Screening Of Antiviral Compounds From Plants- A Review

Sanghai Vaijwade D. N.¹, Kulkarni S. R. ², Sanghai N. N. ³

¹Asst. Professor, Dept. of Pharmacognosy, Sinhgad Technical Education Society’s, Sinhgad Institute of Pharmacy, Narhe Road, Narhe-top, Pune – 411 041. Maharashtra, India.

²Associate Professor,HOD Dept. of Pharmacognosy, Bombay College of Pharmacy, Santacruz (E), Near Mumbai University, Kalina,Mumbai-400 098, Maharashtra, India.

³Asst. Professor ,Dept. of Pharmaceutics, S.T.E. S’s, Sinhgad Institute of Pharmaceutical Sciences, Lonavala – 410 401, Maharashtra, India.

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ABSTRACT
Viruses are one of the main hazards for humans and animals; they enter in human body and redirect body’s metabolism to produce large copies of their genome and proteins. It is difficult to control viral infections with currently available drugs like moroxydine, valaciclovir, ganciclovir, and valganciclovir and they act by inhibiting the virus replication via different mechanisms. The difficulty in drug treatment arises due to their low efficiencies, cytotoxic effect and development of viral resistance against them as viruses are smart organisms which mutate themselves and show resistance to drug therapy. This review is focused on identification of antiviral compounds or fraction from plants as they contains active phytochemicals. Ole is a unique secoiridoid glycoside which was extracted from Jasminum officinale L. var. grandiflorum has reported to show anti-HBV activity. The methanol, butanol and ethyl acetate extracts of Lactuca sativa var. longifolia showed significant anti-coxsackie B3 activity. Aqueous and ethanolic extracts of Propolis exhibited high level so antiviral activity against HSV-2 due to polyphenols, flavonoids and phenyl carboxylic acid content. One report demonstrates the strongest inhibitory action against HIV-1 RT was found in seven plants few includes Quercus infectoria (fruit), Acorus heterophyllus Lam. (seed) and Acorus calamus L. (rhizomes). This paper may serve as a vital link between active Pharmacognosy and Formulation and Development department for using active extracts to be formulated and hence gives the best possible solution to the community to treat viral infections.

Key words: Antiviral, Myristica fragrance, Acorus calamus, Herpes virus, Antiviral phytochemical,

INTRODUCTION
Nature is a silent partner of human beings that provides more reliable source of antiviral agents in the form of phytochemical; almost 40% of currently available drugs are direct or indirect derivatives of plants. Many people suffer from viral infection, especially children. With currently available antiviral compounds it is difficult to get relief; one reason is viral resistance. According to authors since centuries healthcare professionals are observing the battle between virus and antibiotics or drugs, some times virus wins by mutation process to survive sometimes we win by providing correct treatment to viral infections. We strongly believe on plants and phytocconstituents produced by them has great potential to act as antibacterial, anti-inflammatory and antiviral agents. As we know viruses are one of the main hazards for humans and animals, they enter in human body and redirect body’s metabolism to produce large copies of their genome and proteins. Looking at present scenario this review is focused on identification of antiviral compounds or fractions from plants as they are rich source of phytochemical like alkaloids, anthocyanins, flavonoids, saponins, polyphenols and others reported in various journals from 2001 to 2012, to provide useful information to researchers.

Why it is important to study antiviral components from plant origin/natural sources?
• Better understanding of natural antiviral agent’s mode of action and identification of responsible compounds will be helpful to provide a new insight for the development of new antiviral drugs for more effective viral control.
• Chattopadhyay et al, have generated the important information on wide array of ethno- medicinal plants showing high level of antiviral activities with various mechanisms, complementary and overlapping mechanism of action, either inhibiting viral replication or viral genome synthesis. Till date number of compounds with antiviral activity is scarce. This is one of the reasons for present work. (Chattopadhyay et al, 2012).
• A number of ethno-botanical studies trying hard to identify
potential plants for more effective control of health issues related viral infections and hence demonstrate the importance of plant species in health care system.

- Natural compounds, because of their structural diversity, provide a large opportunity for screening anti-HBV agents with novel structure and distinct mechanism of action.
- Traditional healers usually give a mixture of some plants for the treatment of diseases; the mixture could be active due to synergistic effects.

Based on above observations the data is collected comprising name of plant and reported against which type of virus in Table-1.

Table-1 Data collected for various plants that are reported for antiviral activity recently.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Part used</th>
<th>IC_{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamomum loureiro</td>
<td>Stem bark</td>
<td>84.58 ± 5.01</td>
</tr>
<tr>
<td>Quercus infectoria</td>
<td>Fruit</td>
<td>56.08 ± 8.71</td>
</tr>
<tr>
<td>Plumbago indica L.</td>
<td>Root</td>
<td>146.50 ± 3.03</td>
</tr>
<tr>
<td>Artocarpus heterophyllus</td>
<td>Seed</td>
<td>34.69 ± 2.41</td>
</tr>
<tr>
<td>Ocimum sanctum L.</td>
<td>Leaves</td>
<td>72.22 ± 6.04</td>
</tr>
<tr>
<td>Allium sativum L.</td>
<td>Bulb</td>
<td>64.08 ± 1.09</td>
</tr>
<tr>
<td>Acorus calamus L.</td>
<td>Rhizomes</td>
<td>32.96 ± 3.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 3).

