*Int. J. Chem. Sci.: 8(2), 2010, 1101-1109*

# PHYTOCHEMICAL SCREENING OF *RANDIA DUMETORAM* PLANT EXTRACTS FOR THE ANTIOXIDANT ACTIVITY BY DPPH METHOD AND THEIR ANTIBACTERIAL AND ANTIVIRAL ACTIVITIES

**KOKILA A. PARMAR*, SARJU N. PRAJAPATI and AMIT P. JANI**

Department of Chemistry, Hemchandracharya North Gujarat University, PATAN - 384 265 (Guj.) INDIA

## ABSTRACT

*Randia dumetoram* was investigated for preliminary phytochemical analysis and characterization by various instrumental techniques. Methanolic extracts of *Randia dumetoram* fruits bark has very good antibacterial activity and also minimum inhibitory concentrating for different virus using HEL cell cultures, HeLa cell cultures, and Vero cell cultures but MIC of Herpes simplex-1 and 2, vaccinia virus, vesicular stomatitis and Herpes simplecx-1 (TK ACV[l]) were observed. Very good antiviral activity of *Randia dumetoram* fruits bark DMSO extracts and good minimum cytotoxic concentration activity was also observed.

**Key words**: *Randia dumetoram,* Antiviral activity, Analysis, Phytochemical screening.

## INTRODUCTION

*Randia dumetoram* of Rubiaceae family is widely used as anti–inflammatory, anthelminitic and in leprosy, tumors, asthma and bronchitis[1]**.** The *Randia dumetoram* bark has been reported to have good anti-intlammatory and analgesic activities of oleanolic acid 3-/3- glucoside (RDG-1)[2], and also teratogenicity of monocrotophos in rats and rabbits[3]. New biological active triterpene - saponins from *Randia dumetoram*[4] has been isolated. A hemolytic saponin, randianin, triterpenoid, and an iridoid from *Randia dumetoram*[5-7] has also been obtained.

It is a large deciduous armed shrub up to 9.0 mM height with strong straight spines, horizontal rigid branches and dark brown or gray rough scaly bark, leaves simple usually fascicled on the suppressed branches, obviate, obtuse, wrinkled, shining or more or less

*Author for correspondence; E-mail: sarju_11@yahoo.co.in

pubescent above and on nerves beneath, main nerves 6-10 pairs, flowers at first white, later turning yellow, fragrant, solitary or 2-3 together at the ends of short leaf bearing branchless, fruits globose or broadly avoid, smooth or obscurely longitudinally ribbed berries, yellow when ripe, crowned with the large calyx limb, seeds many, angular embedded in the pulp. Preliminary phytochemical screening and analytical investigations were performed to identity the chemical constituents. The present study screens the antiviral activity of DMSO extracts of *Randia dumetoram* fruits bark against the Herpes-simplex-1 and -2, vaccinia virus, vesicular stromatitis and herpes simplex-1 (TK ACV[1]). It shows very good antiviral activity. The cytotoxicity of *Randia dumetoram* was also tested against as above cells.

## EXPERIMENTAL

The following instruments and techniques were used.

(i) Linowate 5 semi auto application with CAL-MAG software.

(ii) HP-TLC plate: silica gel 60 $F_{254}$ (Merck).

(iii) Antioxidant activities of different extracts was recorded on *in vitro* models. Purchased from Himedia ltd. Antiviral activities was done at Rega Institute of Medical Research, Belgium.

(iv) The extract screening for antimicrobial activity was done on different types of gram positive and gram negative strains.

(v) UV visible spectra were recorded on UV-visible spectrophotometer.

### Plant material and preparation of extract

**Extraction:** *Randia dumetoram* fruits of family Rubiaceae were collected from the plant growing in the forest of Sabarkantha District during July month. The fruits were dried at room temperature for 90 days. After collection of fruits bark, these were crushed. The fruits bark powder was extracted in methanol on Soxhlet appartus for 72 hours.

### Preliminary phytochemical screening

Phytochemical screening of extract was carried out for the presence of phenols, tannins, terpenoids, alkaloids, anthraquinones and flavanoids.

