

Antiviral activity of *Bauhinia variegata* extracts against rotavirus *in vitro***¹Mohamed Shaheen, ²Mamdouh El-Gamal, ²Adel Mousa, ³Samy Mostafa and¹Nagwa El-Esnawy**¹*Department of Water Pollution Research, National Research Center, Dokki, Cairo, Egypt.*²*Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt.*³*Department of Medicinal and Aromatic Plants Research, National Research Center, Dokki, Cairo, Egypt.***ABSTRACTS**

This study was designed to determine the anti-rotavirus activities of methanol, chloroform, ethyl acetate, butanol, and aqueous extracts from the leaves of *Bauhinia variegata in vitro*. For this purpose, firstly, different concentrations (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) of each extract were used to determine the cytotoxicity effect of the extracts on GMK cells using MTT colorimetric assay. The non-toxic doses were used to determination the antiviral activity of the extracts by MTT colorimetric assay and TCID50 determination in three different ways. Our results demonstrated that the all extracts of *B. variegata* showed antiviral activity against RV *in vitro* with TI ranged from 0.2 to 23 and reduction in virus titers ranged from 0.25 log₁₀ to 4.75 log₁₀. Our results demonstrated that the methanolic extract was the most potent extract against RV infection followed by ethyl acetate, butanol, chloroform, and aqueous extracts. these findings suggest that these extracts can be used in the treatment of rotavirus induced gastroenteritis.

Key words: Rotavirus, *Bauhinia variegata*, MTT, TCID50, antiviral.**Introduction**

Rotaviruses are the cause of acute gastroenteritis, and disease is widespread amongst infants and young children throughout the world. Rotavirus causes 500 000 deaths and millions of physician visits and hospitalizations per year, with worse outcomes and reduced vaccine efficacy in developing countries (Gray, 2011; Uchiyama *et al.*, 2014). The rotavirus is the most frequent etiologic agent of the acute diarrheic disease in infants and young children worldwide. It is globally estimated that rotaviruses are annually responsible for more than 111 million cases of infantile gastroenteritis and approximately 600,000 deaths. As a result of the high morbidity and mortality, the diarrhea with rotavirus represents a major health problem. The research in the field of the antirotaviral vaccination revealed new possibilities of reducing the frequency of the diarrhea caused by this virus (Singer *et al.*, 2010). Vaccines to prevent RGE were developed subsequent to the recognition that wild-type rotavirus infection induces immunity against subsequent disease. Primary rotavirus infections provide substantial protection against gastroenteritis caused by the same serotype and against severe disease, regardless of serotype. Since 2006, the World Health Organization (WHO) has recommended two oral rotavirus vaccines (RotaTeq® [RV5], Merck & Co.; and Rotarix™; GlaxoSmithKline Vaccines). Both have positively demonstrated safety and efficacy in clinical trials and effectiveness profiles, as well as impact in real world settings (Tu *et al.*, 2011). The disadvantages of the current drugs led us to try to develop a new effective, cheap, and safe drug. Medicinal plants consider as a major source for new antiviral agents and therefore, in the current study, we have tested the antiviral activity of methanol, chloroform, ethyl acetate, n-butanol and aqueous extracts from the leaves of *Bauhinia variegata* plant against RV *in vitro*.

Bauhinia L. (Leguminosae Juss., Caesalpinioideae DC.) is a pantropical legume genus with ca.150—300 species, the number of which depends on the demarcation of the genus (Wang *et al.*, 2014) The *Bauhinia* genus comprises about 500 species of shrubs, small trees, and lianas in the tropics. It can be found in the rainforests and tropical regions of Africa, Asia and Latin America. Many plants of the genus are used in traditional medicine for their interesting biological activities such as analgesic, antidiabetic, antiinflammatory, antimicrobial, astringent and diuretic effects (Braca *et al.*, 2001, Iribarren and Pomilio, 1983). The leaves of *Bauhinia variegata* is reported to have antidiabetic (Thiruvengatasubramaniam and Jayakar 2010), antioxidant (Aderogba *et al.*, 2007), anti-inflammatory (Gale *et al.*, 2007), antimicrobial (Cechinel, 2009), antimicrobial (Gunalan *et al.*, 2011; Rasheed *et al.*, 2013), and anticancer (Mishra *et al.*, 2013), nephroprotective activities (Sharma *et al.*, 2011). Different compounds have been isolated from the leaves of *Bauhinia variegata* such as alkaloids, Glycosides-cardiac, saponinns, Flavonoids, Sugars, tannins, phenolics compounds, s diterpenoids, teroids and sterols (Prusty *et al.*, 2012; Yadava and Reddy, 2001; Modh *et al.*, 2011; Mishra *et al.*, 2013).

