Evaluation of antiviral activity of plant extracts against foot and mouth disease virus *in vitro*

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Abstract: The aim of this study was to evaluate antiviral activity of chloroformic leaves extracts of three plants: *Azadirachta indica, Moringa oleifera* and *Morus alba* against Foot and Mouth disease virus using MTT assay (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). Antiviral and cytotoxic activity of each extract was evaluated as cell survival percentage and results were expressed as Means ± S.D. The concentrations which resulted in cell survival percentages of greater than 50% are considered to be effective antiviral concentrations. From the tested plant extracts, *Moringa oleifera* showed potent antiviral activity ($p<0.05$) while *Azadirachta indica* showed significant antiviral activity in the range of 1-50µg/ml & 12-100µg/ml respectively. In contrast no antiviral activity was observed by *Morus alba* as all the tested concentration resulted in significant reduction ($p<0.05$) in cell survival percentage.

Keywords: Foot and mouth disease virus, plant extract, antiviral activity.

INTRODUCTION

Viral infections are global health problem and so far no considerable antiviral agents are available (Esimone *et al*., 2007). In addition emerging resistance to antiviral agents is also a great problem. Thus development of new and effective antiviral agents is essential in this regard. Various plants are reported to have antiviral properties against RNA and DNA viruses (Naithani *et al*., 2008). *Azadirachta indica* (AI) commonly known as Neem is used for the treatment of several types of diseases includes inflammation, infections, fever, skin diseases and viral infections (Subapriya and Nagini, 2005). Its antiviral activity has been reported for different viruses (Faccin-Galhardi *et al*., 2012, Parida *et al*., 2002, Saha *et al*., 2010). *Moringa oleifera* (MO) commonly known as Sonjna act as diuretic, antipyretic, anticancer, anti-inflammatory, antibacterial and antiviral (Mahmood *et al*., 2010). MO has proved antiviral activity against various viruses (Lipipun *et al*., 2003, Virmani and Garg, 2005), Commonly known as White mulberry. Plant is used as antioxidant, antibacterial, anti-diabetic, anti-hypertensive and antiviral (Butt *et al*., 2008). Its antiviral potential has been reported against different viruses (Du *et al*., 2003, Jacob *et al*., 2007).

FMDV, a picornavirus is positive sense single stranded RNA virus (+ ss RNA virus) and has seven serotypes (A, O, C, Asia 1, SAT 1, SAT2 and SAT3). It causes Foot and mouth disease (FMD) which is economically devastating, transmissible viral infection (Aftosa, 2007). It is endemic in much of Africa and Asia. In case of *FMDV*, antiviral drugs should be arranged as soon as an outbreak is detected and thereby provide a potent adjunct to vaccines for faster control of FMDV (Birtley *et al*., 2005).

MTT assay is a fast and reliable technique. MTT assay is an accurate, safe and sensitive cell viability assay and allow samples to be measured directly in the plate by using a micro titer plate reader or ELISA plate reader (Weyermann *et al.,* 2005).

AI, MO and MA have reported to possess antiviral activity. Moreover they are inexpensive, easily available and quite abundant in Pakistan. This study is an effort to search effective common local plants for antiviral activity against FMDV.

MATERIALS AND METHODS

This project was designed to evaluate antiviral activity of chloroformic leaves extracts of *Azadirachta indica* (AI), *Moringa oleifera* (MO) and *Morus alba* (MA) against FMDV. For this purpose cytotoxic activity of each plant was also evaluated by utilizing *in vitro* cell culture technique on Baby hamster kidney (BHK) -21 cell line by MTT colorimetric assay. Cytotoxic and antiviral activity were evaluated as cell survival percentage (CSP%) and results were expressed as Means ± S.D.

Plant collection

The leaves of plants were collected from district Lahore, Pakistan, air dried under shade and authenticated from herbarium, The University of Punjab Lahore, Pakistan.

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Preparation of extracts and dilutions

Chloroformic extract of each plant was obtained by Soxhlet apparatus (CG-1368) (Davey and Anthony, 2010). A stock solution of 40mg/ml was prepared in cell culture media (M-199) and sterilized by filtration. Twofold serial dilutions of 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12µg/ml, 6µg/ml and 1µg/ml of each plant was prepared in M-199 cell culture media.

