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Full Length Research Paper

Antimicrobial activity of sennosides from *Cassia pumila* Lamk.

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Ayurveda recommends the application of different parts of *Cassia pumila* Lamk. plant species in the forms of paste, powder, juice, and decoction for treatment of various infections. The chloroform active sub-fraction and five sennosides were investigated for their antimicrobial activity by using microdilution method. The reported results indicated that sennoside D demonstrated maximum activity against *Streptococcus pneumoniae* (140 µg/ml) and sennoside B to *Rhizoctonia bataticola* (170 µg/ml). The present data show that *C. pumila* extract has activity against different types of infectious and toxic microorganisms. The active phytoconstituents could potentially be developed for use in the treatment and/or prevention of microbial diseases.

Key words: Caesalpiniaceae, *Cassia pumila*, sennosides, antimicrobial activity, microdilution method.

INTRODUCTION

Despite of tremendous progress in human medicines, infectious diseases caused by the bacteria, fungi, viruses and parasites are still a major threat to the public health. These impact is particularly large in the developing countries due to relative unavailability of medicines, and emergence of wide spread drug resistance (Zampini et al., 2009). *Cassia pumila* Lamk. is a terrestrial, dwarf perennial herb, commonly known as Sarmal, and is found throughout the India, tropical Asia and Australia. The pulp from its fruits called cassia pulp is well known as laxative and purgative, used in treatment of constipation (Kirtikar and Basu, 1955; Bhattacharjee, 1998). The roots of plant is useful in common cold, in case of running nose, smoke from the burning roots can be inhaled (Chopra and Verma, 1952; Jain, 1991). The plant leaves are useful in relieving irritation of the skin and alleviating swellings and pain. Their juice or paste serves as a useful dressing for ring worm and inflammation of the hands or feet caused by exposure to cold (Kirtikar and Basu, 1975; Perry, 1980). According to other reports, the *C. pumila* also possesses anti-inflammatory (Palanichamy and Nagarjan, 1990a; Narnath et al., 2009), antimicrobial (Khan et al., 2001; Abubacker et al., 2008), antitumor (Kumar et al., 2010; Gupta et al., 2000), antioxidant activity (Kaur and Arora, 2010; Bhalodia et al., 2011), analgesic (Palanichamy and Nagarjan, 1990b) and hyperglycemic activity (Palanichamy et al., 1988).

Sennosides and other phytochemicals have been extensively investigated by various workers viz., alkaloids (Viegas et al., 2004; Matsumoto et al., 2010), flavonoids (El-Sayed et al., 1992; Dhandapani and Kadarkarai, 2011), anthraquinones (Jiang et al., 2005; Yadav et al., 2010) and triterpenoids (Khan and Srivastav, 2009). The growing evidence suggests that this plant species has medicinal properties and its extract, purified compounds, could be possibly used as pharmacological and biological interventions in various diseases. In this work, we associated the isolation of pure sennosides and screening for their antibacterial and antifungal activities.

MATERIALS AND METHODS

Plant material

Cassia pumila Lamk. (Caesalpiniaceae) was collected (August, 2008) from Moti Dungari Hills, Jaipur and authenticated by

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Professor R. S. Mishra, and their voucher specimens were deposited in the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India (sheet no. 37779).

General experimental conditions

The melting points of purified compounds were determined on capillary Toshniwal melting point apparatus and are uncorrected. The spectral data were obtained on the following instruments: ir, Perkin-Elmer, 283; ms, Hewlett Packard HP 5930 A; uv, Perkin - Elmer, model - 200 ; nmr, JEOL PS 100 at 300 MHz; hplc, Waters model 501 and adsorbents for TLC (silica gel 60, 230 to 400 mesh for column chromatography and silica gel G used for TLC, Merck); TLC solvent systems – Methyl-ethyl ketone: AcOH: H_2O (300:1.5:150, v/v).

