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Antimicrobial Activity of Bioacive Metabolites Isolated from Selected Medicinal Plants

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Abstract : The Rotenoids and flavonoids isolated from *Derris indica* (L) Benette, ethanolic extract of root of *Withania somnifera* (L) Dunal and fruits of *Terminalia belerica* Roxb were evaluated for antimicrobial activity against two bacteria (*Escherichia coli* and *Enterobacter cloacae*) and two fungi (*Aspergillus flavus* and *Penicillium tubesulum*) using Filter Paper Disc Method. The maximum activity against *E.coli* was recorded in the rotenoid fraction from pods and subsequently from flavonoids extracts of *D. indica* (IZ; 5 & 4 mm, respectively), which was at par with ethanolic extract of root of *W. somnifera* (4 mm). Against the *E. cloacae* rotenoids from pods and stem of *D. indica* gave significant IZ (3 mm). In case of *A. flavus* significant antifungal activity was found in flavonoids and rotenoids (stem and leaves) isolates of *D. indica* (6mm), which was at par with the rotenoids from pods of *D. indica* and ethanolic fraction of fruits of *T. belerica* against *P. tubesulum*. The present study confirms the antimicrobial activity of isolated rotenoids and flavonoids for the first time.

Key words : Antimicrobial Activity, *Withania somnifera* (L), *Derris indica*, Rotenoids and flavonoids

Introduction :

Increasing emergence of resistance to the currently available antibiotics has necessitated continued search for new antimicrobial compounds. Antibiotic principles are distributed widely among higher plants. Many workers have documented antimicrobial screening of plants (Grosvernor *et al*, 1995; Taylor *et al*, 1995). Fabaceae, Brassicaceae and Asteraceae have been reported to possess wider bioactives than other families (Cavallito, 1951) but to determine the broad spectrum antimicrobial is still lacking.

Efforts have been made to develop production of variety of new, useful and important antimicrobial agents (Kurzybaski *et al*, 1967; Cochran & Hahn, 1975; Sharma & Kumar, 2006). Higher plants accumulate defense chemicals and antimicrobially active substances for protecting themselves against microbial infections, have been reviewed both *in vivo* (Dhar *et al*, 1973; Atal *et al*, 1978; Dhawan *et al*, 1980; Darokar *et al*, 1998; Dixit & Trivedi, 2006) and *in vitro* (Misawa *et al*, 1985).

In the present study three medicinal plants viz *Derris indica* (Lam) Benette, *Withania somnifera* L Dunal and *Terminalia belerica* Roxb were investigated.

D. Indica (Fabaceae) The compounds derived from the tree are also attributed in treatment of paromychia, nematicidal, hypoglycemic and agglutinating effect

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(Chopra *et al*, 1986). Mathur and Kamal (1994ab) worked on primary metabolites and rotenoids (*in vivo* and *in vitro*) of this plant.

W. somnifera (Solanaceae) is known to contain 26 alkaloids and steroidal lactones called as withanolides, of which withaferin 'A' seems to be most bioactive and responsible for all the positive activities. Traditionally, all parts of plants are used as medicine but root is most commonly used (Morse, 1997).

T. belerica (Combretaceae) is known for its purgative and antirheumatic property due to the presence of an oil similar to castor oil. The fruit is one of the three constituents of Ayurvedic medicine "*Trifala*" reported to cure anaemia, enlargement of spleen, liver and other disorders (Chadha, 1986).

Materials and Methods

An experiment was conducted in which antifungal and antibacterial properties of rotenoids from different plant parts and callus tissue, free and bound flavonoid of *D. Indica*, ethanolic root extract of *W. somnifera* and ethanolic fruit extract of *T. belerica* were studied

***Rotenoids* :** Different plant parts, tissue samples of *D. Indica* were collected, dried, powdered separately, and subjected to extraction of rotenoids using method of Delfel (1973).

Flavonoids : Aerial plant parts of *D. Indica* were dried, powdered separately, and subjected to extraction of flavonoids using method of Subramanian & Nagarajan (1969).