Cell line and virus

BHK - 21 cell line and identified, purified, characterized FMDV were taken from Quality operation lab (WTO), University of Veterinary and Animal Sciences Lahore. BHK-21 cells were cryopreserved (Day and Stacey, 2007) for further research. The 50% tissue culture infective dose ($TCID_{50}$) for FMDV in BHK-21 cell cultures was calculated by Reed and Munch method (1938) and the virus titre was 10^6 $TCID_{50}$ (Reed and Munch, 1938).

Cytotoxicity assay

BHK-21 confluent monolayers were grown in 96-well cell culture plates. Each dilution of extract was added in triplicate wells. Each plate was covered and incubated at 37° C with 5% CO_2. BHK-21 cells and cell culture media was used as "negative control or cell control" whereas BHK-21 cells, cell culture media and DMSO (20%) were taken as "positive control".

Antiviral assay

80-90% confluent BHK - 21 cells were grown in 96 well cell culture plates. *FMDV* was added to each concentration of extracts at the titre of 10^6 $TCID_{50}$ and then added to each well in triplicate manner. Plates were incubated at 37°Cfor 48 hours with 5% CO_2. "Negative control" was kept as BHK-21 cells and cell culture media while "Positive control" was used as BHK-21 cells, cell culture media and FMDV.

Cytotoxic activity and cells viability were determined by MTT assay (Twenty man and Luscombe, 1987) for cytotoxic and antiviral assay respectively. Each concentration was tested in triplicate wells as described in fig. 1.

STATISTICAL ANALYSIS

Cytotoxic and antiviral activity of each extract was evaluated as cell survival percentage (CSP) and results were expressed as means ± S.D. Statistical analysis was done using Statistical Packages for Social Sciences (SPSS) 19. Results were analyzed by Two way Analysis of Variance (ANOVA) (Jerrold 2007) with post hoc Scheffe test between plants and different concentrations to determine the effective concentration. Significant cut off value was P<0.05.

RESULTS

Eight different concentrations (1, 6, 12, 25, 50, 100, 200 & 400) in µg/ml were investigated for cytotoxic evaluation of each plant extract. In addition the same concentrations were also evaluated for antiviral activity against FMDV. For AI, 12-100µg/ml and for MO the concentrations of 1-50µg/ml with CSP >50% showed significant reduction (p<0.05) in cytotoxic activity. On contrary, all the tested concentrations of MA showed significant (p<0.05) reduction in CSP. Two-way ANOVA showed significant difference between plant-groups (F=2163.82, 2,72, p<0.001). Post-hoc analysis showed significant difference between all the three plants (P<0.001). Comparison of estimated marginal means of cytotoxic and antiviral activity of each plant is graphically represented in figs. 2 and 3 respectively.

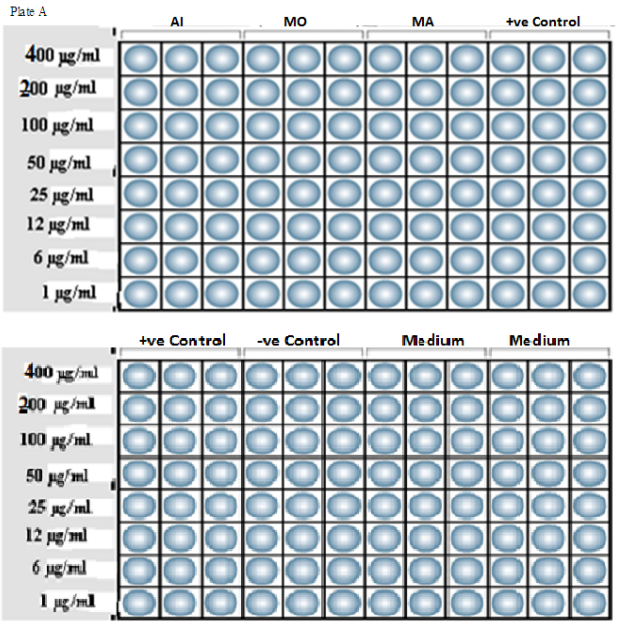

Fig. 1: 96 well cell culture plates showing concentrations in triplicate.