Extraction and characterization

Shade-dried powdered pods of *C. pumila* (10.0 kg) were defatted with chloroform (12.0 l) for 24 h, filtered and resultant residue was further re-extracted with glacial acetic acid (5.0 l) for 72 h, filtered and concentrated (yield-184.418 g). The crude AcOH extract 143.314 g was suspended in 300 ml of H_2O and then treated with Amberlite IR-120-H^+ resin to liberate the free sennosides. The bulk of sennosides is allowed to crystallize from acid solution and is filtered off. The filtrate is fractionated with methyl-ethyl ketone: H_2O (Fraction I, 19.234 g, 1.0 l), Methyl-ethyl ketone - AcOH (3:0.1, v/v; Fraction II, 12.418 g, 800 ml), PrOH: EtOAc (Fraction III, 21.334 g, 700 ml) which were used in subsequent work. The column chromatography of Fraction I with elution by methyl-ethyl ketone–H_2O, 4 fractions A to D; Fraction II with elution by methyl-ethyl ketone– AcOH, 4 fractions E to H; Fraction III with elution by PrOH – EtOAc, 2 fractions I and J were collected (Hietala and Penttila, 1966; Harborne, 1973).

Sennoside A (I) and sennoside B (II)

A portion of A to D fractions were pooled (10.523 g) and re-chromatographed on silica gel G and purified by preparative thin layer chromatography (TLC) with development by methyl-ethyl ketone – AcOH - H_2O (300:1.5:150, v/v); two spots appeared, followed by crystallization with acetone, detection on TLC with 5.0% anisaldehyde sulphuric acid spraying reagent (Sood et al., 2011), yielded I, sennoside A (291 mg), R_f ~ 0.94, yellow spot, mp 200 to 203°C, $C_{42}H_{38}O_{20,}$ $[\alpha]^{20}{}_D$ - 164°(60% acetone), sparingly soluble in MeOH, insoluble in water, benzene, rectangular yellow plates from dilute acetone, sennoside A is slowly isomerized to sennoside B in $NaHCO_3$ and II, sennoside B (338 mg), detection on TLC with 5.0% anisaldehyde sulphuric acid spraying reagent, yellow spot, R_f ~ 0.73, mp 180 to 184°C, $C_{42}H_{38}O_{20,}$ $[\alpha]^{20}{}_D$ - 100° (70% acetone) (Sagara et al., 1987), light yellow prism from dilute acetone, fine needles from water, both sennosides were positive to modified Borntager's test (Srikanth et al., 2011). The isolated compounds were subjected to various physical and spectral studies, and were identical to their standard samples as previously described.

Sennoside C (III)* and *sennoside D (IV)

Fractions E to H combined (8.268 g), re-chromatographed on silica gel G and purified by preparative TLC with development by n-propanol : EtOAc : H_2O : AcOH (3.0: 3.0: 2.0: 1.0, v/v), two spots appeared, III, sennoside C (558 mg), mp 190 to 194°C, $C_{42}H_{40}O_{19}$ (dextrorotatory), R_f ~ 0.61, crystallized with acetone, yellow color spot on TLC sprayed with 5.0% anisaldehyde sulphuric acid reagent and IV, sennoside D (447 mg), R_f ~ 0.46, light yellow color spot on TLC, $C_{42}H_{40}O_{19,}$ mp 196 to 198°C, optically inactive, yellow color needles, insoluble in benzene (Shah et al., 2000).