Material and methods

Plant collection:

Bauhinia variegata leaves were collected from the National Research Centre (NRC) Botanical Garden during May and June 2011 and was kindly identified by, Mrs. Tersea Labib, taxonomist at Orman Botanical garden, Giza and Dr. Mona Marzok, Researcher in NRC.

Preparation of Extracts:

Bauhinia variegata leaves were air dried in shade, ground and powdered. The leaves powder was separately extracted by methanol in percolator at room temperature till exhausted, and evaporated till dryness in rotary evaporator at 40 °C. The methanolic extract of each plant was dissolved in hot distilled water to give suspension and was partitioned with chloroform, ethyl acetate and n-butanol several times in separating funnel till complete extraction, the all extracts, in addition to the residue remained in water, concentrated till dryness in rotor vapor at 40 °C and the percentage of produced extract from plant leaves was recorded then kept in refrigerator until use. 100 mg of every dried extract was dissolved in 500 µl of dimethylsulphoxide (DMSO) then the volume was made up to 10 ml with cell culture medium to obtain 10mg/ml as stock solution. After filtration with Millipore 0.22 µm, the stock solutions were kept at 4 °C until used.

Cell culture and virus stock:

MA 104 was grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of heat inactivated fetal bovine serum (FBS, Gibco BRL) and 1% of antibiotics PSA (100 IU/ml penicillin G, 100 µg/ml streptomycin and 0.025 mg/ml amphotericin B; Gibco BRL) at 37°C in a humidified 5% CO₂ atmosphere. Test medium used for cytotoxicity assay was with the above mentioned antibiotics and only 2% FBS which replaced by 1% trypsin in medium (infectious medium) for antiviral assay. The stock of rotavirus SA11 strain was propagated in MA 104 cells and was tittered by using karber method (Karber, 1931), and expressed as 50% tissue culture infectious doses/ml (TCID₅₀/0.1ml). The titrated virus stock was kept in small aliquots at -80 °C until used.

In vitro Experiments:

Cytotoxicity assay:

The cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method as described previously by Nabil *et al.* (2012). Briefly, MA 104 cells were seeded in 96-well plates at concentration of 5×10^3 and 5×10^3 cells/well. The seeded cells were incubated for 24 h at 37 °C in a 5% CO₂ humidified atmosphere. Then the cells were treated with the various concentration of the extracts (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) followed by an additional 48 h incubation at 37 °C. The cells were checked daily under light microscope to determine the minimum concentration of extract that induced changes in cell morphology. After that, the medium was removed from wells and replaced by 100 µL of MTT solution (5 mg/mL) for 4 h at 37°C. MTT solution was then removed and replaced by 50 µL dimethyl sulfoxide (DMSO) to dissolve insoluble formazan crystal, followed by incubation the plates for 30 min in CO₂ incubator at 37 °C. Optical density (OD) was measured by using a Spectrophotometer reader at 540 nm. Data were obtained from triplicate wells. The percentage of cytotoxicity was calculated as $[(A - B) / A] \times 100$, where A, the mean of optical density of untreated cells and B, the mean of optical density of treated cells. The cytotoxicity was assessed and expressed as CC₅₀ (concentration that reduced the absorbance of treated cells by 50% when compared to control - untreated cells).

Study of antiviral activity with mode of action of plant extracts on RV SA11 by MTT method:

MA104 cells were grown in 96-well plates in concentration of 5×10^3 cells/well and after 24 h incubation at 37 °C the medium was removed from the wells and the antiviral assay was carried out in three different ways:

Virucidal assay:

Viral suspension containing 10⁶ TCID₅₀/0.1 ml was incubated with an equal volume of medium containing three different non-toxic concentrations of extract separately for 1 h at 37 °C. Then 100 µ of mixed suspension was added to subconfluent monolayer cells. After 1 h incubation, the mixed suspension was removed and

replaced by 200 μ l of infectious medium. The cells were incubated under CO₂ atmosphere at 37 °C for 48 h or until typical CPE was visible.