In case of antiviral activity, two-way ANOVA shows significant difference between groups-plants at 1µg/ml (F=690.6 (2,48), P<0.001), 6µg/ml (F=616.2 (2,48), P< 0.001), 12µg/ml (F=589.5 (2,48), P<0.001), 25µg/ml (F=705.2 (2,48), P<0.001), 50 µg/ml (F=605.7 (2,48), P <0.001), 100 µg/ml (F=365.4 (2,48), P<0.001), 200µg/ml (F=23.6 (2,48), P<0.001). Post- hoc analysis showed significant difference between all the three plants (P<0.001).

DISCUSSION

From the tested extracts, MO extract was found to be most active against FMDV as compared to AI and MA extracts. Antiviral potential for MO leaves have been reported for Equine herpes virus (Double stranded DNA

virus), Herpes Simplex virus (Double stranded DNA virus), Epstein Bar virus (Double stranded DNA virus), Hepatitis virus (ds DNA virus), Rhinovirus (+ sense ss RNA virus), HIV (Retro RNA virus) Newcastle Disease Virus (Chollom *et al*., 2012, Virmani and Garg, 2005). Lipipun *et al*. (2003) reported *in vitro* and *in vivo* anti-HSV-1 activity of MO ethanolic extract indicating EC_{50} (Effective concentration for 50% plaque reduction) of 100µg/ml (Lipipun *et al*., 2003). However in the present study antiviral concentration is found to be 1-50µg/ml on BHK-21 cells indicating more potent antiviral activity against FMDV might be due to difference in type of extract, assay method and virus. Glycosides, flavonoids and caffeoyl acids are found in MO as described by Bennett *et al*. (2003)(Bennett *et al*., 2003). Quercetin and kaempferol are major compounds of phenolics present in MO (Siddhuraju and Becker, 2003). In addition, isothiocyanate type compounds are also reported in MO (Eilert *et al*., 1981, Faizi *et al*., 1994) and these isothiocynates as well as their different modified constituents have been shown to inhibit different viruses (Karakuş *et al*., 2009, Tajima *et al*., 2007) during initial stages of viral replication cycle. Kaseem and co workers isolated kempferol glycosides from Diplotaxis harra and investigated their antiviral activity by inhibition of FMDV-mediated cytopathic effect (Kassem *et al*., 2013). For kaempferol different antiviral mechanistic actions have been suggested that includes inhibition of: internal ribosome entry site (IRES) activity (Tsai *et al*., 2011), virus attachment to the cell membrane, virus entry and viral polymerase (Behbahani *et al*., 2013), viral ion channels (Schwarz *et al*., 2014).

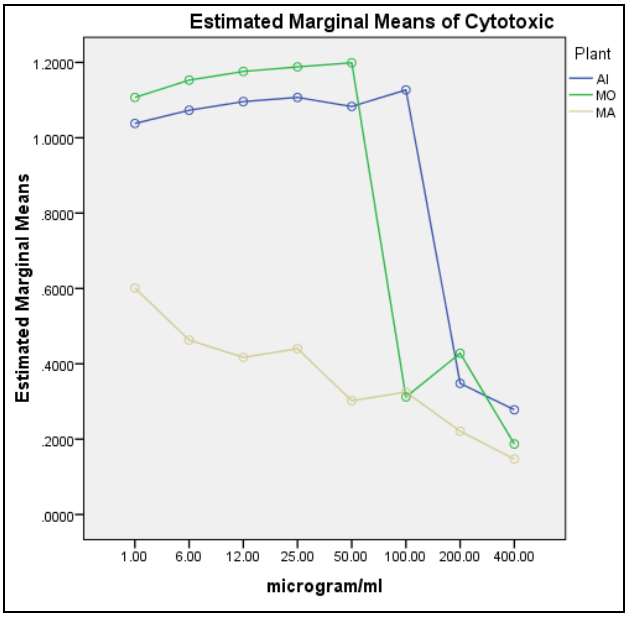

Fig. 2: Comparison of Estimated marginal means of Cytotoxic activity of *Azadirachta indica*, *Moringa oleifera* & *Morus alba* against FMDV ANOVA, Post hoc Scheffe test; p<0.05.