Rhein-8-O-glycoside (V)

Fractions I and J combined (4.467 g), re-chromatographed on silica gel G and purified by preparative TLC with development by 1-PrOH : EtOAc : H_2O (4.0: 4.0: 3.0, v/v), crystallized with acetone, V, rhein-8-O-glycoside (635 mg) orange powder; melting point 260 to 263°C. UV light absorption MeOH: 318, 377, 365, 430 sh IR : νcm^{-1}/ max KBr: 3630 (glycoside), 3420 (O–H), 1700 (C = O), 1600, 1610, 1560, 1510, 1450, 1400 (aromatic), 1385, 1310 ^{1}HNMR(300MHz, $CDCl_3$): 2.45 (H_1), 6.69 (H_2), 2.35 (H_3), 6.89 (H_4), 7.87 (H_5), 7.14 (H_6), 6.94 (H_7), 3.88 (H_8), 1.96 (H_9), 1.79 (H_{10}), 1.66 (H_{11}), 1.06 (H_{12}), 3.76 (H_{13}), 1.02 (H_{14}), 1.06 (H_{15}) ^{13}C NMR(300MHz, $CDCl_3$): 172.0 (C_1), 135.3 (C_2), 126.9 (C_3), 134.0 (C_4), 143.6 (C_5), 23.84 (C_6), 142.6 (C_7), 132.1 (C_8), 126.2 (C_9), 128.4 (C_{10}), 129.9 (C_{11}), 142.8 (C_{12}), 142.7 (C_{13}), 126.6 (C_{14}), 669.7 (C_{15}), 33.8 (C_{16}), 32.3 (C_{17}), 35.1 (C_{18}), 72.6 (C_{19}).

HPLC analysis

The HPLC determination of sennosides in *C. pumila* (aerial parts) was achieved on a Millipore Waters model 501, fitted with pump solvent delivery system, injector (model 6 UK) by using in µ Bondapak C_{18} column (30 cm × 3.9 mm; temperature 24 ± 2°C). The mobile phase used for the separation was MeOH: H_2O: AcOH: tetrahydrofuran (60-38-2-2; HPLC grade Merck) and flow rate was adjusted to 0.7 ml/min (eluent program: 40 min, 100% MeOH). The effluent was monitored by UV absorption at 254 nm with a detector adjusted at an attenuation of 0.5 AUFS (Lambda - max model 481 LC spectrophotometer, detector - Waters). Before use, the columns were washed with methanol. The fractions were pretreated, filtered, dissolved in 50 µl of the mobile phase and a 10 µl volume of the sample was injected (chart speed: 0.5 cm/min). The calibration graph was constructed by plotting the ratio of the peak area of determination (Verma et al., 1996).

Sources of microorganisms

Pure cultures of bacteria, *Escherichia coli* (ATCC - 5922), *Pseudomonas aeruginosa* (ATCC - 25928), *Salmonella typhi* (ATCC - 25922), *Staphylococcus aureus* (ATCC – 25923), *Bacillus subtilis* (ATCC - 10031), *Streptococcus pneumoniae* (ATCC - 10032), (obtained from S.M.S. Medical College, Jaipur) were grown on nutrient agar culture medium at 37°C for 24 h and fungi, *Aspergillus niger*, *Aspergillus flavus*, *Rhizoctonia bataticola* (from Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur), were grown on potato dextrose agar (PDA) medium at 27°C for 48 h and *Candida albicans* (obtained from Superior Diagnostic Center, Jaipur) was cultured on Sabouraud dextrose agar (SDA) medium at 30°C for 5 days (Chang and Cury, 1991).

Antimicrobial activity

The antifungal and antibacterial activity was evaluated by minimum inhibitory concentration (MIC) determined by microdilution method (Jones and Barry, 1987). The organisms to be tested were grown in nutrient broth (Difco Co.) at 37°C for bacteria and Sabouraud dextrose broth (Difco Co.) at 30°C for fungi, respectively. After 24 h, 1 ml of culture broth from culture was transferred to 10 ml of the same medium and further incubated for 6 h, and each culture was