Compound treatment before virus infection (Pre-treatment):

Three non-toxic concentrations from each extract were incubated separately with the cells for 24 h at 37°C in 5% CO₂ atmosphere. The extract dilutions were removed and the cells challenged with 10⁶ TCID₅₀/0.1ml of virus. After 1 h incubation, unabsorbed virus was removed and replaced with 200 μ l of infectious media. The cells were incubated under CO₂ atmosphere at 37 °C for 48 h or until typical CPE was visible.

Compound treatment after virus infection (Post-treatment):

Confluent cell monolayers were challenged with 10⁶ TCID₅₀/0.1ml virus for 1 h. then the unabsorbed virus was discarded and replaced with infectious media containing various non-toxic concentrations. The cells were incubated under CO₂ atmosphere at 37 °C for 48 h or until typical CPE was visible.

Viral control (virus suspension without extract) and cell control (only test medium) were included in all assays. The obtained data are results of experiments performed in triplicate. The percentage of protection was determined spectrophotometrically by MTT method as described above for evaluation of extract cytotoxicity and it was calculated as $[(A-B)/(C-B) \times 100]$, where A, the mean absorbance of treated cells; B, the mean absorbance of virus control; C, the mean absorbance of cell control. The 50% inhibitory concentration (IC₅₀) was defined as the concentration required to reduce 50% cytoprotection against viral infection.

Virus yield reduction assay in vitro:

Rotavirus SA11 were defrosted and after activation, different dilutions of viral suspension (10⁻⁴ to 10⁻⁹) was prepared in FBS free DMEM. Virus dilutions with extract at concentration of 500 μ g/ml or without extract was added into four parallel wells in three different ways as described above with MTT for evaluation of antiviral activity. All plates were incubated at 37°C in CO₂ incubator for 5 days then the CPE was observed under light microscope and virus titration was calculated and expressed as 50% tissue culture infectious dose (TCID₅₀/0.1 ml) using Kärber method (Karber, 1931). The inhibition percentage was calculated as described by Bastos *et al.*, (2013) using the formula: $(IP) = (1 - T/C) \times 100$, where T is the extract-treated viral titers and C is the control viral titers.

Data analysis:

The 50% cytotoxic (CC₅₀) and 50% effective (IC₅₀) concentrations were calculated by linear regression analysis. Then the therapeutic index (TI) of each extract was calculated as the ratio of CC₅₀ / IC₅₀.

Results:

The methanol, ethyl acetate, and aqueous extracts of *B. variegata* plant didn't show cytotoxicity on MA104 cells at 1 mg/ml whereas the 50% cytotoxic concentrations of chloroform and butanol extracts on MA 104 cells were 881 and 554 μ g/ml respectively (Table 1). The methanol extract exhibited higher protective activity against virus infection when it was added to cells after infection (TI=23 and 4.75 log₁₀ reduction in virus titers). This protective activity against virus was significantly decreased when it was added to cells before virus infection (TI=12.5 and 2.5 log₁₀ reduction in virus titers). This extract was less effective against virus infection when it was pre-incubated with virus prior to infection (TI=4 and 0.5 log₁₀ reduction in virus titers). The chloroform extract inhibited the virus replication three times during virucidal, pre-treatment, and post-infection with TI= 5.5 and 0.75 log₁₀ reduction in virus titers; TI=5 and 0.75 log₁₀ reduction in virus titers; and TI= 3.3 and 0.25 log₁₀ reduction in virus titers, respectively. The ethyl acetate showed higher antiviral activity when the virus was pre-incubated with virus prior to infection (TI= 19 and 4 log₁₀ reduction in virus titers). This antiviral activity was significantly decreased when the cells was pre-treated with the plant extract (TI=5 and 0.75 log₁₀ reduction in virus titers). This extract showed less effect against virus when it was added to cells after infection (TI=2.5 and 0.25 log₁₀ reduction in virus titers). The butanol extract was shown to be approximately twice more protective against virus infection when it was pre-incubated with virus prior to infection (TI=6 and 0.75 log₁₀ reduction in virus titers) and when it was pre-incubated with cells prior to infection (TI=7.5 and 1.5 log₁₀ reduction in virus titers) whereas it didn't show significant protective effect when it was added to cells after virus infection (TI=0.2). The Aqueous extract exhibited three times protective effect against RV during virucidal, pre-treatment, and post-infection with TI=4.5 and 0.5 log₁₀ reduction in virus titers; TI=2.6 and 0.25 log₁₀ reduction in virus titers; and TI=5.3 and 0.75 log₁₀ reduction in virus titers, respectively (The results are summarized in Tables 1 and 2; Figures 1 and 2).