Table-2- Plants with anti-HIV properties.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of author/ Year</th>
<th>Plant</th>
<th>Active extract/ Components</th>
<th>Virus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zhao et al.; 2009</td>
<td>Jasminum officinale L. extract</td>
<td>Hydro-alcoholic extract. Ole is a natural. secorioid glycoside isolated from this plant Methanol, butanol and ethyl acetate extracts</td>
<td>Hepatitis B Virus</td>
<td>03</td>
</tr>
<tr>
<td>2</td>
<td>Edziri et al.; 2011</td>
<td>Lactuca sativa var.</td>
<td>Phenolic compounds like flavonoids and tannins Tannins seem to prevent HSV entry into host cells as well as antiviral effects of proanthocyanidins</td>
<td>Anti-coxsackie B3</td>
<td>04</td>
</tr>
<tr>
<td>4</td>
<td>Nolkemper et al.2010</td>
<td>Propolis</td>
<td>The cytotoxicity, antitherpetic effect against HSV-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Rombaut et al.; 2010</td>
<td>Smallest naturally occurring intact antigen-binding domains, abundantly present at the outer surface of polioviruses. Variable domains of the heavy chain antibiotics (VHHs) or Nanobodies.</td>
<td></td>
<td>Potential antiviral agent; HIV-1 RT, Poliovirus type-1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Velmurugan et al.,2012</td>
<td>Psidium guajava Linn. root bark</td>
<td>Flavonoids, resins, coumarins, phenols, quinines, alkaloids, tannins. Ethyl acetate extract was active as antiviral agent and successfully control Methanolic extract; polyphenols help to boost immunity. Apoptosis in a Human Leukemia Cell Line through SIRT 1 mRNA Downregulation</td>
<td>WSSV.</td>
<td>08</td>
</tr>
<tr>
<td>7</td>
<td>Chirathaworn et al.; 2007</td>
<td>Myristica fragrans Houtt.</td>
<td></td>
<td>Human leukemia</td>
<td>09</td>
</tr>
<tr>
<td>8</td>
<td>Letidal and Tahir et al.2010</td>
<td>Plant material collection from Sudan region-23 plants; Zizyphus spinosa christi</td>
<td>Proteanaceous substances or polypeptides.</td>
<td>Newcastle disease Virus</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>Silprasit et al.;2001</td>
<td>Asian medicinal plants; Acorus calamus Linn. (AC) and Artocarpus heterophyllus Lam. (AH)</td>
<td>Hexane extracts with IC50 of 32.96 (AC) and 34.69mcg/ml (AH) respectively.</td>
<td>Anti-HIV-1 reverse transcriptase</td>
<td>11</td>
</tr>
</tbody>
</table>
Lot of efforts has been made to find antiviral agents from natural sources and various plants have been traditionally used to treat infections. Selection of plant was based upon use of traditional Chinese medicine, which has long been used in folk treatment of chronic hepatitis in China. Many such plants are now being collected and examined in an attempt to identify possible sources of antiviral. In the last decades, as an alternative to conventional chemical agents, a large number of phytochemicals have been recognized as a way to control infections caused by viruses. Natural compounds, because of their structural diversity, provide a large opportunity for screening anti- HBV agents with novel structure and distinct mechanism of action. The findings are the hydro-alcoholic extract of flowers of JOG showed preferable antiviral efficacy against HBV. For this study cell line was used. The HepG2 2.2.15 cells were routinely cultured in Dulbecco’s modified Eagle’s medium. The HepG2 2.2.15 cell line was used. The HepG2 2.2.15 cells were routinely cultured in Dulbecco’s modified Eagle’s medium. The HepG2 2.2.15 cell line was used. The HepG2 2.2.15 cells were routinely cultured in Dulbecco’s modified Eagle’s medium.

Ole is a unique secoiridoid glycoside present in natures, which possess wide range of pharmacological activity and health promoting properties like anti-inflammatory, anti-rheumatic, diuretic and antiviral. In this report, authors attempted to demonstrate the efficient anti-HBV activity of Ole which was extracted from JOG both in vitro and in vivo. Two lines of evidence support this conclusion. First; Ole blocks effectively HBsAg secretion with an IC50 value of 23.2 microgram/ml in HepG2 2.2.15 cells. Second, Ole (80 mg/kg, twice daily) reduced viremia in DHBV-infected ducks. The mechanism of how Ole suppresses HBsAg gene expression in the HepG22.2.15 cells is not yet clear (Zhao et al; 2009).