### Instrurnental analysis

### Gas chromatography

From the gas chromatography analysis of *Randia dumetoram*, it was observed that

fruits bark of *Randia dumetoram* comprised of 6 components as it showed 6 peaks at 8.03, 8.48, 9.66, 10.02, 10.10 and 10.32 min of time. Relative data and graph shows that tannins, terpenoids, anthraquinones, flavanoids, alkaloids, and phenol were present in *Randia dumetoram* fruits bark.

## Pharmacological evaluation

### Antioxidant activity

Various diseases are associated with free radicals and hence, free radicals scavengers are well known for their therapeutic activity. A number of anti-oxidants like ascorbic acid, pyrogallol, vitamin E, curcumins etc. have been shown to effectively quench these radicals and hence, these were found to be very beneficial in prophylaxis of the diseases. There are three types methods to observe the activities of free radicals, which cause diseases in humans. These are antiradical, superoxide scavenging and nitric oxide scavenging activities.

### Antiradical activity

Antiradical activity is measured by decrease in absorbance at 516 nm, of methanolic solution of colored DPPH[8,9]. Decrease in absorbance in the presence of test compound at different concentrations was measured after 15 minutes. The $EC_{50}$ is the concentration of the test solution that can bring about 50% decrease in absorbance. In this study, pyrogallol was used as a reference standard. The antiradical activity of the test compound are shown in Table 1.

**Table 1: Antiradical activity of *Randia dumetoram* fruits bark**

| Compound | Concentration (µg/mL) | % Inhibition | $EC_{50}$ (µg/mL) |
|---|---|---|---|
| Standard Pyrogallol | 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 | 22, 30, 36, 44, 54, 62 | 1.7 |
| *Randia dumetoram extract* | 10 | 18.22 | 37 |
| | 20 | 31.78 | |
| | 40 | 53.56 | |
| | 60 | 71.00 | |
| | 80 | 90.06 | |

### Superoxide anion activity of *Randia dumetoram* fruits bark

Superoxide radicals generated in riboflavin–light–NBT system were observed[10]. The

reaction mixture contains 50 mM phosphate buffer pH 7.6, 20 µg riboflavin, 12 mM EDTA and NBT 0.1 mg/3 mL. These were added in the sequence. The reaction was started by illuminating the reaction mixture with different concentrations of the test solution and then absorbance was measured at 590 nm and $EC_{50}$ was calculated. Ascorbic acid was used as a standard antioxidant. The superoxide anion activity of test compound is shown in Table 2.

**Table 2: Superoxide anion activity of *Randia dumetoram* fruits bark observed with riboflavin light-NBT system**

| Sample | Concentration (µg/mL) | % Inhibition | $EC_{50}$ (µg/mL) |
|---|---|---|---|
| Standard Ascorbic acid | 5, 10, 15, 20, 25 | 24.06, 40.34, 58.04, 75.24, 92.55 | 12.5 |
| *Randia dumetoram* | 3 | 20.06 | 8.4 |
| | 6 | 37.09 | |
| | 9 | 52.13 | |
| | 12 | 63.69 | |
| | 15 | 84.30 | |

## Nitric oxide scavenging activity

Nitric oxide is responsible for inflammation, cancer and other pathological conditions[11,12]. The procedure for estimating nitric oxide scavenging activity is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrites ions that can be estimated using 0.5 mL Greiss reagent (1%; N-(1-napthyl ethylenediamine, dihydrochloride)[13]. The absorbance of the chromophore formed was read at 546 nm using curcumin as a positive control. The nitric oxide activity of *Randia dumetoram* observed with Griess reagent is shown in Table 3.

**Table 3: Nitric oxide activity of *Randia dumetoram* fruits bark observed with Griess reagent**

| Sample | Concentration (µg/mL) | % Inhibition | $EC_{50}$ (µg/mL) |
|---|---|---|---|
| Standard Curcumin | 5, 10, 15 20, 25 | 37.56, 47.30, 58.33, 65.38, 77.30 | 11.2 |

Cont…

| Sample | Concentration (µg/mL) | % Inhibition | $EC_{50}$ (µg/mL) |
|---|---|---|---|
| *Randia dumetoram* | 5 | 26.28 | 16.8 |
| | 10 | 31.53 | |
| | 15 | 48.46 | |
| | 20 | 54.23 | |
| | 25 | 68.84 | |

## Antiviral activity

In antiviral activity studies, live cell cultures were used. In this method, cell was grown on solid media. Any cytopathic effect was checked with comparing uninoculated cell line. Various cell lines were used for the study of antiviral activity of drug e.g. HEL cell cultures, Hela cell cultures, Vero cell cultures, etc.