Table 1: Results of Cytotoxicity and anti-rotavirus of *B. variegata* extracts with the mode of action on MA104 cells determined by MTT method.

Bauhinia Extracts	CC ₅₀ (µg/ml) ^a	Virucidal		Treatment before infection		Treatment after infection	
		IC ₅₀ (µg/ml) ^b	TI ^c	IC ₅₀ (µg/ml) ^b	TI ^c	IC ₅₀ (µg/ml) ^b	TI ^c
Methanol	4016	963	4	321.7	12.5	174.5	23
Chloroform	881	158	5.5	175	5	266.8	3.3
Ethyl acetate	3594	188	19	699.8	5	1455.5	2.5
Butanol	554	93.16	6	73.66	7.5	2455	0.2
Aqueous	6924	1544.8	4.5	2625	2.6	1292.6	5.3

^a: 50% cytotoxic concentration; ^b: 50% inhibitory concentration; ^c: therapeutic index (CC₅₀/ IC₅₀).

Table 2: Results of antiviral activity of *B. variegata* extracts against rotavirus with the mode of action on MA104 cell line by TCID₅₀ / 0.1ml determination.

Extracts	Virucidal			Treatment before infection			Treatment after infection		
	without extract	with extract	Reduction in virus titer ^a	without extract	With extract	Reduction in virus titer ^a	without extract	with extract	Reduction in virus titer ^a
Methanol	10 ^{6.25}	10 ^{5.75}	10 ^{0.5}	10 ⁶	10 ^{3.5}	10 ^{2.5}	10 ^{6.5}	10 ^{1.75}	10 ^{4.75}
Chloroform	10 ^{6.25}	10 ^{5.5}	10 ^{0.75}	10 ⁶	10 ^{5.25}	10 ^{0.75}	10 ^{6.5}	10 ^{6.25}	10 ^{0.25}
Ethyl acetate	10 ^{6.25}	10 ^{2.25}	10 ⁴	10 ⁶	10 ^{5.25}	10 ^{0.75}	10 ^{6.5}	10 ^{6.25}	10 ^{0.25}
n-Butanol	10 ^{6.25}	10 ^{5.5}	10 ^{0.75}	10 ⁶	10 ^{4.5}	10 ^{1.5}	10 ^{6.5}	10 ^{6.5}	0
Aqueous	10 ^{6.25}	10 ^{5.75}	10 ^{0.5}	10 ⁶	10 ^{5.75}	10 ^{0.25}	10 ^{6.5}	10 ^{5.75}	10 ^{0.75}

^a Reduction in virus titer when compared with virus control (without extract).

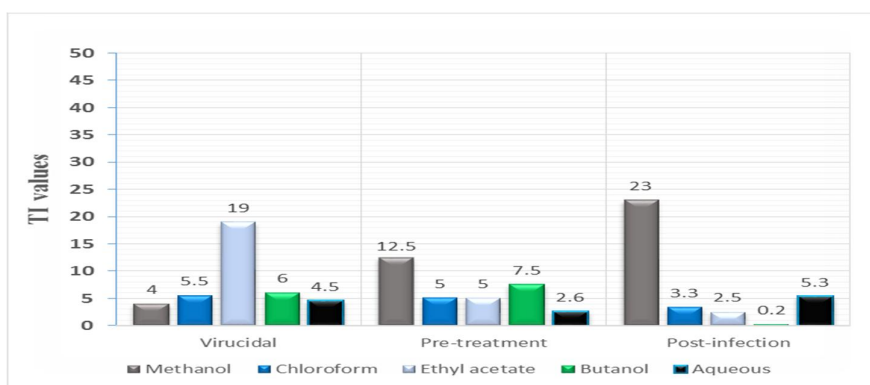


Fig. 1: Antiviral activity of *B. variegata* plant extracts against rotavirus *in vitro* determined by MTT method.