Edziri et al has reported antioxidant, antibacterial and antiviral effects of aqueous and methanol extracts of Lactuca sativa var. longifolia leaves (Asteraceae). This plant is richly flavored romaine lettuce is firm and crisp enough to be heated and served in warm salads, and is an important component of Tunisia salad, in Mexican and American caesar salad. Various reported activities include; anti-convulsant and sedative-hypnotic effects for the leaves of this plant, seeds extract had analgesic and anti-inflammatory activity in rats. The objective of the study was to identify and characterize the extract for abovementioned activities. Concentration of total phenolic contents was more in methanolic extract than water extract which may be due to the methanol capacity to solubilize flavonoid components from Lactuca sativa plant. The methanol extract of Lactuca sativa exhibited a significantly greater hydroxyl radical-scavenging activity (IC50 = 3.5 microgram/ml) than the aqueous extract (IC50 = 4.1 microgram/ml) by DPPH assay method. The methanol extract was the most effective extract with the lowest MIC (2.5 mg/ml) against all Gram negative bacteria (Escherichia coli, Klebsilla pneumonia, Enterobacter cloacae, Serratiamarcenscens, Acinetobacter baumannii) and Gram negative bacteria (Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium and Corynebacterium spp.), and this might also be attributed to the high quantity of polyphenols, which are known to possess efficient antibacterial activity. The antiviral activity was estimated on the basis of cytopathic effect (CPE) of the virus-infected confluent monolayer of MRC-5 cells. The mean IC50, CC50 and SI (CC50/IC50) values. Conclusion says that all extracts were not toxic against MRC-5 cells (IC50 > 500 microgram/ml). Methanol and aqueous extracts showed anti-coxsackie B3 and anti-HCMV activity with IC50 values of 200 microgram/ml and with selective index (SI) upper to 2.5. They have reported that methanol, butanol and ethyl acetate extracts showed significant anti-coxsackie B3 activity. The observed anti-coxsackie B3 activity may be due to the higher amount of phenolic compounds particularly flavonoids and tannins. Final conclusion states that methanolic extract of Lactuca sativa var. longifolia plant can be used

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<th>Virus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.</td>
<td>Silpratit et al.2001</td>
<td>Swertia macroperma</td>
<td>Swertiabixanthone-1</td>
<td>Anti- HIV agents</td>
<td>11</td>
</tr>
<tr>
<td>15.</td>
<td>Lin et al.2003</td>
<td>Taiwan plant called Caesalpinia pulcherima Swartz. (Leguminosae).</td>
<td>Aqueous extract of stem and leaf; fruit &amp; seed &amp; quercetin.</td>
<td>Flavones</td>
<td>HSV-1</td>
</tr>
<tr>
<td>17.</td>
<td>Hagezi et al.2001</td>
<td>Egyptian propolis</td>
<td>Ethanolic extract Caffeic acid.</td>
<td></td>
<td>Avian reovirus (ARV) and Infectious bursal disease virus (IBDV)</td>
</tr>
<tr>
<td>19.</td>
<td>Wang et al. 2009</td>
<td>Coffee beans, a single cup may contain 70-350 mg of chlorogenic acid.</td>
<td>Polyphenols: Compounds chlorogenic acid, quinic acid and caffeic acid.</td>
<td></td>
<td>Hepatitis B Virus (HBV)</td>
</tr>
</tbody>
</table>
as natural antioxidant and as a possible food supplement or in pharmaceutical industry and it can be dispensed in suitable tablet of capsule dosage form for maintaining health or as a cream preparation for skin to show antioxidant effect (Edziri et al; 2011).

Geschera et al. carried out the antiviral activity against Herpes simplex virus type 1 by using plant extract from the aerial parts of the South African resiniferous plant Myrothamnus flabelifolia.

Their rationale quoted behind this study was some antiahesive drugs/ compounds interact with bacterial or viral outer surface proteins responsible for the specific attachment of the pathogen to the host cell membrane. The specific recognition and adhesion process is the first important step responsible for infection of host cells and tissue; inhibition of this process may therefore prevent or decrease the severity of infection. Plant extracts, especially tannin-enriched preparations could thus be suitable candidates for the study. How this virus acts: first step is viral entry into the orolabial epithelial cells, and this is mediated by the viral glycoprotein gB, gC, gD, gH and gL. After successful entry into the host cell, the viral gene expression and replication starts, followed by rapid cell to cell spread within the epithelium. Also, the virus rapidly reaches trigeminal ganglia by infecting sensory neuritis intervening the infected epithelium, and establishes life-long latency within infected sensory neurons. Therapy includes; treatment with DNA polymerase inhibitors like acyclovir, ganciclovir, penciclovir and their prodrugs valaciclovir, valganciclovir and famciclovir respectively, brivudine, cidofovir and fascarnet. Resistance was observed by HSV-1 against acyclovir and structurally related compounds is mostly due to mutations abolishing or strongly diminishing the expression of a functional thymidine kinase protein while point of mutations in the viral DNA polymerase are less frequently responsible for resistance. Literature survey says plant derived polyphenols exhibit anti-HSV activity by influencing the early phases of virus infections. Tannins seem to prevent HSV entry into host cells as well as antiviral effects of protocyanandins has been described against Influenza-A virus (Geschera et al., 2011). Nolkemper et al. (2010) reported that aqueous and ethanolic extracts of propolis were analyzed by phytochemical methods, different polyphenols, flavonoids and phenyl carboxylic acids were identified as major constituents. The aqueous propolis extract revealed relatively high amount of phenyl carboxylic acids and low concentrations flavonoids when compared to the ethanolic special extract GH2002. The cytotoxic and anticerherpetic effect of propolis extracts against HSV-2 was analyzed in cell culture, and revealed moderate cytotoxicity on RC-37 cells. The 50% inhibitory concentration (IC50) of aqueous and ethanolic GH2002 propolis extracts for HSV-2 plaque formation was determined at 0.0005% and 0.0004%, respectively. Both propolis extracts exhibited high level so antiviral activity against HSV-2 in viral suspension tests, infectivity was significantly reduced by 499% and a direct concentration-and time-dependent ant herpetic activity could be demonstrated for both extracts. In order to determine the mode of virus suppression by propolis, the extracts were added at different times during the viral infection cycle. Addition of these drugs to uninfected cells prior to infection or to herpes virus-infected cells during intracellular replication had no effect on virus multiplication. However both propolis extracts exhibited high anticerherpetic activity when viruses were pretreated with these drugs prior to infection. Selectivity indices were determined at 80 and 42.5 for the aqueous and ethanolic extract, respectively, thus propolis extracts might be suitable for topical therapy in recurrent herpetic infection (Nolkemper S. et al 2010).