The minimum concentration of extract, which inhibits viral activity or reduced virus induced cytopathogenicity by 50% is good for drug. There is a corelationship between MIC (minimum inhibition concentration) and MCC (minimum cytotoxic concentration). Higher MCC and lower MIC indicate usefulness of drug.

## Different viruses infect different cell lines

MIC of different virus using HEL cell cultures were determined. Here, brivudin, ribavirin, acyclivir and ganciclovir were used as control. MIC of Herpes simplex-1 and 2, vaccinia virus, vasicular stomatitis and Herpes simplex-1 (TK $ACV^{I}$) were observed and very good antiviral activity of *Randia dumetoram* DMSO extract was observed. *Randia dumetoram extract* has good antiviral agent because their MIC for all five viruses was less then 10 µg/mL and their MCC was less than 50 µg/mL. The data are given in Table 4.

*Randia dumetoram* plant extracts for the antiviral activity viruses were studied using Hela cell cultures. Here also brivudin, ribavirin, acyclovir and ganciclovir were used as control. MIC for vesicular stomatitis, coxsackie virus and respiratory syncytial virus show good antiviral activity of *Randia dumetoram* DMSO extracts and good minimum cytotoxic concentration (MCC) activity. *Randia dumetoram extract* has good antiviral activity because their MIC for there viruses are less than 10 µg/mL. and their MCC is less than 50 µg/mL. The respective data are given in Table 5.

Cytotoxicity and antiviral activity of vero cell cultures were negligible in case of *Randia dumetoram* plant extract.

**Table 4: Cytotoxicity and antiviral activity in HEL cell cultures**

| Compounds. | Minimum cytotoxic concentration[a] (µg/mL) | Minimum inhibitory concentration[b](µg/mL)) | | | | |
|---|---|---|---|---|---|---|
| | | Herpes simplex virus-1 (KOS) | Herpes simplex virus-2 (G) | Vaccinia Virus | Vesicular stomatitis Virus | Herpes simplex virus-1 TK KOS ACV[r] |
| Standard | | | | | | |
| Brivudin (µM) | >250 | 0.08 | 0.8 | 6 | >250 | 250 |
| Ribavirin (µM) | >250 | 250 | 250 | 50 | 15 | 250 |
| Acyclovir (µM) | >250 | 0.4 | 0.16 | >250 | >250 | 150 |
| Gancicolvir (µM) | >100 | 0.032 | 0.096 | >100 | >100 | 4 |
| *Randia dumetoram* | >50 | >10 | >10 | >10 | >10 | >10 |

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathologenicity by 50%.

**Table 5: Cytotoxicity and antiviral activity in HeLa cell cultures**

| Comp. | Minimum cytotoxic concentration[a] (µg/mL) | Minimum inhibitory concentration[b](µg/mL) | | |
|---|---|---|---|---|
| | | Vesicular stomatitis virus | Coxsackie virus B4 | Respiratory syncytial virus |
| Standard | | | | |
| Brivudin (µM) | >250 | 250 | >250 | >250 |
| (S)-DHPA (µM) | >250 | 150 | >250 | >250 |

Cont…

| Comp. | Minimum cytotoxic concentration[a] (µg/mL) | Minimum inhibitory concentration[b](µg/mL) | | |
|---|---|---|---|---|
| | | Vesicular stomatitis virus | Coxsackie virus B4 | Respiratory syncytial virus |
| Ribavirin (µM) | >250 | 30 | 150 | 10 |
| *Randia dumetoram* | >50 | >10 | >10 | >10 |

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathologenicity by 50%

## Antibacterial activity

Antibacterial activity was determined by the agar cup plate method. Petri plates containing 20 mL nutrient agar medium (pH 7.2-7.4) were seeded with 17 cultures of the bacterial strains. Extract of fruits bark of *Randia dumetoram* plant has good antibacterial activities against different grams (on the basis of Gram's reaction). The extract of *Randia dumetoram* also show notable antifungal activity, against yeast *saccharomyces cerevisiae*, and also good activity against *E. coli*. It was 25 µg/mL for both organisms.