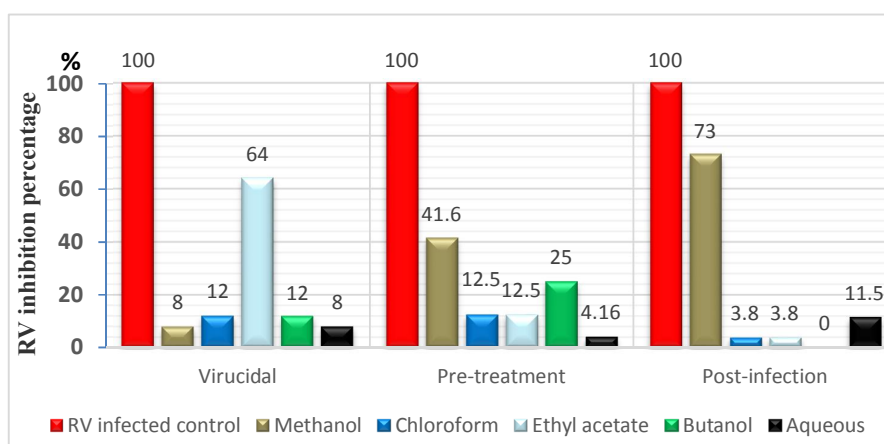


Fig. 2: Inhibitory potential of *B. variegata* extracts on rotavirus infection of MA 104 cells. The red line show to maximum infection with RV (titer of virus without treatment as virus control) and was set to 100% and inhibition percentage of virus by extracts treatment was calculated as a percentage against maximum infection.

Maximum infection with RV (titer of virus without treatment as virus control) and was set to 100% and inhibition percentage of virus by extracts treatment was calculated as a percentage against maximum infection.

Discussion:

Rotavirus (RV) still the most common pathogen of diarrheal disease worldwide, affecting almost all children under age of 5 (Parashar *et al.*, 1998 and 2006). In spite of the great efforts to prevent and/or control RV infections, it is still represent a current major threats worldwide with high morbidity and mortality particularly in children of developing countries (Gu *et al.*, 2000). In the present time, there are two vaccines in the market for clinical use and they can prevent rotavirus infection however, they are specific against certain strains of rotavirus beside high cost and side effect which may occurred in immune compromised persons. Thus cheap, safe, and effective drugs are desired and we think that the natural compounds from herbal extracts are ideal candidates for use as antiviral agents against RV infection.

In the present study, five extracts from leaves of four plants of *B. variegata* were investigated against simian rotavirus SA11 infection *in vitro*. We initially used a simple and rapid staining method (MTT) to identify the cytotoxicity effects of the *B. variegata* extracts on MA104 cells followed by the antiviral activity by MTT method which confirmed by TCID50 determination in three different ways to identify if the extract affect on the viral capsid, viral receptor, or viral replication cycle.

Our results demonstrated that the higher activity of methanol extract was shown twice when pre-incubated with cells prior to infection and when was added to cells after infection to inhibit the virus infectivity by 41.6% and 73% when compared with virus control respectively. This result suggested that this extract may inhibit the virus replication by blocking the viral receptor on host cell with affecting on one or more steps of viral replication cycle after entry into host cell. Whereas chloroform and butanol extracts showed the higher inhibitory effect against RV infection when they pre-incubated with cells prior to infection inhibiting the virus titers by 12.5% and 25% when compared with virus control respectively. This result suggested that the both extracts may be inhibited the virus by affecting on the viral receptor of the host cell.

We have observed also that the ethyl acetate extract showed its higher activity against RV infection when pre-incubated with virus prior to infection inhibiting the virus infectivity by 64% when compared with virus control. This result hypothesis that this extract inhibited the virus infectivity by affecting on the viral capsid with viral receptor of the host cell.

The aqueous extract was observed to be more active against RV infection when added to cells after infection and reduced the virus infectivity by 11.5% when compared with virus control. This result suggested that this extract may be inhibited the virus infectivity by affecting on one or more steps of viral replication cycle after entry into host cell.

