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Pharmacological Studies on *Prosopis juliflora*

KEYWORDS

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INTRODUCTION

Prosopis juliflora (Spanish: ***bayahonda blanca***) is a shrub or small tree in the Fabaceae family, a kind of mesquite. It is native to Mexico, South America and the Caribbean. It has become established as an invasive weed in Africa, Asia, Australia and elsewhere.***Prosopis juliflora*** (SW.) Dc. (Syn.***) Acacia cumanensis, Algarobia juliflora*** (swartz) Benth is known as velikathan in tamil. It mesquite tree is one of the major invasive alien species of India and it has been used to treat eye problems, open wounds, dermatological ailments and digestive problems by the native tribes of many countries [(1)]. It has soothing, astringent, antiseptic, antibacterial and antifungal properties[2]. Its roots are able to grow to a great depth in search of water: in 1960, they were discovered at a depth of 53 meters (175 feet) at an open-pit mine near Tucson, Arizona, putting them among the deepest known roots.

Prosopis juliflora, is found in arid and semi-arid regions of India. It has been used as a folk remedy for catarrh, cold, diarrhoea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat and in healing of wounds [(3)]. In Tamil Nadu, in Tamil language it is known as ***cheemai karuvel*** (சீமகைகரூவலேை), a quite literal equivalent of ***vilayati babul***. A vernacular Tamil name is ***velikathan***(வலேிகாத்தான்), from ***veli*** (வலேி) "fence" + ***kathan*** (காத்தான்) "protector", for its use to make spiny barriers. In Andhra Pradesh, in the Telugu language it is known as ***mulla tumma*** (ముల్ల తుమ్మ) or ***sarkar tumma***. The flowers are in 5–10 cms. long green-yellow cylindrical spikes, which occur in clusters of 2 to 5 at the ends of branches.

The extracts of ***P. Juliflora*** seeds and leaves were well studied for several ***in vitro*** pharmacological effects such as antibacterial, antifungal and anti-inflammatory properties[(4-7)]. The flavonoid, patulitrin have already been isolated from its flowers and fruits[(8)]. With a view to locating additional flavonoids, the flowers of ***P. juliflora*** have been investigated and the results are presented hereunder.

The fresh flowers of ***P. juliflora*** have been found to contain cryptostrobin and heperidin (hesperitin 7-O-glucoside).

The UV spectrum of the aglycone from Et_2O fraction exhibited one major absorption peak and two shoulders 240sh, 295 and 360sh indicating a flavanonoe skeleton. The absence of decreasing intensity, on the addition of NaOMe indicates the absence of o-dihydroxyl grouping in A – ring of the compound. A shift from 295 nm to 330 nm , on the addition of NaOAc showed the presence of 5,7-dihydroxy flavanone. The absence of catechol type of B – ring as well as the A – ring can be inferred from the fact that its NaOAc spectrum was unaffected on the addition of H_3BO_3. The $AlCl_3$ spectra of the aglycone consists of a major absorption peak and two shoulders which indicated the presence of a free 5 –OH group in the aglycone. It was also confirmed by a bathochromic shift of 26 nm (band II), On the addition of $AlCl_3$ – HCl. The absence of ortho-dihydroxyl grouping was confirmed from the unaffected spectrum of $AlCl_3$ from that of $AlCl_3$ – HCl.

In the ^1H – NMR spectrum (400 MHz, DMSO – d6, TMS) of the aglycone, the CH_3 protons of C – 8 resonate at δ 2.0 ppm as a distinct singlet. The trans and cis protons of H – 3 appears at δ2.7 and 2.6 ppm respectively. The H – 2 appears at δ 5.2 ppm as a triplet which is characteristic feature of cryptostrobin. The protons at 2′ and 6′ due to symmetry appear at doublet at δ 7.2 ppm. The protons at 3′,5′ and 4′ resonate at δ 7.43 ppm as a multiplet.

Additional evidence for the structure of the aglycone was provided by the ^{13}C – NMR (100 MHz, DMSO – d_6, TMS) spectral data. The UV spectrum of the glycoside showed an absorption maximum at 283 nm (band II) and a low intensity band at 326 nm (band I) showing a flavanone skeleton. The shift of +23 nm (band II) on the addition of $AlCl_3$ – HCl reveals the presence of 5 –OH group. Since the B – ring has no conjugation with the major chromophore, the presence of 3′,4′ – dihydroxyl groups could not be detected by $AlCl_3$ and NaOAc – H_3BO_3 spectra. A bathochromic shift of + 35 nm observed in the NaOAc spectrum (band II) of the aglycone obtained after hydrolysis of the glycoside suggests that the site of glycosylation could be at C - 7.