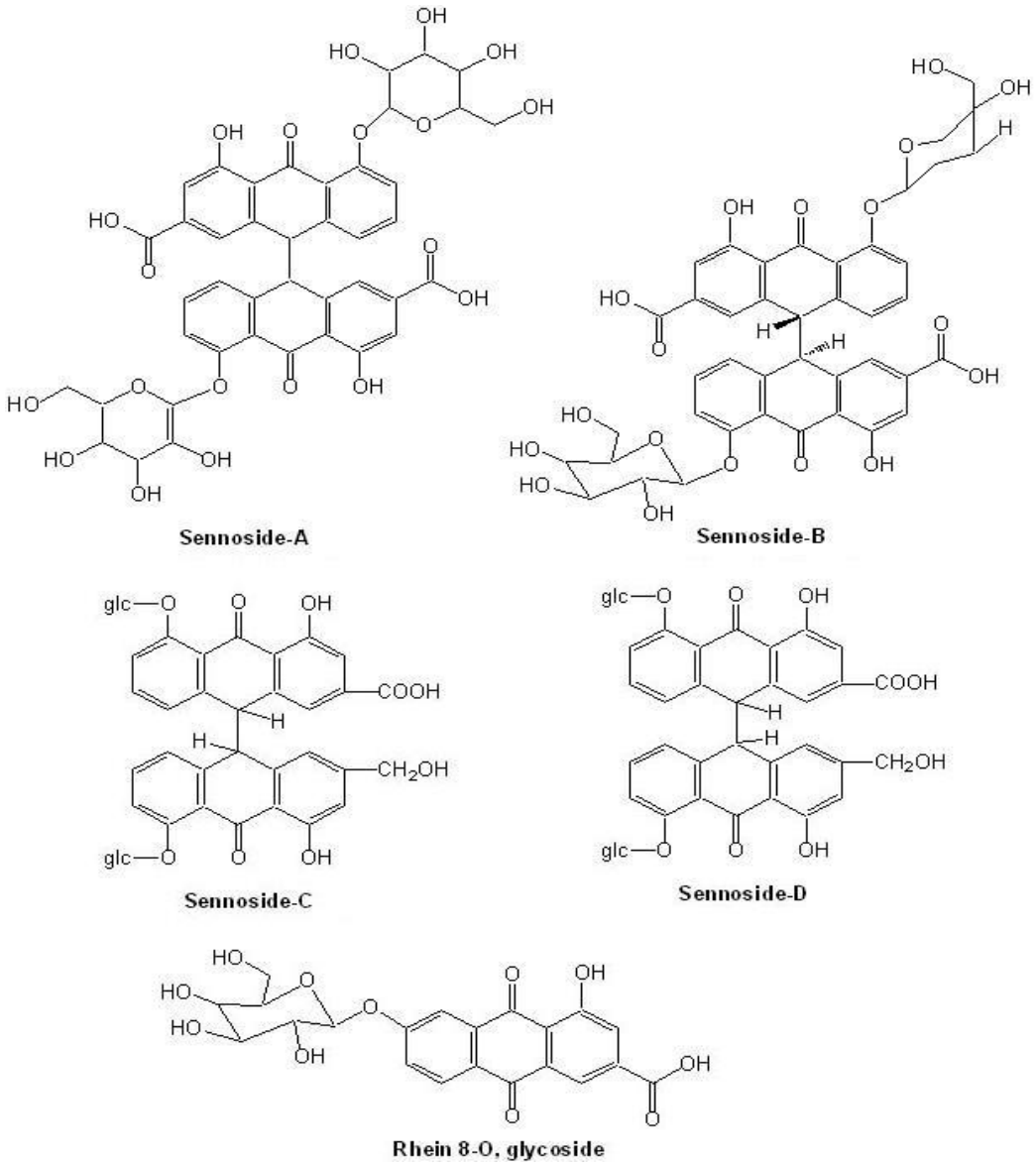

Figure 1. Structures of isolated sennosides from *Cassia pumila* Lamk.

adjusted with nutrient broth or Sabouraud dextrose broth to obtain 0.1 ml of cell culture and was inoculated in tubes with 0.9 ml of nutrient broth or Sabouraud dextrose broth supplemented with different concentrations of the crude extract, isolated and standard compounds which were dissolved in dimethyl sulphoxide. Culture with dimethyl sulphoxide (0.5%) was used as solvent control, and culture supplemented with tetracycline, gentamycin and nystatin was used as positive control, respectively. The MIC was defined as the lowest concentration able to inhibit and show microbe growth, and was determined by measure of cell growth OD after 48 h cultivation. All data are presented as mean values of triplicate of each microorganism.

