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FLAVONOIDS FROM SOME MEDICINAL PLANTS *IN VIVO* AND *IN VITRO*

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ABSTRACT
Unorganized cultures of medicinally useful plants *Aegle marmelos*, *Cocculus pendulus*, *Moringa oleifera* and *Tinospora cordifolia* were established on MS medium supplemented with suitable combinations and concentrations of growth regulators, using seeds as explant in the case of *A. marmelos* and *M. oleifera*, nodal explant in *C. pendulus* and inflorescence explant in *T. cordifolia*. Plant parts as well as calli of all selected plant species were analyzed for antimicrobial principle. TLC, Spectrophotometry and infra-red spectral studies were used for qualitative and quantitative estimation of flavonoids. Presence of Kaempferol and Quercetin in all four selected plant species was confirmed with significantly higher amount in calli than plant parts in all. Crude extract of flavonoids, extracted from selected plant species and calli, were tested for their antimicrobial activity against Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae* and fungal pathogen *Candida albicans*. Flavonoid extracted from calli of all plant species showed greater antimicrobial activity against microorganisms because of higher concentrations of flavonoids in them.

INTRODUCTION

Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites but restricted to specific taxonomic genera of plant kingdom and specific part of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological, pharmaceutical.

Flavonoids, one of the secondary metabolites, are water soluble phenolic glycosides imparting color to flowers and fruits of higher plants. They have multiple biological effect including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. Their contribution to physiological functions such as seed maturation and dormancy has already established (Brenda, 1998).Presence of flavonoids has been reported from many plant species like *Citrullus colocynthis, Corchorus depressus, Fagonia cretica, Lysium barbarum* (Harsh *et al*., 1983), *Arachis hypogea* (Pratt and Miller, 1984), *Passiflora palmeri* (Ulubelen *et al.,* 1984), *Heliotropium species* (Sethia, 1988), *Sophora griffithi* and *Goebelia pachycarpa* (Muminova *et al.,* 2006), *Aconitum naviculare* (Shreshtha *et al.,* 2006) and *Bauhinia forficate* (Pinheiro *et al.,* 2006).

The vital role of flavonoids in defense against micro-organisms, due to antimicrobial activity has been discussed by Skinner (1955). Nickell (1959) reported the presence of antibiotic substances in 147 plant families out of 174 families of vascular plants surveyed. Isolated flavonoids from plants and tissue cultures of *Lysium barbarum, Seetzenia orientalis* (Grover, 1984), *Zygophllum simplex* (Shekhawat, 1985), *Calligonum polygonoides* (Bhojak, 1991), *Gossypium cultivars* (Kaur, 1997), *Peganum harmala* (Badia, 1999), *Withania somnifera* (Bains, 2002), *Cassia angustifolia* (Reddy, 2005), *Balanites aegyptiaca* (Bedawat, 2006) have been screened for antimicrobial activity against microorganisms.

The present paper deals with the *in vivo* and *in vitro* identification, isolation and estimation of four medicinal plants for their flavonoids and to speculate the antimicrobial potential of these medicinal plants *Aegle marmelos* (Bael tree) Rutaceae; *Cocculus pendulus* (Falor) Menispermaceae; *Moringa oleifera* (Drumstick tree) Moringaceae; *Tinospora cordifolia* (Heartleafmoo-onseed) Menispermaceae for their flavonoids and to speculate the antimicrobial potential.

MATERIALS AND METHODS

Plants namely *Aegle marmelos* (Bael), *Cocculus pendulus* (Falor), *Moringa oleifera*(Drumstick) and *Tinospora cordifolia*(Heartleafmooonseed) were collected from the local areas, plant parts (stem, leaves, flowers and fruits) separated, dried and powdered for analysis of flavonoids by Subramanian and Nagarajan (1969) method.

For *in vitro* studies various explants were used to initiate callusing. Explants presoaked in 0.1% liquid detergent for 30 minutes, were washed with running tap water and then surface sterilized with 0.1% (w/v) mercuric chloride for 3 minutes followed by two or three rinses of sterile distilled water.

Murashige and Skoog's medium (1962) supplemented with various concentrations and combinations of growth hormones were used. Calli were maintained for six months by frequent subculturing at interval of 6 to 8 weeks at 26 $\pm$1ºC, 55% relative humidity and diffused light conditions (3000 lux). Growth Indices (GI) of tissues were calculated at 2,4,6,8 and

10 weeks time intervals.

Analysis of flavonoids

Different plant parts as well as tissue samples (at maximum GI) of selected plant species were air dried, weighed, powdered, soxhlet extracted separately with 80% hot ethanol on a water bath for 24 hr and filtered. Filtrate was re-extracted with petroleum ether (Fr I), ethyl ether (Fr II) and ethyl acetate (Fr III) in succession. Fraction II was analyzed for free flavonoids while the fraction III was hydrolyzed with 7% H_2SO_4 for 2 hr. The mixture was filtered, the filtrate extracted with ethyl acetate was neutralized with 5% NaOH and then dried in vacuum and analyzed for bound flavonoids.