In the ^1H – NMR spectrum of the glycoside (400 MHz, DMSO – d6, TMS) , the proton at C – 2 appears as a quartet at δ 5.5 ppm (J = 5.0, 11 Hz) as a result of coupling of C – 2 proton with the two C – 3 protons [(11)]. The C – 3 protons couple with each other in addition to their spin – spin interaction with the C – 2 proton, thus giving rise to two overlapping quartets at 2.7 ppm as a weak signal [(12)]. The signal at δ 6.92 ppm (d, J = 3 Hz) corresponds to the protons at C – 2′ and C – 6′. The proton at C – 5′ appears at δ 6.14 ppm , whereas those of C – 6 and C – 8 resonate respectively at δ 5.4 ppm and 6.12 ppm. The - OH protons at C – 5 and C – 3′ appear as a distinct singlets at δ 12.08 and 9.10 ppm respectively. The intense signal at δ 3.77 ppm corresponds to the - OCH_3 protons at C – 4′. H – 1″ of glucose resonate at δ 5.18 pm while that of H – 1‴ of rhamnose at δ 4.8 ppm (d, J = 2.5 Hz). The signal appears at δ 0.8 ppm (J = 6 Hz) correspond to the C – 6‴ protons (methyl protons of rhamnose) and is clearly reminiscent of the presence of rutinoside.

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Additional evidence for the structure of the glycoside was provided by the ^{13}C – NMR (100 MHz, DMSO – d_6, TMS) spectral data. Due to glycosylation at C – 7, the signals of C- 6 and C -8 appear at δ 96.4 ppm and 95.5 ppm respectively. The appearance of the carbonyl carbon (C-4) at δ 196.9 ppm (much downfield), is due to the hydrogen bonding with 5 –OH in the absence of C – 3 –OH in addition to the missing of olefinic double bond between C -2 and C – 3. The rutinosyl carbons except C – 1" ,C – 1"' and C – 6"' carbons appear at δ 99.5 , 100.6 and 17.8 respectively. The O – methyl carbon at C – 4' resonates at 55.7ppm.

The glycoside could thus be characterized as hesperitin – 7 – O – rutinoside) in comparison with an authentic sample of hesperidin isolated from ***Buddleja asiatica*** [9].

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DPPH radical scavenging activity (DPPH assay):

The antioxidant activity of the plant extract was estimated using a slight modification of the DPPH radical scavenging protocol given by (Chang ***et al***)[14]. DPPH free radical scavenging is one of the generally accepted mechanisms against lipid oxidation. Difference between DPPH free radical binding method and the other method is the short run time allowing rapid determination of the radical scavenging. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. The antioxidant activity of plant extracts were calculated according to the percentage inhibition in DPPH assay. It was optimized to be able to use microtiter plates, a multichannel pipet, and an ELISA reader, which makes it possible to analyze large numbers of samples in a run. With this method it was possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. As a result of the colour changing from purple to yellow the absorbance is decreased when the DPPH radical is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH-H molecule[15] . For a typical reaction, 2mL of 100 μM DPPH solution in ethanol was mixed with 2mL of 100 μg/mL of plant extract. The effective test concentrations of DPPH and the extract were therefore 50 μM and 50μg/mL, respectively. The reaction mixture was shaken vigorously and allowed to stand at room temperature and incubated in the dark for 15min and thereafter the optical density was recorded at 517nm against the blank. For the control, 2mL of DPPH solution in ethanol was mixed with 2mL of ethanol, and the optical density of the solution was recorded after 15min. The assay was carried out in triplicate. Then the absorbance was measured at 517 nm in an ELISA reader. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity[16] . The decrease in optical density of DPPH on the addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical:

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radical scavenging (%) = $\frac{\text{(A control – A sample)}}{\text{A control}} \times 100$ (10)

The polyphenolic compounds and their percentage of inhibition of oxidation are listed in table.

TABLE
ANTIOXIDANT ACTIVITY OF THE ISOLATED FLAVONOID GLYCOSIDES

S.No	Grouping	Absorption λ at 517 nm	% of inhibition of oxidation
1	Ascorbic acid	0.04	98%
2	CB	2	--
3	Hesperitin	0.07	96.50%
4	Cryptostrobin	0.24	88%

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