Quercetin has been investigated as antiviral compound against equine herpes virus type 1 (EHV-1), adenoviruses (AdV-3, AdV-8, and AdV-11) and bluetongue virus (BTV) (Tharanath *et al*., 2013). It has been reported that quercetin targets the initial stages of virus replication cycle of porcine epidemic diarrhea virus (PEDV)(Choi *et al*., 2009). The occurrence of isothiocynates, kaempferol and quercetin in MO leaves could be associated with observed antiviral activity in the present study.

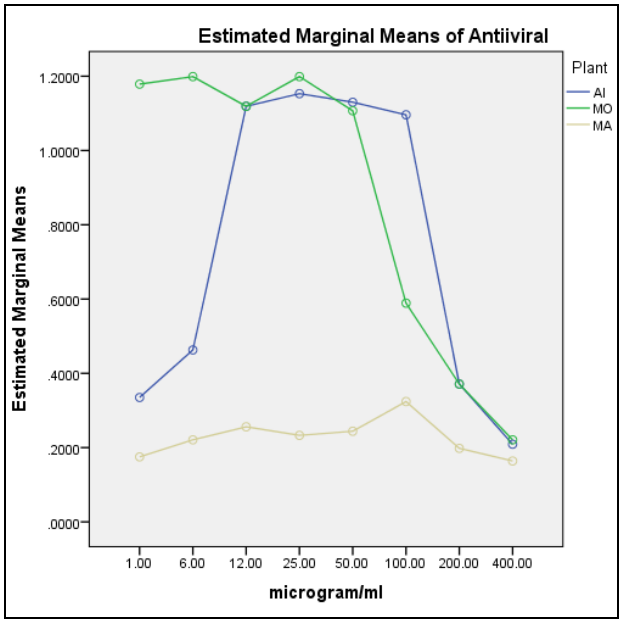

Fig. 3: Comparison of Estimated marginal means of Antiviral activity of *Azadirachta indica*, *Moringa oleifera* & *Morus alba* against FMDV ANOVA, Post hoc Scheffe test; p<0.05.

AI leaves have been reported for antiviral activity against chikungunya virus (Gogate and Marathe, 1986) chicken pox (DNA), poxvirus (DNA)(Kaij-a-Kamb *et al*., 1991), coxsackie B type of viruses (Badam *et al*., 1999), dengue virus (Parida *et al*., 2002) smallpox (DNA), infectious bursal disease virus(Virmani *et al*., 2009), herpes simplex type 1 virus (Amer *et al*., 2010, Bharitkar *et al*., 2014) bovine herpes virus type-1 (Saha *et al*., 2010) and duck plague virus (Xu *et al*., 2012). In a study, (Faccin-Galhardi *et al*., 2012) evaluated antiviral activity of polysaccharides present in AI leaves against poliovirus type 1. A peptide named Meliacine (isolated from *Melia azedarach* L leaves) inhibited the process of uncoating of FMDV in BHK-21 cells by preventing vacuolar acidification (Wachsman *et al*., 1998). Like FMDV, poliovirus also belongs to family Picornaviridae and observed antiviral activity of AI in the present study could be associated to Meliacine or any other peptide. Additionally, this plant contains general class of natural products called triterpenes; more specifically, limonoids which had been reported as antiviral against influenza A and herpes simplex type 1 virus (Khan *et al*., 2005). One

of the limonoid, 1-cinnamoyl-3,11-dihydroxymeliacarpin, derived from leaves of *Melia azedarach* L inhibited vesicular stomatitis (VSV) and herpes simplex (HSV-1) viruses, through induction of IFN-*γ* and TNF-*α* (Alché *et al*., 2003)**.** Thus presence of triterpenes in AI could be linked to anti-FMDV activity.