The in-vitro antiviral activity was carried out against poliovirus type-1 by using variable domains of the heavy chain antibodies (VHHs) or Nanobodies by Rombaut et al. VHHs are smallest naturally occurring intact antigen-binding domains, abundantly present at the outer surface of polioviruses. VHHs typically exhibit a superior stability as compared with conventional antibodies, they can be formulated as long shelf-life, ready-to-use solutions and for therapy these small formats can be beneficial in various other aspects not mentioned above such as bio-distribution, renal clearance, tissue penetration and target retention. They have confirmed the antiviral activity observed with the cytopathic effect reduction assay, also performed an infectious virus yield reduction assay on poliovirus type-1-infected cell cultures treated with increasing amounts of VHHs and found out that the antiviral activities of PVSP6A and PVSP29F VHHs have been in the nanomolar range, which is comparable to the neutralizing efficiencies of monoclonal antibodies against poliovirus.

This study demonstrates the proper use of VHHs as a novel class of antiviral drugs and provides, at least two neutralizing VHVs, for the development of a potentially efficacious antiviral drug for the treatment and prophylaxis of poliomyelitis induced by poliovirus type-1 at a low, economic cost per dose. The result of this study can be interpreted as these two VHVs were PVSP6A and PVSP29F, the two VHVs with the most potent antiviral activity (Thys and Rombaut et al., 2010).

Velmutugan et al., has selected Psidium guajava Linn. root bark for antiviral activity against white spot syndrome virus (WSSV). The plant is commonly known as Peru in Marathi language. The leaves were traditionally used for many years in treatment of diarrhea, stomachache and hepatic disorders. Pharmacological activities reported includes; antimicrobial, anti-diabetic, hypoglycemic and hypotensive, anticancer, anti-inflammatory, anti plaque, anti-allergic, cardioprotective, antioxidant and antimutagenic activities. The plant contains terpenoids, flavonoids, tannins and reported components includes; ursolic acid (pentacyclic triterpenoids), 2-alpha hydroxyursolic acid, three of oleanane series namely; oleanolic acid, maslinic acid, arjunolic acid. Base of this study includes WHO guidelines for study of medicinal plants (here Psidium guajava) in terms of identification of chemical components and its biological activity, to develop alternative therapy to control white spot syndrome virus, to screen antiviral compounds against WSSV through in-vitro and in-vivo delivery of Indian white shrimp.
The root bark powder of *Psidium guajava* (100 gm) were serially extracted with 100ml of non-polar solvent hexane, mid polar extract ethyl acetate and polar solvent methanol by percolation method. extract was filtered by Whatman no. 1 filter paper and filtrate was condensed by rotary evaporator under reduced pressure at a temperature 50°C. This study revealed that presence of high amount of flavonoids, coumarins, phenols, quinones, resins, tannins and alkaloids ethyl acetate extract of *Psidium guajava* Linn. and it is responsible for the antiviral activity and successfully control the WSSV replication. Further six compounds were identified includes; asarone, phthalic acid-butyl dodecyl ester, phyitol, phenol, 2,5-bis (1,1-dimethyl ethyl), diethyl phthalate, 1,2-benzenedicarboxylic acid mono (2- ethylhexyl) ester (Velmurugan et al., 2012).

Chirathaworn et al. have carried out the study on *Myristica fragrans* Houtt. Methanolic extract and its effect on apoptosis in a Human Leukemia Cell Line through SIRT 1 mRNA Downregulation. The *Myristica fragrans* Houtt. known as nutmeg (Jaiphal in Marathi) is a commonly found Asian medicinal plant with many bioactive compound like; myristic acid, elemicin, isoelemicin, camphene, eugenol, isoeugenol, methoxyeugenol, pinene and lignan derivatives. Various reported activities of *Myristica fragrans* Houtt. are antibacterial, antiviral against rotavirus. When this extract was administered into hyperlipidaemic rabbits, it reduced the level of blood lipoprotein lipids. The myristicine has cytotoxic and apoptotic effects. The base of study is medicinal plants have been used since long time to treat disease and maintain health, currently, the search for active / bioactive compounds in these plants has magnified. Various reports support the activity of phenolic compounds as anticancer, antioxidant, antimicrobial and boost the immune system. Hence present study was carried out to investigate the effects of *Myristica fragrans* Houtt, methanolic extract as anti-cancer agent by using Jurkat leukemia T cell line. RNA was extracted from these Jurkat cells using Trizol reagent and SIRT 1 (silent information regulatory 2) gene expression was studied at various time intervals. The result showed that the *Myristica fragrans* Houtt. methanolic extract inhibited Jurkat cell proliferation through apoptosis induction. Since this plant extract was known to have phenolic compounds and it has been reported that polyphenols stimulate deacetylase activity of SIRT 1 protein, also the extract (10μg/ml and 20 μg/ml) inhibited a Jurkat-T cell leukemia line proliferation and induced SIRT 1 mRNA downregulation. The minimum quantity of extract 10 μg/ml can induce SIRT 1 (silent information regulatory 2) gene expression that can b detected by RTPCR. It means the potent activity was observed (Chirathaworn et al., 2007).

