h. Hyposaline(0.25%, 2ml), phosphate buffer (0.15u, pH =7.4, 1ml)and SRBC (1%, 0.5ml) were taken in seven tubes. Solutions of different concentrations of drug were added to six of the tubes. The seventh tube served as the control in which instead of the drug, isosaline (0.85%, 1ml) was added. The contents in all the seven tubes were incubated at 37°C for 30 min and then centrifuged. The intensity of colour of the supernatant which was due to hemoglobin was measured at 560nm. The results are depicted in table 3 and the fig. 1.

Table 3: Effect of EtOAc fraction of *Vitex negundo* HRBC membrane stabilization studies

S. No	Conc. of. Drug in µ gram	Transmission nm
1	10	65
2	25	61
3	50	65
4	75	68
5	100	65
6	200	72

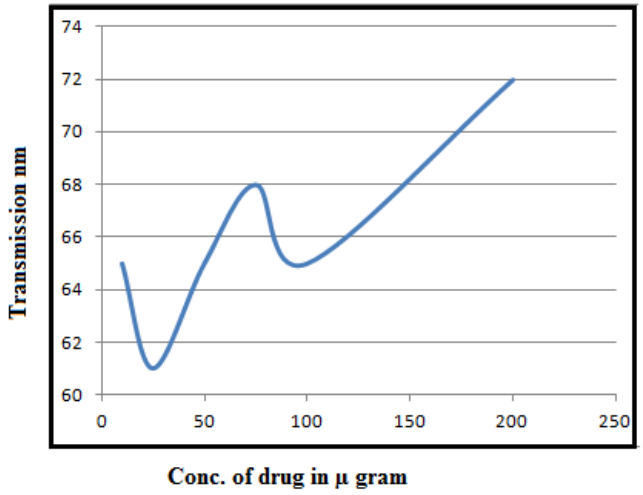

Fig. 1: HRBC Membrane Stabilization Studies

4. CONCLUSION

The isolated compounds were identified as Fisetin3-o-glucoside by chromatography, UV and NMR studies. The hypotonicity induced hemolytic property of the EtOAc soluble has been investigated for their screening anti inflammatory studies. The percentage protection of SRBC Membrane initially decreases and then rises to get a maximum at 75µ gram. Further increase in concentrations makes the curve to declaim to a minimum. The profile once again rises with increase in concentration to reach a maximum at 200µ gram. Beyond this, the curve becomes parallel to x-axis (ie) the equilibrium is reached, Therefore the anti inflammatory activity may be due to the Fisetin3-o-

glucoside present in the extract of *V. negundo* and it can be used as a potent antiinflammatory agent. In future extensive investigation and development work is needed to exploit their therapeutic utility against diseases and feasibility of being healthy supplements.

Conflict of Interest

None declared

5. REFERENCES

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