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ANTIMICROBIAL ACTIVITY OF FLAVONOIDS OF MEDICINALLY IMPORTANT PLANT *CASSIA ANGUSTIFOLIA IN VIVO* AND *IN VITRO*

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Isorhamnetin and kaempferol have been identified and isolated from different plant parts of *Cassia angustifolia* collected in summer and winter as well as from unorganised tissues raised and maintained on MS medium supplemented with 10 mg/l IAA, 5 mg/l BAP and 10 mg/l 2,4-D and screened for their antimicrobial activity against Gram positive, Gram negative and fungal pathogen.

Keywords : *Cassia angustifolia;* Isorhamnetin; Kaempferol; Tissue culture.

Introduction

Flavonoids are water soluble phenolic glycosides imparting colour to flowers and fruits of higher plants. Their contribution to physiological functions such as seed maturation and dormancy has already been esteblished[1]. Presence of flavonoids has been reported from many plant species like *Citrullus colocynthis, Citrullus depressus, Fagonia cretica, Lycium barbarum*[2], *Arachis hypogea*[3], *Passiflora palmer*[4], *Heliotropium* species[5]. *In vitro* studies have also shown their occurence in *Crotolaria juncea*[6], *Calendula officinalis, Crotolaria burhia* and *Papaver rhoeas*[7], *Peganum harmala*[8], *Tribulus alatus*[9] and *Dolichs lablab, Glycine max, Pisum sativum*[10].

The vital role of flavonoids in defence against microorganisms, due to antimicrobial activity has already been discussed by Skinner[11]. Nickell[12] reported the presence of antibiotic substances in 147 plant families out of 174 families surveyed. Antimicrobial substance has been isolated from different parts of plant species and tested for their antimicrobial activity against Gram positive and Gram negative bacteria as well as fungal pathogen[2,13]. Their activity has also been reported from tissue culture[8,9,14,15].

Present study deals with the isolation, identification and quantitative estimation of flavonoids for observing antimicrobial activity from medicinally important plant *Cassia angustifolia* commonly called as senna of family Caesalpiniaceae *in vivo* and *in vitro*.

Material and Methods

Fresh plants of *Cassia angustifolia* were collected in summers and winters from fields of Agriculture Research Station, Beechwal, Rajasthan Agriculture University, Bikaner. Plant parts were separated, dried and analysed for estimation of flavonoids. Unorganised cultures of *C. angustifolia* were established from germinating colyledones on MS medium supplemented with 10 mg/l IAA, 5 mg/l BAP and 10 mg/l 2,4-D for six months by frequent subculturing at interval of six to eight weeks. Cultures were maintained at 26±1° C temperature, 55% relative humidity and diffused light conditions (300 lux). Growth indices were calculated at time interval of 2, 4, 6, 8 and 10 weeks and harvested at age of maximum growth index for present studies.

Test organisms *Escherichia coli, Staphylococcus aureus* and *Candida albicans* were procured from Department of Microbiology, Medical College, Bikaner. The growth medium used for *S. aureus* and *E. coli* was nutrient broth and for *C. albicans* Sabourd's liquid medium. The inoculum was prepared by adjusting the concentration of micro organisms at 40% transmittance for bacteria and 65% for *C. albicans*[16] using spectronic 20 colorimeter (Bausch and Lomb) set at 630 nm.

Isolation of flavonoids : Dried, weighed and powdered plant parts as well as 8 weeks old tissue of *C. angustifolia* were soxhlet extracted with 80% hot ethanol on a water bath for 24 hours and filtered[17]. The filterate was concentrated and then re-extracted with petroleum ether, ethyl ether and ethyl acetate in succession. The ethyl ether fraction was analyzed for free flavonoids while the ethyl acetate fraction was hydrolyzed with 70% H_2SO_4 for 2 hours. The mixture was filtered, the filtrate extracted with ethyl acetate, neutralized with 5% NaOH, then dried in vacuo and analyzed for bound favonoids.