As per Letidal and Tahir et al., the infectious diseases are the world’s leading cause of premature deaths. So there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action. The rationale quoted behind this study was viral infections are responsible for a variety of infectious diseases ranging from common cold to uniformly fatal rabies and AIDS. In contrast to the enormous amount of antimicrobial drugs, very few, effective antiviral drugs are available. One of the most important reasons for the lack of success in developing antiviral drugs is due to the nature of the infectious viral agents, which depends upon the cell they infect for their multiplication and survival. Base of their study is, since many of the existing disinfectants and antiseptics fail to kill all pathogenic viruses, the demand for new antiviral agents is great and needs all possible approaches towards the development of new antiviral drugs. One of the possible pharmacognostic methodology used for the discovery of bacteri(al and antiviral agents principals is the screening of selected plant extracts for the activity followed by bioassay guided fractionation of active extracts leading to the isolation of the pure constituents.

The method includes; plant material collection from Sudan region (23 plants), drying, and preparation of extracts. The extracts are prepared by taking 100 gm of sample defattening with petroleum ether and chloroform, drying of extracts, followed by extraction with methanol. Then different concentrations 100-200 mcg/ml was prepared for antibacterial activity. In case of antiviral assay the dried extracts were redissolved in Hank’s balanced solution to prepare the test concentrations 100-200 mcg/ml. bacteria used were Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, for in-vitro antiviral assay allantoic sac or chlorio-allantoic membrane (CAM) of developing chick embryos was selected. The highest activity was recorded for Zizyphus punicum against S. aureus strain K. pneumonia. Result says 50% of the extracts exhibited some antiviral activity against Newcastle disease Virus. Seven of these active plants extracts had shown complete inhibition as recorded; also 11 plant extracts exhibited high activity against fowl pox virus. The justification given for the said activity; as the activities demonstrated by the various extracts may be attributed to the diversity of structures and or the uneven distribution of chemical constituents within these extracts. Also it may be due to the fact that, most of the plants showing antiviral activity were found to contain some proteaceous substances or polypeptides as reported. As per Hudson et al in 1994, antiviral phytochemicals were profoundly affected by presence of serum components. It is possible that there are other ingredients in a plant preparation that help to control the virus by additional effect, such as immune modulations, tissue-healing etc. Virus infections are frequently accompanied by disturbances in immune functions and other important metabolic pathways, thereby, influencing multiple physiological parameters. Furthermore, the maximum beneficial effect of a medicinal plant preparation may require the synergistic contribution of antiviral in addition to the other activities. Traditional healers usually give a mixture of some plants for the treatment of diseases; the mixture could be active due to synergistic effects (Letidal and Tahir et al., 2010).

Houtt. methanolic extract as anti-cancer agent by using

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Silprasit et al., has reported the Anti-HIV-1 reverse transcriptase activities of hexane extracts from selected Asian medicinal plants and found that the hexane crude extracts from Acorus calamus Linn and Artocarpus heterophyllus Lam. contained potent activity against HIV-1 RT, with IC50 of 32.96 and 34.69 microgram/ml respectively.

The root bark powder of *Psidium guajava* (100 gm) were serially extracted with 100ml of non-polar solvent hexane, mid polar extract ethyl acetate and polar solvent methanol by percolation method. extract was filtered by Whatman no. 1 filter paper and filtrate was condensed by rotary evaporator under reduced pressure at a temperature 50°C. This study revealed that presence of high amount of flavonoids, coumarins, phenols, quinones, resins, tannins and alkaloids ethyl acetate extract of *Psidium guajava* Linn. and it is responsible for the antiviral activity and successfully control the WSSV replication. Further six compounds were identified includes; asarone, phthalic acid-butyl dodecyl ester, phyitol, phenol, 2,5-bis (1,1-dimethyl ethyl), diethyl phthalate, 1,2-benzenedicarboxylic acid mono (2- ethylhexyl) ester (Velmurugan et al., 2012).
Base of study was HIV-1 is a retrovirus, reverse transcription is the first process for viral propagation in host cells of animal. HIV-1 reverse transcriptase (HIV-1 RT) is responsible for transcription of viral RNA into DNA, which is later integrated into the host cell and carries the information for the synthesis of new viral particles. This enzyme HIV-1 reverse transcriptase is one of the main targets for **inhibiting the reproduction of HIV**. Till date, there are two known types of HIV-1 RT inhibitors; the first is nucleoside HIV-1 RT inhibitor (NRTI), a nucleoside analog that resembles the polymerase.