Identification of flavonoids

The isolates were identified by TLC (silica gel G coated plates) along with standard reference compounds – Apigenin, Isorhamnetin, Kaempferol, Luteolin, Scutellarein and Quercetin. The plates were developed in n-butanol, acetic acid and water (4:1:5 upper layer) and sprayed with 5% ethanolic $FeCl_3$ solution. Each of the isolates was purified by preparative TLC in similar solvent system. Isolates were eluted with acetate, crystallized by $CHCl_3$ and further confirmed by melting points, UV maxima on spectrophotometer and infra red spectral studies. Quantitative estimation of the identified flavonoids was carried out colorimetrically following method of Kariyon *et al.,* (1953).

Flavonoids identified by TLC, mp and UV spectra in all four selected plant species were Kaempferol and Quercetin only.

Testing of flavonoids for antimicrobial activity

Test was performed in the Department of Microbiology, Tanveer Malawat College, Bikaner. Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae* and fungal pathogen *Candida albicans* were selected for testing antimicrobial activity of flavonoids (antimicrobial principle). The growth medium used for bacteria was nutrient broth and Sabouraud's liquid medium for *Candida albicans*. The inoculum was prepared by adjusting the concentration of microorganisms at 40% transmittance for bacteria and 65% for *C. albicans* using spectronic 20 calorimeter (Bausch and Lomb) set at 630 nm (Khanna and Staba, 1968).

RESULTS AND DISCUSSION

As the occurrence of secondary metabolites is very specific in plants and even in the plant parts, all the plant parts of selected plant species were analyzed separately to estimate comparative amount of secondary metabolites.

Plant parts of selected four plant species, compared individually for their quantitative estimation of flavonoids, showed that maximum amount lies in flowers in *A. marmelos* and *M.oleifera* while in *C. pendulus* and *T. cordifolia* it was maximum in stem. Minimum amount was found in leaves of *A. marmelos* and *M.oleifera* whereas in flowers of *C. pendulus* and *T. cordifolia*. Analyzed unorganized tissues (calli) of all plant species showed significantly high percentage of flavonoids even than maximum amount present in plant parts. Comparing all four plant species for flavonoid content, minimum amount was observed in *C. pendulus* and maximum in *A. marmelos* (Table 1).

Flavonoids isolated from flowers and calli of *A. marmelos* and *M.oleifera* and stem and calli of *C. pendulus* and *T. cordifolia* were tested for antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae* and *Candida albicans*. Antimicrobial test against five microorganisms showed that maximum activity of selected plants was reported against *B. subtilis* except *T. cordifolia* where it was against *S. aureus*. Activity of *A. marmelos, M.oleifera* and *T. cordifolia* was minimum against *C. albicans* and that of *C. pendulus* against *E. coli*. *C. pendulus* and *T. cordifolia* did not show any activity against *K. pneumoniae* and *C. albicans* and *K. pneumoniae* respectively (Table 2).

Activity of four plants was shown in following decreasing order-

A. marmelos - B. subtilis > E. coli > S. aureus > K. pneumoniae > C. albicans

C. pendulus - B. subtilis > S. aureus > E. coli

M.oleifera - B. subtilis > S. aureus > E. coli > K. pneumoniae > C. albicans

T. cordifolia - S. aureus > B. subtilis > E. coli > C. albicans

Table 1: Flavonoid content (mg /100 g.d.w.) of selected plant species

	Plant Species																			
	Aegle marmelos					*Cocculus pendulus*					*Moringa oleifera*					*Tinospora cordifolia*				
Flavo.	L	S	Fl	Fr	C	L	S	Fl	Fr	C	L	S	Fl	Fr	C	L	S	Fl	Fr	C
Kaem.	0.38	0.47	0.59	0.49	0.61	0.31	0.38	0.21	-	0.41	0.36	-	0.48	0.33	0.58	0.35	0.42	0.23	-	0.44
Quer.	0.3	0.4	0.52	0.44	0.54	0.27	0.35	0.19	-	0.37	0.34	-	0.45	0.3	0.55	0.31	0.38	0.2	-	0.41

L = leaves, S = stem, Fl = flowers, Fr = fruits, C = callus, Flavo = flavonoids, Kaem. = Kaempferol, Quer. = Quercetin

Table 2: Antimicrobial activity of selected plant species

Plant	Plant parts	BS	SA	EC	KP	CA
Aegle marmelos	Flower	9.5	7.8	8.4	7.0	6.7
	Callus	9.8	8.0	8.6	7.2	6.9
Moringa oleifera	Flower	10.5	9.0	8.6	8.0	6.5
	Callus	10.8	9.3	8.8	8.5	6.7
Cocculus pendulus	Stem	8.5	8.2	7.8	-	-
	Callus	8.8	8.5	8.0	-	-
Tinospora cordifolia	Stem	8.8	8.9	8.1	-	7.2
	Callus	9.2	9.3	8.2	-	7.4

Values represent diameter of zone of inhibition in mm including diameter of paper disc (6 mm). Experiment was repeated five times. The values represent the average diameter; SA = *Staphylococcus aureus*, EC = *Escherichia coli*, BS = *Bacillus subtilis*., KP = *Klebsiella pneumoniae* , CA = *Candida albicans*.

Calli of all plant species showed little more activity against microorganisms than plant parts (having maximum flavonoid content).

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