Identification of flavonoids : The isolates were identified by TLC (silica gel G coated plates) along with standard reference compounds, apigenin, isorhamnetin, kaempferol, luteolin and quercetin. The plates developed in n-butanol, acetic acid and water (4:1:5, upper layer) were sprayed with 5% ethanolic $FeCl_3$ solution. Each of isolates were purified by preperative TLC insimilar solvent system. Isolates were eluted with ethyle acetate and crystallized from $CHCl_3$. Quantitative estimation of the identified flavonoids was carried out colorimetrically following the method of Kariyone *et al.*[18] Substances

Table 1. Flavonoids (mg/g dw) from *Cassia angustifolia in vivo* and *in vitro*.

Plant Parts	Amount of Flavonoids					
	Winter Season			Summer Season		
	Isorhamnetin	Kaempferol	Total	Isorhamnetin	Kaempferol	Total
Stem	0.36	0.42	0.78	0.44	0.49	0.93
Leaves	0.88	0.98	1.86	0.91	0.99	1.90
Flowers	0.81	0.89	1.70	0.85	0.92	1.77
Fresh Pods	0.57	0.62	1.19	0.62	0.69	1.31
Seeds	0.45	0.49	0.94	0.49	0.53	1.02
Callus	1.02	1.25	2.27	1.02	1.25	2.27

Table 2. Antimicrobial screening of isolated flavonoids.

Isolated Flavonoids	Test microorganisms				
	Staphylococcus		*Escherischia coli*		*Candida albicans*
	I/C[a]	I/P[a]	I/C[a]	I/S[a]	I/M[a]
Isorhamnetin	0.21	0.13	0.25	0.19	0.15
Kaempferol	0.26	0.17	0.27	0.12	0.11

a=ratio of the diameter of the inhibition zone of the isolated substances(10mg) under observation (I) to the inhibition zone of the reference disc.

Average inhibition zone: (C)=Chloremphenicol(30mg) against *S. aureus*=17mm and *E. coli*=15mm; (P)= Penicillin (10units) against *S. aureus*=22mm; (S)= Streptomycin (10mg) against *E. coli* =20mm; (M)= Mycostatin (100 units) against *C. albicans* =21mm.

isolated from plant parts and unorganised callus were identified and confirmed as Isorhamnetin and Kaempferol.

Testing of isolated flavonoids for antimicrobial activity : Sterilized petri plates were prepared with 10 ml of growth agar medium and 4 ml of inoculum in case of *S. aureus* and *E. coli* while 6.5 ml of inoculum in case of *C. albicans*[14,16]. Paper discs measuring 6 mm diameter, absorbing near about 0.1 ml of the test sample and known quantity of standard reference antibiotics were used. Inoculated plates were kept at 5°C for 45-55 min and then incubated at 35-37°C for 18 hours. The inhibition zones were measured and compared with those of standard reference antibiotics.

Results and Discussion

Unorganised callus of *C. angustifolia* was fragile and creamish green. Growth indices of tissue showed a linear increase up to eighth week but declined later.

Active principles isolated were identified and confirmed as Isorhamnetin (Rf 0.85, greenish brown) and kaempferol (Rf 0.93, brownish) in free and bound form respectively. The characteristic IR peaks of isolated and authentic samples were identical. Among different plant parts of *C. angustifolia* maximum amount of flavonoids has been found in leaves of summer season (0.99 mg/g dw) and minimum in stem of winter season (0.36 mg/g dw) as given in Table 1. Flowers also presented high amount of flavonoids but slightly less than leaves. On comparing the plant parts of two different seasons, all parts showed lettle higher amount of flavonoids in summer season than winters, which may be due to stress conditions. Amount of free Isorhamnetin was slightly less than bound kaempferol in all plant parts collected in both the seasons. Unorganised cultures showed maximum amount of flavonoids than all *in vivo* parts.

Both the isolated flavonoids showed antimicrobial activity against all the three microorganisms tested (Table 2).

It is already reported that *C. angustifolia* commonly called as senna is valued medicinally for its

cathartic properties. It is useful in habitual constiveness by increasing the peristaltic movements of colon. Hence it can be concluded that besides medicinal importance due to presence of anthroquinones, it has antimicrobial activity also because of antimicrobial principle (flavonoids).

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