Substrate and the second type of inhibitor is non-nucleoside HIV-1 RT inhibitor (NNRTI). Because of their hydrophobic property, these inhibitors bind to the HIV-1RT hydrophobic pocket (allosteric site) which is far away from the catalytic position. NNRTI show very high selectivity, and bind specifically to HIV-1 RT only. Long-term treatment with commercial marketed drugs can cause the propagation of drug-resistant strains of HIV-1. This is one of the reason why Kiattawee et al decided searching for new inhibitors to overcome this problem which is an area of innovative research. The biodiversity of plant species makes them a promising source of new drugs. The history of folklore medicines demonstrates the potential of plants as sources of lead compounds. Examples of numerous anti- HIV agents have been discovered from plants includes; calanolide A, a series of coumarins originating from Calophyllum lanigerum Miq. var. austrocoriaceum, swertiabixanthone-1 from Swertia macropersea, swertipunicoside from Swertia punicea, swertifrancheside from Swertia franchetiana and new glucopyranosides and flavonoids (4) discovered from the stem-bark of Jugalans mandshurica. The other reason was to identify the mechanism of these plant extracts, various Asian medicinal plants (40) for inhibition of HIV-1 RT were selected. **The hydrophobic property of most HIV-1 RT inhibitors was reported; hence hexane extraction was used for recovery of aromatic and hydrophobic substances.** This was first attempt to search for a hydrophobic HIV-1 RT inhibitor that can be obtained from selected plants. Inhibitory assays were performed by the fluorescence method.

All fresh materials were dried in hot air oven, powdered and 100 mg of each material was extracted with 80ml of hexane in a Soxhlet extractor for 8 hours. The hexane extracts were dried under vacuum using a rotary evaporator at 50°C. 1 ml of DMSO was added as a solvent to dissolve the crude compounds, which were then diluted to 16 mg/ml in 50% DMSO-containing buffer. Samples were kept ready at 4°C until further study. One can say that from the testing of hexane extracts from 40 traditional Asian medicinal plants, seven of the extracts showed a high HIV-1RT inhibitory effect. This strong inhibitory effect was confirmed by their IC50 values. Details of plants are depicted in Table- 2

The results showed that these plants contained anti-HIV properties, which was in accordance with previous reports in which the plants A. calamus L. and P. indica L. exhibited potent antiviral activity against the Herpes simplex viruses HSV-1 and HSV-2. A. sativum was reported to be effective against HIV infection by inhibiting virus replication, specifically by interfering with viral reverse transcriptase activity. O. sanctum L. was found to demonstrate antibacterial, antifun-gal and antiviral activity; this report also showed that the plant possessed an anti-HIV property through inhibition of viral reverse transcriptase activity. Based on this data a daily diet of food item can be prepared by using these seven extracts in the form of wafers, microemulsion for HIV-1 infected patients which ill help in inhibiting reproduction of HIV (Silprasit et al. 2011).

Ching et al., reported action of aqueous extract and quercetin against herpes simplex viruses HSV-1 and suggested the potential use of this compound to treat the infections. Selected plant was a common medicinal herb in Taiwan called Caesalpinia pulcherrima Swartz. (Leguminosae). This plant has been used in common remedies for treatment of various infections like, pyrexia, menoxenia, wheeving, merial infection and bronchitis etc. Flower contains lupeol, lupeol acetate, myricetin, quercetin and rutin. This study was carried out with one of the object that despite the great advances in the synthetic nucleoside analogues or cysteine protease inhibitors for anti-adenoviral replication; currently there is no proven chemotherapy treatment that interrupts this viral infection. Also to demonstrate the power of naturally occurring flavonoids from traditional Chinese herb, to inhibit the multiplication of HSV and adenovirus.

Among the different parts of this medicinal herb tested (flower, stem and leaf, fruit and seed), the flower extract appeared to possess the strongest anti-HSV activity ($P < 0.05$). The flower contains quercetin-3-rutinoside, flavones. The extract of stem and leaf, fruit and seed has showed the strongest activity against adenovirus (ADV-8). This study shows that three crude drugs from C. pulcherrima including flower, stem and leaf, fruit and seed possess anti-adenoviral activity; the strongest anti-ADV-3 activity was associated with flower extract with an SI value of 8; strongest anti-ADV-8 activities were stem and leaf, and fruit and seed with SI values of 52.1 and 83.2, respectively. The mode of action of quercetin was found to be at early stage of multiplication with SI value greater than 20. One can conclude this study as the effect of these drugs on adenoviruses is worthy of further investigations, also find more potent natural components from Caesalpinia pulcherrima to treat this virus infection and come up with tablet formulation containing the said active extract (Ching et al., 2003).

Li et al., have studied the antiviral activities of flavonoids and some organic acids by using Trollius chinensis Burge. flower extracts (Li et al., 2002). The flowers are used to treat upper respiratory tract infec-

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### Table 2- Seven extracts with HIV-1RT inhibitory effect with IC50 values