**Table 6: Antibacterial activity of methanolic extracts of *Randia dumetoram* fruits bark on different bacteria**

| Genus | Species | *Randia dumetoram* methanol extracts |
|---|---|---|
| *Proteus* | *mirabilis* | 25 µg/mL |
| *Proteus* | *Vulgaris* | 200 µg/mL |
| *Staphylococcus* | *Aureus* | 100 µg/mL |
| *Micrococcus* | *Luteus* | 200 µg/mL |
| *Mycobacterium* | *smegmatis* | 25 µg/mL |
| *Bacillus* | *Cereus* | 200 µg/mL |
| *Clostridium* | *sporegenes* | 200 µg/mL |

Cont…

| Genus | Species | *Randia dumetoram* methanol extracts |
|---|---|---|
| *Klebsiella* | *pheumoniae* | 150 µg/mL |
| *Salmonella* | *typhimurium* | 200 µg/mL |
| *Shigella* | *Flexneri* | 150 µg/mL |
| *Vibrio* | *parahaemolyticus* | 50 µg/mL |
| *Pseudomonas* | *aeruginosa* | 200 µg/mL |
| *Bacillus* | *pumilus* | 25 µg/mL |
| *Staphylococcus* | *epidermidis* | 200 µg/mL |
| *Escherichia* | *Coli* | 50 µg/mL |
| *Escherichia* | *Coli* | 25 µg/mL |
| *Saccharomyces* | *cerevisiae* | 50 µg/mL |

## RESULTS AND DISCUSSION

Herbal standardization of *Randia dumetoram* was performed and the following results were observed using various chemical, instrumental and analytical techniques. Preliminary phytochemical analysis of methanolic extracts of *Randia dumetoram* showed the presence of phenol, tannins, terpenoids, alkaloids and flavonoids. The instrumental analysis of extract was carried out using various analytical techniques such as UV, TLC, GC, and HPLC, which showed presence of phytochemicals. The 50% effective concentration for inhibition of HSV-1 and 2, using HEL and HeLa Cell cultures was obtained. The MIC that reduced the viruses is less then 10 µg/mL and their MCC is less than 50 µg/mL.

## ACKNOWLEDGEMENTS

The authors are thankful to Department of Chemistry, Hem. North Gujarat University, Patan for providing necessary facilities for this work and also thankful to Surat for antimicrobial screenig and RIMR, Belgium for antiviral activity studies.

## REFERENCES

1. N. D. Prajapati, S. S. Purohit, A. K. Sharma and T. Kumar, A Hand Book of Medicinal Plants, Agrobios (2003).

2. D. Ghose, P. Thejomoorthy and Veluchamy, Indian J. Pharmacol., **15(4)**, 331 (1983).

3. A. Janardhan, P. Sisodia and P. Pentiah, Indian J. Pharmacol., **15(4)**, 293 (1983).

4. M. A. Dubois, S. Benze and H. Wagner, Planta Med., **56(5)**, 451 (1990).

5. S. Sotheeswaran, M. Bokel and W. Kraus, Phytochem., **28(5)**, 276 (1989).

6. Y. L. N. Marty and M. A. Jairaj, Phytochem., **28(1)**, 276 (1989).

7. O. P. Sati and D. C. C. Chaukiyal, Phytochem., **25(11)**, 2658 (1989).

8. C. M. Navarro, M. P. Montilla, A. Martin, J. Jimenez and M. P. Utrilla, Planta Medica, **59**, 312 (1993).

9. T. Vani, M. Rajani, S. Sarkar and C. J. Shishoo, Int. J. Pharmacognosy, **35(5)**, 313 (1997).

10. C. Beauchamp and I. Fridovich, Anal. Biochem., **44**, 276 (1961).

11. A. Moncada, R. M. J. Palmer and E. A. Higgs, Pharmacol. Rev., **43**, 109 (1991).

12. L. Marcocci, L. Packer, M. T. Droy-Lefaiz, A. Sekaki and F. M. Albert, Methods in Enzymol., **234**, 462 (1994).

13. M. N. A. Rao Sreejayan, J. Pharmacol., **49**, 105 (1997).

*Accepted : 12.11.2009*