***Ethanolic extract* :** Dried root and fruit powder of *W. somnifera* and *T. belerica* were soxhlet extracted on water bath for 72 h (Uma Devi 1989).

Antimicrobial Activity

Culture and Maintenance of Bacteria :

Pure cultures of *Escherichia coli* and *Enterobacter cloacae* obtained from SMS Medical College, Jaipur, India was used as indicator organisms. These bacteria were grown in Nutrient Broth medium (prepared by autoclaving 8% Nutrient Broth of Difeco - Laboratories, Detroit, USA, in distilled water at 15 lbs psi for 25-30 min) and incubated at 37oC for 48 h. Each bacterial culture was further maintained on the same medium after every 48 h of transferring.

A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay (Isenberg, 1992).

Culture and Maintenance of Fungi:

Aspergillus flavus and *Penicillium pubesulum* obtained from the Laboratory of Microbiology and Mycology, Department of Botany, University of Rajasthan, Jaipur, India, were used as indicator fungi. Fresh potato dextrose agar (PDA) medium was prepared using method of Isenberg (1992) .

***Rotenoids* :** Different plant parts, tissue samples of *D. Indica* were collected, dried, powdered separately, and subjected to extraction & estimation for Rotenoids (Delfel, 1973) were used for the experiment.

Determination of Antimicrobial activity

The 'Filter paper disc method' (Gould & Bowie, 1952; 6 mm in diameter) saturated with the extract (0.04 ml and the known quantity of standard reference antibiotics (mycostatin) separately, the standard disc 10 µg/ml (SMS Medical College, Jaipur) were used in each plate as a control.

For anti-fungal screening, spore suspension (5 ml) of each test organism (72h culture) was added to 100 ml of sterilized PDA medium at 35-40°C by thorough shaking.

The petri plates were seeded with this mixture and the paper discs of the extract(s) and the reference antibiotic (mycostatin, 100 units/ml).

Five replicates in each experiment were run and their average was computed.

Results and Discussion

The results of bactericidal and fungicidal efficacy of ethanolic extracts of *W. somnifera* and T. belerica, flavonoids and Rotenoids of *D. Indica* are presented (Table).

Bactericidal Activity

The bactericidal efficacy of ethanolic extracts of with *W. somnifera* and T. belerica, flavonoids and Rotenoids of *D. Indica* showed positive antibacterial activity against E. coli and E. cloacae.

Diethyl ether fraction of flavonoids from *D. Indica* showed 2.5 mm inhibition zone (IZ) against E. coli and 2 mm inhibition zone against E. cloacae whereas the ether to alcoholic fraction showed maximum 4mm IZ and minimum 2 mm against E. coli and 1.5 mm against E. cloacae. Petroleum ether showed 2 mm IZ against both the bacteria sps.

Rotenoids from leaves and stem of *D. Indica* showed maximum (4 mm) IZ against E. coli, whereas minimum was (3mm) in pods. Against E. cloacae pods of *D. Indica* showed 3 mm IZ that was at par with that of the leaves and stem.

The ethanolic root extract of *W. somnifera* showed 1 mm IZ against E. cloacae. The ethanolic fruit extract of *T. belerica* showed 3 mm IZ against E. coli and 1.5 mm against E. cloacae.

Between the three plant sps the bactericidal activity against E. coli and E. cloacae was found to be slightly superior in case of *D. Indica* (flavonoids) over *W. somnifera* and *T. belerica* (Table 1).

Various parts of Derris sps viz D. elliptica, *D. Indica* and D. trifoliata on fractionation with a number of solvents (petrol, dichloromethane, ethyl acetate, butanol and methanol) gave fractions that demonstrated a varied level of broad-spectrum antibacterial activity. Good activity was exhibited by the methanol fractions of the leaves and root heart-wood, petrol, butanol and methanol fractions of the root bark of *D. Indica* and petrol and ethyl acetate fractions of D. trifoliata. None of the plants showed antifungal activity (Khan *et al*, 2006).