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plants</th>
<th>IC50 (µg/ml)</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cinnamomum loureiroi</td>
<td>84.58 ± 5.01</td>
<td>Stem bark</td>
</tr>
<tr>
<td>2</td>
<td>Quercus infectoria</td>
<td>56.08 ± 8.71</td>
<td>Fruit</td>
</tr>
<tr>
<td>3</td>
<td>Plumbago indica L.</td>
<td>146.50 ± 3.03</td>
<td>Root</td>
</tr>
<tr>
<td>4</td>
<td>Artocarpus heterophyllus Lam.</td>
<td>34.69 ± 2.41</td>
<td>Seed</td>
</tr>
<tr>
<td>5</td>
<td>Ocimum sanctum L.</td>
<td>72.22 ± 6.04</td>
<td>Leaves</td>
</tr>
<tr>
<td>6</td>
<td>Allium sativum L.</td>
<td>64.08 ± 1.09</td>
<td>Bulb</td>
</tr>
<tr>
<td>7</td>
<td>Acorus calamus L.</td>
<td>32.96 ± 3.17</td>
<td>Rhizomes</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 3).
tions, pharyngitis, tonsillitis and bronchitis in Chinese folk medicine. Base of this study was to demonstrate antiviral activity of flower extract of mostly used plant *Trollius chinensis* Bunge. The flowers were dried and extracted with 60 % ethanol by refluxing three times for 1.5 hours. The solution was filtered and evaporated to obtain crude extract (brown color, 342 gm). Further 1 gm of this extract was partitioned by polyamide column by using water, methanol. The methanol fraction was collected and evaporated to get yellow colored powder (106.5 mg.) which contained flavonoids; it was confirmed by TLC for 1.5 hours. The solution was filtered and evaporated to obtain extract of mostly used plant.

The ethanol extract and total flavonoids of the flowers of *T. chinensis* showed antiviral activities against Para 3 with IC50 value of 184.2 microgram/ml. Proglobeflowery acid (Fig.1) showed weak antiviral activity against Para-3 with IC50 value of 184.2 microgram/ml. The term adequate nutrition means a diet that provides nutrients (vitamins, proteins, carbohydrates, fats and minerals) in sufficient amounts to satisfy particular organic needs of body. One concept says that optimal nutrition means besides above, the potential of food to promote health, improve general well-being and reduce the risk of developing certain illnesses. This is the area where nutraceuticals, functional food, designed diet, therapeutic foods, super foods or medicinal foods play an important role and such products are available in market and their sell is increasing annually at a rate 15-20 %. One may include all those originating in the bee hive: propolis, royal jelly and of course honey.

Propolis is a resinous substance that is collected by bees from the exudates of plants and they use to seal the holes in bee hive. Propolis is a part of traditional medicine, and chemical analysis says that it contains about 300 components. The secondary metabolites includes; resin (50 %), wax (30 %) pollens (05 %) essential oils (10 %), organic compounds (05 %). organic compounds includes; phenolic, flavonoids (flavones, flavonols, flavonones etc.) terpenes, beta-steroids, aromatic aldehydes and alcohols etc. Amongst them Caffeic acid phenyl ester (CAPE) is biologically active ingredient of propolis with activities like apoptosis, metastatis.

Phenolic compounds are found to be main compounds responsible for the functional properties associated with many foods like antioxidant capacity, cardio protective effects, antibacterial agent, antiviral agent, anti-inflammatory agent and prevention of enzymatic browning. Among these foods, one can include propolis, royal jelly and honey (Viuda-Martos et al., 2008).

Hegazi et al., (2001) have reported the antiviral activity of four different propolis Austria, Egypt, France and Germany against avian reovirus (ARV) and infectious bursal disease virus (IBDV) and found that Egyptian propolis showed highest antiviral activity.

Propolis (bee glue) is a sticky resinous bee-hive product. It consists of exudates from plants mixed with beeswax and used by bees as glue for general-purpose, sealer and draught-excluder for beehives. It is collected by honey bees from various areas. The reported use of propolis was seen for a long time as early as 3000 BC. Propolis showed variable biological activities: antiviral activity against Newcastle Disease Virus, Rift Valley Fever Virus, anti small-pox, anti-influenza virus, antibacterial, antiulcer and anti-tumour etc. The basic objective was to study antiviral activity of the four different propolis samples.

Chemical composition found by GC/MS method includes aromatic acids and majorly caffeic acid found in all samples. They have identified various components for first time; 2-Phenyl-2-hydroxyacrylic acid identified in Austrian and German samples, 4-methylmandelic acid identified in Austrian, German and French samples. Aromatic acids and significant amounts of esters of these acids are present in Propolis. Phenyl ethyl caffeate appeared in all samples. Common components found are; Flavonoids found was Pinocembrin, 4-methylmandelic acid, benzyl-trans-4-coumarate, cinnamyl-trans-4-coumarate, cinnamylisoferulate, 3-methyl-3-butenyl trans-caffeate, 3-methyl-2-butenyl trans-caffeate, Prenylated caffeate esters and cinnamyl caffeate.

The paper reports that each propolis is different a little bit like: French propolis contains benzyl caffeate and Egyptian propolis is characterized by the presence of unusual esters of caffeic acid with C12- C16 fatty acids mainly saturated. It also contains a series of triterpenes known as lanosterol. Whereas German propolis contains galangin, butanly-caffeate and phenylethyl- caffeate isomer. Result of this study says that all four samples reduces the viral infectivity in varied degree, amongst them Egyptian propolis showed the highest antiviral activity against ARV and IBDV. Egyptian propolis contains a series of triterpenoids, fatty acids viz.; linoleic acid, etradecyl caffeate, high quantity of glucose and animal sterol precursor was identified. Activities of all samples were similar in spite of the differences in their chemical composition. It is evident that the all propolis samples showed significant qualitative similarities (Hegazi et al., 2001).
Jasim et al (2010) has reported the antitumor activity of Iraqi propolis and its chemical composition. Since ancient time bee products have been studied and proved to possess medicinal properties like antibacterial, antifungal, cytostatic. Propolis in one such resinous compound collected by honeybees from various plant flowers. It contains array of phytochemical, mostly mixture of polyphenols, flavonoid aglycones, phenols and ketones (Jasim et al., 2010).