In conclusion, the methanolic extract of *B. variegata* was the most active against RV infection followed by ethyl acetate, butanol, chloroform, then aqueous extracts. The higher activity of crude (methanol) extract may be due to that the crude extract contains many different compounds such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, and sterols. The combination of these compounds can result in synergistic action and produce greater antiviral activity than the purified individual compound (Delaquis *et al.*, 2002). On the other hand, it has been reported that the phenol and flavonoid compounds have antiviral activity against several RNA viruses, among them, coxsackie virus B3 (Nabil *et al.*, 2012; Yin *et al.*, 2014; Zhu *et al.*, 2009) and rotavirus (Bae *et al.* 2000). In previous study, we have determined the presence of these compounds in the methanolic leave extract of *B. variegata* and we have found that this extract contains 28.67 mg of phenol/ 100 mg plant leaves and 4.19 mg of flavonoid/100 mg of plant leaves (Shaheen *et al.*, 2014). Thus the higher inhibitory effect of the methanolic leave extract against RV infection may attribute to presence of these compounds.

ACKNOWLEDGEMENTS

We are grateful to Dr. Mamta C. Sarker, professor of virology at National Institute of Cholera and Enteric diseases (NICED), Kolkata, India, for her partially supervision on this project. Partially financial support for this research was provided INSA – JRD TATA fellowship.

References

- Aderogba, M.A., L.J. McGaw, A.O. Ogundaini and J.N. Eloff, 2007. Antioxidant activity and cytotoxicity study of the flavonol glycosides from *Bauhinia galpinii*. *Natural Product Research*, 21: 591-599.
- Bae, E.A., M.J. Han, M. Lee and D.H. Kim, 2000. *In vitro* inhibitory effect of some flavonoids on rotavirus infectivity. *J. Biol Pharm Bull*, 23(9): 1122-1124.