Although antiviral activity of *Morus alba* (MA) has been reported against various viruses like HSV-1, murine norovirus-1 (MNV-1) and feline calicivirus-F9 (Du *et al*., 2003, Lee *et al*., 2014). Kayo *et al*. (2001) also reported antiviral flavonoids like Moralbanone, flavane and isoquercetin in MA leaves. On contrary, MA in the present study did not exhibit antiviral activity rather all tested concentrations were observed to be cytotoxic. This discrepancy might be due to difference in solvent, virus, cell line, or assay technique (Asano *et al*., 2001). Moreover phenolic acids are reported to exhibit cytotoxicity by affecting DNA synthesis, RNA reductase and microsomal mixed-function oxidase (Picardo *et al*., 1987) and the presence of these compounds in MA leaves could be associated with its observed cytotoxicity.

At present few therapeutic drugs are available against FMDV. Different studies have been conducted and still going on in search of effective anti-FMDV agents. In this regard, different steps and particular protein/enzymes of viral replication cycle could be possible targets. An inhibitor of viral polymerase enzyme has been indicated to overcome FMD virus. Different Silencing RNA (siRNA) techniques have been widely investigated to combat FMDV(Kim *et al*., 2008). Nevertheless more advancement is required for effective delivery and to prevent viral escape.

Comparison of chloroformic extract of *Azadirachta indica*, *Moringa oleifera* and *Morus alba* showed that *Moringa oleifera* extract had potent antiviral activity while *Azadirachta indica* indicated considerable antiviral activity in the range of 1-50µg/ml & 12-100µg/ml respectively. At higher concentrations the extracts were found to be cytotoxic.

CONCLUSION

In accordance, these plants could be helpful in the development of effective and economical antiviral agents. This is a preliminary *in vitro* study reporting antiviral potential of plant extracts. Further research including *in vivo* studies is required to elucidate the antiviral phytochemicals with their mechanism of action.

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REFERENCES

Aftosa F (2007). Foot and Mouth Disease, Iowa State University. College of Veterinary Medicine. *The Center for Food Security and Public Health*. **120**:50-62.

Alché LE, Assad Ferek G, Meo M, Coto CE and Maier MS (2003). An antiviral meliacarpin from leaves of Melia azedarach L. *Z. Natur. C.,* **58**: 215-219.

Amer H, Helmy WA and Taie H (2010). *in vitro* Antitumour activities of seeds and leaves Neem (Azadirachta indica) extracts. *Int. J. Acad. Res.,* **2**: 165-171.

Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS and Ryu KS (2001). Polyhydroxylated alkaloids isolated from mulberry trees (Morus alba L.) and silkworms (Bombyx mori L.). *J. Agr. Food. Chem.,* **49**: 4208-4213.

Badam L, Joshi S and Bedekar S (1999). *In vitro* antiviral activity of neem (Azadirachta indica. A. Juss) leaf extract against group B coxsackieviruses. *J. Comm. Dis.,* **31**: 79-90.

Behbahani M, Shanehsazzadeh M, Shokoohinia Y and Soltani M (2013). Evaluation of anti-herpetic activity of methanol seed extract and fractions of Securigera securidaca *in vitro*. *J. Antivir. Antiret.,* **5**: 072-076.

Bennett RN, Mellon FA, Foidl N, Pratt JH, Dupont MS, Perkins L and Kroon PA (2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera L. (horseradish tree) and Moringa stenopetala L. *J. Agri. F. Chem.,* **51**: 3546-3553.

Bharitkar Y, Bathini S, Ojha D, Ghosh S, Mukherjee H, Kuotsu K, Chattopadhyay D and Mondal N (2014). Antibacterial and antiviral evaluation of sulfonoquinovosyldiacylglyceride: A glycolipid isolated from Azadirachta indica leaves. *Letters in App. Micro.,* **58**: 184-189.

Birtley JR, Knox SR, Jaulent AM, Brick P, Leather barrow RJ and Curry S (2005). Crystal Structure of Foot-and-Mouth Disease Virus 3C Protease new insights into catalytic mechanism and cleavage specificity. *J. Bio. Chem.,* **280**: 11520-11527.

Butt MS, Nazir A, Sultan MT and Schroën K (2008). Morus alba L. nature's functional tonic. *Trends. Food. Sci. Tech.,* **19**: 505-512.