RESULTS AND DISCUSSION

The pods of *C. pumila* were defatted with chloroform and resultant residue was further re-extracted with glacial acetic acid, filtered and concentrated (yield-184.418 g), and partitioned with methyl-ethyl ketone – H_2O (Fraction I), methyl-ethyl ketone - AcOH (Fraction II) and PrOH - EtOAc (Fraction III). The silica gel TLC of the fractions showed the presence of five indole sennosides (I to V) after spray of 5.0% anisaldehyde reagent (I, 291 mg; II, 338 mg; III, 558 mg; IV, 447 mg; V, 635 mg).

The antimicrobial activity of chloroform extract and isolated five compounds (Figure 1) were carried out by microdilutiom method against selected fungal and bacterial microorganisms, and compared with commercially available antibiotics (Table 1). Tetracycline, gentamycin and nystatin showed antimicrobial activity at all tested concentrations (10 to 30 μg/ml). The isolated

Table 1. MICs (µg/ml) of active sub-fraction, reference compounds and isolated sennosides against selected bacteria and fungi.

Crude extract or isolated compounds	MIC (µg/ml)										
	Ec	Pa	St	Sa	Bs	Sp	An	Af	Rb	Fm	Ca
Active sub-fraction											
Chloroform	>400	>350	450	540	>480	450	>380	>500	370	>360	550
Standard compounds											
Tetracycline	10.75	12.50	14.75	>18.00	19.75	17.50	-	-	-	-	-
Gentamycin	26.50	24.50	25.75	28.00	23.00	23.75	-	-	-	-	-
Nystatin	-	-	-	-	-	-	27.00	27.50	26.00	28.70	26.75
Isolated compounds											
Sennoside A	150	>280	340	>200	280	>330	>350	220	330	260	>390
Sennoside B	300	>360	260	>290	>380	>290	340	300	>170	290	>260
Sennoside C	>400	270	>380	>190	340	180	290	>230	340	>270	>180
Sennoside D	>240	320	240	330	>120	140	>200	200	260	>190	370
Rhein – 8 – *O* - glycoside	220	200	270	>350	>400	370	320	300	280	200	230

Used microorganisms: Ec = *Escherichia coli*; Pa = *Pseudomonas aeruginosa*; St = *Salmonella typhi*; Sa = *Staphylococcus aureus*; Bs = *Bacillus subtilis*; Sp = *Streptococcus pneumoniae*; An = *Aspergillus niger*; Af = *Aspergillus flavus*; Rb = *Rhizoctonia bataticola*; Fm = *Fusarium moniliforme*; Ca = *Candida albicans*.

compounds were tested at various doses from 100 to 400 µg/ml. The strong antibacterial activity was showed by sennoside D at 140 µg/ml against *S. pneumoniae* and sennoside B exhibited maximum antifungal activity against *R. bataticola* at the dose of 170 µg/ml. The moderate antibacterial and antifungal activity was exhibited by all the isolated compounds.

World Health Organization (WHO) encourages countries to examine traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for different diseases (Akinyemi et al., 2002; Uwumaronngie et al., 2007). The investigated results obtained from this study support the WHO recommendations as it provides scientific evidence that the sub-fractions of aerial parts of *C. pumila* have antimicrobial activity. The sennosides isolated from this species have demonstrated powerful effects against selected bacteria and fungi.

There are still many *C. pumila* sennosides and their derivatives, whose pharmacological activities have not yet been investigated. It is possible that they may contain beneficial pharmacological properties. Therefore, *in vivo* and *in vitro* investigations regarding their effects could provide insight into the benefits of *C. pumila* for future clinical management of many human diseases.

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