- Bastos, S.C.J., K.K. Luciana, F. Fabiana, A.P. Marina, F.F. Eduardo, P.S. Bárbara, B.A.M. Cláudia and W.A. Clarice, 2013. Antiviral Activity of *Bacillus* sp. Isolated from the Marine Sponge *Petromica* against Bovine Viral Diarrhea Virus, a Surrogate Model of the Hepatitis C Virus. *Viruses*, 5: 1219-1230.
- Braca, A., N.D. Tommasi, L.D. Bari, C. Pizza, M. Politi and I. Morelli, 2001. Anti-oxidant principles from *Bauhinia tarapotensis*. *Journal of Natural Products*, 64: 892-895.
- Cechinel, F.V., 2009. Chemical composition and biological potential of plants from the genus *Bauhinia*. *Phytother Res.*, 23: 1347-54.
- Delaquis, P.J., K. Stanich, B. Girard and G. Mazza, 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Intl. J. Food Microbiol.*, 74: 101.
- Gale, G.A., K. Kirtikara, P. Pittayakhajounwut, S. Sivichai, Y. Thebtaranonth, C. Thongpanchang and V. Vichai, 2007. In search of cyclooxygenase inhibitors, anti-*Mycobacterium tuberculosis* and anti-malarial drugs from Thai flora and microbes. *Pharmacology and Therapeutics*, 115: 307-351.
- Gray, J., 2011. Rotavirus vaccines: safety, efficacy and public health impact. *J. Intern. Med.*, 270(3): 206-214.
- Gu, Y., Q. Gu, H. Kodama, W.E. Mueller and H. Ushijima, 2000. Development of antirotavirus agents in Asia. *Pediatr. Int.*, 42: 440-447.
- Gunalan, G., A. Saraswathy and K. Vijayalakshmi, 2011. Antimicrobial Activity of Medicinal Plant *Bauhinia variegata* Linn. *International J. of Pharmacy and Biological Sciences*, 1: 400-408.
- Tu, H.A., H.J. Woerdenbag, H.J. Kane, M.H. Rozenbaum, S.C. Li and M.J. Postma, 2011. Economic evaluations of rotavirus immunization for developing countries: a review of the literature.
- Iribarren, A.M. and A.B. Pomilio, 1983. Components of *Bauhinia candicans*. *Journal of Natural Products*, 46(5): 5-753.
- Karber, G., 1931. 50% end point calculation. *Archiv für Experimentelle Pathologie und Pharmakologie*, 162: 480-483.
- Modh, M.K., T.P. Purvi, P. Bibhuranjan, S.A. Indermeet and N.P. Chhagan, 2011. Pharmacognostical and Phytochemical Evaluation of Leaves of *Bauhinia variegata* Linn. *Pharmacognosy Journal*, 3: 45-49.
- Mishra, A., S.A. Kumar, S. Kumar, A.K. Saxena and A.K. Pandey, 2013. *Bauhinia variegata* Leaf Extracts Exhibit Considerable Antibacterial, Antioxidant, and Anticancer Activities. *BioMed Research International*, 1-10.
- Nabil, B.S.A., R. Zyed, A.L. Mohamed, S. Souad and A. Mahjoub, 2012. Assessment of the cytotoxic effect and in vitro evaluation of the anti-enteroviral activities of plants rich in flavonoids. *Journal of Applied Pharmaceutical Science*, 02(05): 74-78.
- Parashar, U.D., C.J. Gibson, J.S. Bresse and R.I. Glass, 2006. Rotavirus and severe childhood diarrhea. *Emerg Infect Dis.*, 12: 304-6.
- Parashar, U.D., J.S. Bresee, J.R. Gentsch and R.I. Glass, 1998. Rotavirus. *Emerg Infect Dis.* 4: 561-70.
- Prusty, K.B., B. Harish and C.H. Mamatha, 2012. Evaluation of nephroprotective activity of the methanolic extract of Leaves of *Bauhinia variegata* Linn, (Family-Caesalpinaceae). *J. of PharmaSci Tech.*, 2(1): 16-19.
- Rasheed, E.M., M.H. Hussain and A.M. Janet, 2013. Evaluation of activity of alcoholic extract of *Bauhinia variegata* against some G+ve & G-ve. *Euphrates J. of Agriculture Science*, 5(1): 36-43.
- Shaheen, M., M. El-Gamal, A. Mousa, S. Mostafa and N. El-Esnawy, 2014. Anti-Rotaviral Effects of *Bauhinia variegata* methanolic extract in Mice with Rotavirus Diarrhea. *Middle East Journal of Applied Sciences*, 4(3): 555-562.
- Sharma, R.K., G.P. Rajani, V. Sharma and N. Komala, 2011. Effect of ethanolic and aqueous extracts of *Bauhinia variegata* Linn. on gentamicin-induced nephrotoxicity in rats. *Ind J. Pharm Edu Res.*, 45(2): 192-198.
- Singer, C., P. Stancu, S. Coşoveanu, L. Osiac, C. Grigorie and A. Botu. 2014. Diarrhea with Rotavirus in Children. *Current health sciences journal*, 36(4)2010 updates, 40(2): 240-244
- Thiruvengkatasubramaniam, R. and B. Jayakar, 2010. Anti-Hyperglycemic and Anti-Hyperlipidaemic Activities of *Bauhinia variegata* L on Streptozotocin Induced Diabetic Rats. *Der Pharmacia Lettre*, 2(5): 330-334.
- Uchiyama, R., B. Chassaing, B. Zhang and A.T. Gewirtz, 2014. Antibiotic treatment suppresses rotavirus infection and enhances specific humoral immunity. *J Infect Dis.*, 15; 210(2): 171-182.
- Wang, Q., Z. Song, Y. Chen, S. Shen and Z. Li, 2014. Leaves and fruits of *Bauhinia* (Leguminosae, Caesalpinioideae, Cercideae) from the Oligocene Ningming Formation of Guangxi, South China and their biogeographic implications. *BMC Evol Biol.*, 24: 14:88.
- Yadava, R.N. and V. M. Reddy, 2001. A New Flavone Glycoside, 5-Hydroxy 7,3',4',5'-Tetra-Methoxyflavone 5-O-β-D-Xylopyranosyl-(1→2)-α-L-Rhamnopyranoside from *Bauhinia variegata* Linn. *J. of Asian Natural Products Research*, 3(4): 341-346.

- Yin, D., J. Li, X. Lei, Y. Liu, Z. Yang and K. Chen, 2014. Antiviral Activity of Total Flavonoid Extracts from *Selaginella moellendorffii* Hieron against Coxsackie Virus B3 *in vitro* and *in vivo*. *Evid Based Complement Alternat Med.*, 950817.
- Zhu, H.Z. Yuanyuan, Y. Guan, L. Zhixiong, Z. Pei and H. Chenggang, 2009. *In vivo* and *in vitro* Antiviral Activities of Calycosin-7-O- β -Dglucopyranoside against Coxsackie virus B3. *Biol. Pharm. Bull.*, 32(1): 68-73.