Fungicidal Activity

Ethanolic extract of *T. belerica* and *W. somnifera*, flavonoids of *D. Indica* and Rotenoids from *D. Indica* plant parts showing positive antifungal activity against *A. flavus* and *P. tubesulum*.

The maximum IZ (6 mm) was observed in diethyl ether fraction of flavonoids, Rotenoids from stem and leaves of *D. Indica* while, among ethanolic root and fruit extracts of W.somnifera and T.belerica the maximum IZ (5 mm) was observed in fruit extract which was not worthy than flavonoids fraction and Rotenoids stem and leaves of *D. Indica* (Table 1).

To evaluate the biological and pharmacological importance bactericidal and fungicidal activities of Rotenoids from different plant parts, and flavonoids from *D. Indica*, and ethanolic root and fruit extract

Table 1 : Antibacterial and antifungal activities of rotenoids from different plant parts, flavonoids from leaves of Derris indica, ethanolic root extract of Withania somnifera and ethanolic fruit extract of Terminalia belerica

Plant species / Microrganism	*D.indica*						*W.somnifera* .	*T. belerica*
	Flavonoids			***Rotenoids***			***Ethanolic Extract*** *Roots*	***Ethanolic Extract*** *Fruit*
	Pet.Ether	*EtAc*	*Diethyl-ether*	*Stem*	*Leaf*	*Pods*		
Fungi *Aspergillus flavus*								
IZ	Residual toxicity	5	6	6	6	5	3	5
AI		0.33	0.4	0.4	0.6	0.5	0.3	0.5
Penicillium tubesulum								
IZ	4	3	6	5	4	6	5	6
AI	0.4	0.3	0.6	0.33	0.26	0.4	0.33	0.6
Bacteria *Escherichia coli*								
IZ	2	4	2.5	3	4	5	4	3
AI	0.4	0.4	0.25	0.3	0.4	0.5	0.4	0.6
Enterobacter cloacae								
IZ	2	1.5	2	3	2.5	3	1	1.5
AI	0.2	0.15	0.2	0.3	0.25	0.3	0.1	0.1

IZ= Inhibition zone (in mm) excluding the diameter of disc (6mm)
AI= Activity index = Inhibition zone of the sample/ inhibtion zone of the standard

from *W. somnifera* and *T. belerica* have been investigated, against the indicator human pathogenic bacteria (*E. coli* & *E. cloacae*) and fungi (*A. flavus* & *P. tubesulum*).

While assessing the antimicrobial activity it was observed that all the different plant extracts showed positive activity. The maximum activity against *E. coli* was recorded in the rotenoid fraction from pods and subsequently from flavonoids extracts of *D. Indica* (IZ; 5 & 4 mm, respectively), which was at par with ethanolic extract of root of *W. somnifera* (4 mm). Against the E. cloacae Rotenoids from pods and stem of *D. Indica* gave significant IZ (3 mm). In case of *A. flavus* significant antifungal activity was found in flavonoids and Rotenoids (stem and leaves) isolates of *D. Indica* (6mm), which was at par with the Rotenoids from pods of *D. Indica* and ethanolic fraction of fruits of *T. belerica* against *P. tubesulum*.

Similarly, in case of *A. flavus* significant antifungal activity was found in flavonoids and Rotenoids (stem and leaves) isolates of *D. Indica* (6mm), which was at par with the Rotenoids from pods of *D. Indica* and ethanolic fraction of fruits of *T. belerica* against *P. tubesulum*.

Sharma and Kumar (2006) showed remarkable activity of *Ageratum conizoides* L with maximum IZ in EtOH fraction of flavonoids against *Fusarium semitectum* and AI (Activity Index) against *Aspergillus niger* . Dixit and Trivedi (2006) reported antimicobial effect of plant extracts against *Helicobactor pylori* of gastric mucosa.

The present study confirms the antimicrobial activity of isolated Rotenoids and flavonoids for the first time.

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