Choi HJ, Kim JH, Lee CH, Ahn YJ, Song JH, Baek SH and Kwon DH (2009). Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. *Antivir. Res.,* **81**: 77-81.

Chollom S, Agada G, Gotep J, Mwankon S, Dus P, Bot Y, Nyango D, Singnap C, Fyaktu E and Okwori A (2012). Investigation of aqueous extract of Moringa oleifera lam seed for antiviral activity against Newcastle disease virus *in vivo*. *J. Med. Plants Res.,* **6**: 3870-3875.

Davey MR and Anthony P (2010). *Plant cell culture: Essential Methods*, John Wiley & Sons. 1-62.

Day JG and Stacey G (2007). *Cryopreservation and freeze-drying protocols*, Springer Science & Business Media.

Du J, He Z-D, Jiang R-W, Ye W-C, Xu H-X and But PP-H (2003). Antiviral flavonoids from the root bark of Morus alba L. *Phytochemistry,* **62**: 1235-1238.

Eilert U, Wolters B and Nahrstedt A (1981). The antibiotic principle of seeds of Moringa oleifera and Moringa stenopetala. *Planta. Med.,* 55-61.

Esimone CO, Ofokansi KC, Adikwu MU, Ibezim EC, Abonyi DO, Odaibo GN and Olaleye DO (2007). *In vitro* evaluation of the antiviral activity of extracts from the lichen Parmelia perlata (L.) Ach. against three RNA viruses. *J. Infection in Developing Countries.,* **1**: 315-320.

Faccin-Galhardi LC, Yamamoto KA, Ray S, Ray B, REC and Nozawa C (2012).The *in vitro* antiviral property of Azadirachta indica polysaccharides for poliovirus. *J. Ethnopharmacol.,* **142**: 86-90.

Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K and Gilani A-uH (1994). Isolation and structure elucidation of new nitrile and mustard oil glycosides from Moringa oleifera and their effect on blood pressure. *J. Nat. Prod.,* **57**: 1256-1261.

Gogate S and Marathe A (1986). Differential cytocidal effect of neem (Azadirachta indica Juss.) extract on normal and tumor originated cells *in vitro*.

Jacob JR, Mansfield K, You JE, Tennant BC and Kim YH (2007). Natural iminosugar derivatives of 1-deoxynojirimycin inhibit glycosylation of hepatitis viral envelope proteins. *J. Mcrobiol., (Seoul, Korea),* **45**: 431-440.

Kaij-a-Kamb M, Amoros M and Girre L (1991). Search for new antiviral agents of plant origin. *Pharm. Acta. Helv.,* **67**: 130-147.

Karakuş S, Küçükgüzel ŞG, Küçükgüzel İ, De Clercq E, Pannecouque C, Andrei G, Snoeck R, Şahin F and Bayrak ÖF (2009). Synthesis, antiviral and anticancer activity of some novel thioureas derivedfrom N-(4-nitro-2-phenoxyphenyl)-methanesulfonamide. *Eur. J. Med. Chem.,* **44**: 3591-3595.

Kassem ME, Afifi MS, Marzouk MM and Mostafa MA (2013).Two new flavonol glycosides and biological activities of Diplotaxis harra (Forssk.) Boiss. *Nat. Prod. Res.,* **27**: 2272-2280.

Khan MTH, Ather A, Thompson KD and Gambari R (2005). Extracts and molecules from medicinal plants against herpes simplex viruses. *Antivir. Res,* **67**: 107-119.

Kim SM, Lee KN, Park JY, Ko YJ, Joo YS, Kim HS and Park JH (2008). Therapeutic application of RNA interference against foot-and-mouth disease virus *in vitro* and *in vivo*. *Antivir. Res.,* **80**: 178-184.

Lee JH, Bae SY, Oh M, Kim KH and Chung MS (2014). Antiviral effects of mulberry (Morus alba) juice and its fractions on foodborne viral surrogates. *Food borne Path. Dis.,* **11**: 224-229.

Lipipun V, Kurokawa M, Suttisri R, Taweechotipatr P, Pramyothin P,Hattori M and Shiraki K (2003). Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection *in vitro* and *in vivo*. *Antivir. Res.,* **60**: 175-180.

