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Research Article

PHYTOCHEMICAL INVESTIGATION AND HYPOTONICITY INDUCED MEMBRANE STABILIZATION STUDIES OF *PROSOPIS CHILENSIS*

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ABSTRACT

The first and foremost step in *in vitro* drug designing was the screening of phytochemical constituents of the traditional plants. Literature survey on traditional plant indicated that, *Prosopis chilensis* was a frequently used 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 *Prosopis chilensis* were collected, dried and extracted with various solvents according to solubility index, and finally five extracts were studied. The isolated compounds were identified by chromatography, UV and NMR studies. In the present study, phytochemical investigation and hypotonicity induced membrane stabilization studies of *Prosopis chilensis* was carried out.

Keywords: *In vitro*, *Prosopis chilensis*, chromatography, Hypotonicity, UV, NMR.

1. INTRODUCTION

Most interest in the flavonoids stemmed from their ability to inhibit tumor initiation and growth. This was especially true of quercetin and naringenin, but also seen with hesperidins and the isoflavone, genistein. Quercetin is important and serves as the backbone for other flavonoids, and is the most active of the flavonoids [1]. Many medicinal plants have significant quercetin content. Studies on quercetin in experimental models indicate significant influence against diabetic complication, viral infection, inflammatory and allergic condition and cancer. The leaves of *Trifolium repens* [2] have been reported to contain quercetin 3-0-glucoside (isoquercitrin). The flowers of *Delonix elata* have been reported [3] to contain isoquercitrin also. There is a recent report on the isolation of quercetin 3-0-glucoside from the flowers of *Acacia leucophloea*. In the present study phytochemical investigation and hypotonicity induced membrane stabilization studies of *Prosopis chilensis* was carried out.

2. MATERIAL AND METHODS

2.1. Plant material

The plant material used in this study was the whole flower of *Prosopis chilensis* collected from coastal area of Nagapattinam district, Tamilnadu, India during Aug 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 *Prosopis chilensis* were initially rinsed with distilled water and dried on paper towel in laboratory at (30±1°C) for 48 h 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

The extracted compound was evaluated by UV, 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 (40 mg). 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 [6, 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 indomeythacin 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 hemolysis 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 *Gassia alata* has been reported to show anti-inflammatory activity [18].Anti-inflammatory and anti-histaminic activities of *Datura stramoniun* contaning kaempferol and quercetin has been reported [19]. The chloroform extract of *Myristrica fragrans* (nutmeg) showed anti-inflammatory, analgesic and antithrombotic effects in rodents [20]. Anti-inflammatory, analgesic and acute toxicity studies of *Sivanar Amrutham* have been investigated [21].

The EtOAc fraction concentration of *P. chilensis* was used for SRBC membrane stabilization. Fresh sheep 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 concentration 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.

3. RESULTS AND DISCUSSION

3.1. UV spectral analysis

The UV spectrum of the aglycone exhibited two major peaks at 370 nm (Band I) and 255 nm (Band II) to reveal 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 spectrom 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 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 Spectral analysis

In the ^{1}H NMR spectrum (400 MHz DMSO-d6 TMS) the 5-OH proton resonates as a distinct singlet at 12.500 ppm. The sharp singlet at 10.779 ppm can be traced to the OH at C-7[6]. The C-5^{1} proton appears as a doublet at 6.893 ppm (J-8Hz). The signals at 6.196 ppm (d, J=2Hz) and 6.415 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 7.538 ppm (d, J=2Hz). The C-2^{1} proton shows up at 8.043 ppm.

Supporting evidence for the structure of the aglycone is also provided by the analysis of ^{13}C NMR data (100 MHz, DMSO-d6, and TMS). The ^{13}C NMR spectral data for quercetin as collected from the literature are also listed for easy comparison. From the observation the glycoside from EtOAc fraction on acid hydrolysis yielded Quercetin and Galactose (1:1 molar quantities). The compound isolated from diethyl ether fraction was processed NMR study gave following table II. On this basis the isolated pigments could be characterized as Quercetin and its 7-O-galactoside.

Table 1: R_f (X 100) values of sugar from the glycoside of *P. chilensis*

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

(Whatmann No: 1 / Ascending 30+ 2°C)

Table 2: ^{13}C- NMR Spectral data of the constituents of the flowers of *P. Chilensis* 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	147.7	135.7	175.8	160.7	98.2	163.9	93.3	156.1	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	115.5	145	146.8	115.6	120			
Authentic	122	115.3	145	146.9	115.6	120			

3.4. Significance for membrane stabilization study

The hypotonicity induced haemilytic property of the EtOAc soluble has been investigated for their screening anti inflammatory studies. The Flavonoids isolated from *P.Chilensis* is encouraging as it shows biphasic property. While concentration increased the capacity also increases to reach a maximum at 50 gram. Beyond this concentration, the stabilization declines. Such kind of biphasic property is quite prevalent in literature. The results are depicted in table 3 the fig. 1

Table 3: Effect of EtOAc fraction of *P. chilensis* SRBC membrane stabilization studies

S.No	Conc. of. Drug in µ gram	Transmission nm
1	10	65
2	25	63
3	50	67
4	75	64
5	100	63
6	200	63

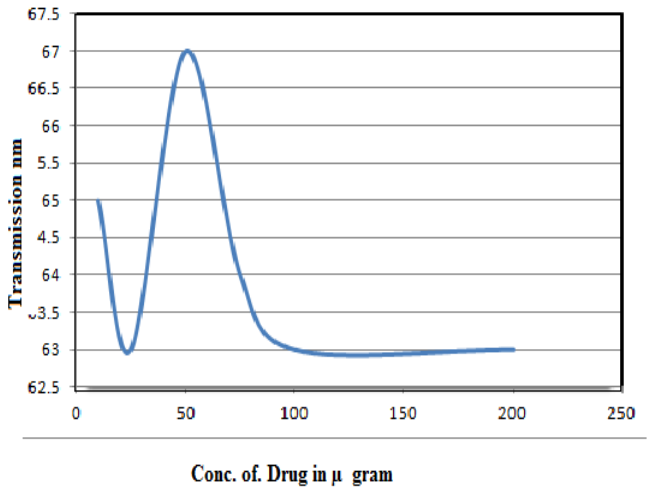

Fig. 1: HRBC Membrane Stabilization Studies

4. CONCLUSION

The isolated compounds were identified as Quercetin and its 7-O-galactoside by chromatography, UV and NMR studies. The hypotonicity induced haemilytic property of the EtOAc soluble have been investigated for their screening of anti inflammatory studies and the

flavonoids isolated from *P. Chilensis* is encouraging as it shows biphasic property. While concentration increased, the capacity also increases to reach a maximum at 50µg. Beyond this concentration, the membrane stabilization declines. Therefore the anti inflammatory activity may be due to the flavonoids present in the extract of *P. Chilensis* and it can be used as a potent antiinflammatory agent.

Conflict of interest

The authors wish to declare that there are no conflict of interest associated with this research work and similarly and there has been no financial support involved in this work that could affect its outcome.

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