Mahmood KT, Mugal T and Haq I (2010). Moringa oleifera: A natural gift-A review. *J. Pharmac. Sci. Res.,* **2**: 775-781.

Naithani R, Huma LC, Holland LE, Shukla D, McCormick DL, Mehta RG and Moriarty RM (2008). Antiviral activity of phytochemicals: A comprehensive review. *Mini reviews Med. Chem.,* **8**: 1106-1133.

Parida M, Dash P, Upadhyay C, Saxena P and Jana A (2002). Serological & virological investigation of an outbreak of dengue fever in Gwalior, India. *Indian. J. Med. Res.,* **116**: 248-254.

Picardo M, Passi S, Nazzaro-Porro M, Breathnach A, Zompetta C, Faggioni A and Riley P (1987). Mechanism of antitumoral activity of catechols in culture. *Biochem. Pharmacol.,* **36**: 417-425.

Reed I and Munch H (1938).Toxicity studies on experimental animals. *Am. J. Hygne.,* **27**: 493-498.

Saha S, Galhardi LC, Yamamoto KA, Linhares REC, Bandyopadhyay SS, Sinha S, Nozawa C and Ray B (2010). Water-extracted polysaccharides from Azadirachta indica leaves: Structural features, chemical modification and anti-bovine herpesvirus type 1 (BoHV-1) activity. *Int. J. Biol. Macromol.,* **47**: 640-645.

Schwarz S, Sauter D, Wang K, Zhang R, Sun B, Karioti A, Bilia AR, Efferth T and Schwarz W (2014). Kaempferol derivatives as antiviral drugs against the 3a channel protein of coronavirus. *Planta Med.,* **80**: 177-182.

Siddhuraju P and Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *J. Agr. Food Chem.,* **51**: 2144-2155.

Subapriya R and Nagini S (2005). Medicinal properties of neem leaves: A review. *Curr. Med. Chem.,* **5**: 149-156.

Tajima H, Nakamoto Y and Taketo A (2007). Effect of synthetic hydroxy isothiocyanates on a bacterial virus and DNA. *Biosci. Biotech. Biochem.,* **71**: 1094-1097.

Tharanath V, Peddanna K and Kotaiah DVSG (2013). Flavonoids Isolated from Foeniculum vulgare (Fennel) have Virostatic Efficiency Against Bluetongue Virus. *Int. J. Pharma. Soc.,* **23**: 237-242.

Tsai FJ, Lin CW, Lai CC, Lan YC, Lai CH, Hung CH, Hsueh KC, Lin TH, Chang HC and Wan L (2011). Kaempferol inhibits enterovirus 71 replication and internal ribosome entry site (IRES) activity through FUBP and HNRP proteins. *Food Chem.,* **128**: 312-322.

Twentyman P and Luscombe M (1987). A study of some variables in a tetrazolium dye (MTT) based assay for

cell growth and chemo sensitivity. *Brit. J. Cancer.,* **56**: 279.

Virmani M and Garg S (2005). *In vitro* antiviral activity of plant extracts against equine herpes virus-1. *Indian J. Microbiol, Immunol. Infect. Dis.,* **26**: 89-91.

Virmani M, Kapoor S, Garg SL and Virmani N (2009). *In vitro* antiviral activity of plant extracts against infectious bursal disease virus. *J. Immunol. Immunopath.,* **11**: 48-52.

Wachsman M, Castilla V and Coto C (1998). Inhibition of foot and mouth disease virus (FMDV) uncoating by a plant-derived peptide isolated from Melia azedarach L leaves. *Arch. Virol.,* **143**: 581-590.

Weyermann J, Lochmann D and Zimmer A (2005). A practical note on the use of cytotoxicity assays. *Int. J. Pharm.,* 288: 369-376.

Xu J, Song X, Yin Z, Cheng A, Jia R, Deng Y, Ye K, Shi C, Lv C and Zhang W (2012). Antiviral activity and mode of action of extracts from neem seed kernel against duck plague virus *in vitro*. *Poultry Sci.,* **91**: 2802-2807.