Liu et al carried out study of structure of polysaccharide PD from Polygonatum cyrtonema Hua. and the antitherpetic activity of its hydrolyzed fragments. The PD was isolated from the traditional Chinese medicinal herb, Polygonatum cyrtonema Hua. Five fragments were isolated by Bio-Gel P4 chromatography from hydrolysates of PD.

The roots of P. cyrtonema Hua. were washed and cut into thin pieces, dried at 60 °C to yield brown powder. After homogenized and refluxed in 85% ethanol. The powder was firstly extracted with 50% ethanol and subsequently precipitated with 100% ethanol to yield the crude extract. The antitherpetic activity was determined by cytopathic effect (CPE) inhibition assay, Neutral red (NR) dye uptake assay and Plaque formation inhibition assay.

It was shown that PD was a branched fructan with average DP of 28 and average molecular weight 2000-5000. It simply means it is a complex polysaccharide molecule and B3 fragment showed antitherpetic activity. Authors would like to suggest that one may formulate eye drop formulation to treat herpes simplex keratitis infection (Liu et al., 2004).

Wang et al., stated that several hundreds of plants, trees, herbs have been reported to have potential antiviral activity and a wide variety of phytochemicals are identified; out of these chlorogenic acid is an important polyphenols widely distributed in leaves and fruits of dicotyledons plants like coffee beans, a single cup may contain 70-350 mg of chlorogenic acid.

Chemically plant polyphenols like chlorogenic acid is the ester of caffeic acid with quinic acid; the later acids are powerful antioxidants and have multi-antiviral activities. The antiviral activity of chlorogenic acid, caffeic acid and quinic acid against Hepatitis B Virus (HBV) was carried out by using HepG2.2.15 cell line and in DHBV-infected duckling model by using crude coffee extract. In the cell model, all the three compounds chlorogenic acid, quinic acid and caffeic acid inhibited HBV-DNA replication as well as HBsAg production.

Thus the result suggests that the chlorogenic acid as well as caffeic acid and quinic acid are long known as antioxidant, and might contribute to its anti-carcinogenic properties (Wang et al., 2009).

Sohail et al., have reported various plants that show antiviral activity against prominent viruses viz., Herpes virus, Human Immunodeficiency Virus (HIV), Influenza and Hepatitis virus. Viruses are one of the main hazards for humans and animals. They enter in human beings and redirect body’s metabolism to produce large copies of their genome and proteins. It is difficult to control viral infections with currently available antiviral drugs like moroxydine, ganciclovir, valganciclovir; valaciclovir which inhibits the virus replication via different mechanisms. The difficulty in drug treatment arises due to their low efficiencies, cytotoxic effect and development of viral resistance against them.

Plants are rich source of phytochemical like alkaloids, anthocyanins, carotenoids, flavonoids, isoflavones, lignans, monoterpenes, organosulfides, phenolic acids, saponins and many more. These phytochemical have been proved to be responsible for their antimicrobial, antihyper tensive anti-diabetic, antioxidant, hepatoprotective, cardioprotective and other biological and pharmacological activities. Base of this study was to analyze the previously reported antiviral plants, its component and their potential mode of action (Sohail et al., 2011).

**Natural supporting medicines for viral infections.**

Common cold and flu, allergies, and dermatitis may be caused due to virus entry and their multiplication in human body. In one of the review important data was collected for natural drug antiviral means the preparations that are used for enhancing immune function, promoting healing and lowering cholesterol and are non-FDA reviewed or approved, natural alternatives, to be used for cold and flu, allergies and dermatis are given below. Marketed preparations are depicted in Fig. 2.

1) Bee fense (60 caps) Defence formula [Benefits of this product includes; immune system, winter defense, prevents infection, vitality and well-being]
2) Rutin (50tabs 500 mg) [Enhances the power of Vit. C]
3) Alluna sleep (28 tabs) [Boosts immunity, protection against Flu and cold]
4) Wellness Fizz (10 wafers) [Better defense against cold and flu viruses]
5) Cold Flu (1 fl. Oz.) [Relieves cold and flu symptoms; achiness, chills, headache, fever].

![Fig.2- Natural Medicines; Bee fense, Rutin, Alluna sleep, Wellness Fizz, Cold Flu.](image)

**Summary**

Plants are true friends of human beings. They provide all necessary phytochemical which in turns fight against microbes and help us to maintain our health. One has to get closer to plants, feel them and then use them for therapy and see the magic of this positive power. It is now clear that phytochemicals derived from plants like polyphenols, secoiridoid glycoside, flavonoids, tannins, flavonoids, coumarins, quinones, quinines, resins, phenyl ethyl caffeate and al-
kaloids shows antiviral activity. According to author mankind has observed beneficial health effects of crude drug when taken orally or other means, this has developed zest for identification of that component showing the activity, further they isolated active component and studied their individual effect against virus and of course they got positive result. This not necessarily means to get antiviral activity one needs to take separated phytochemical from that plant in tablet, capsule and wafer dosage forms. One may adapt naturopathy like daily intake of some important plants like Ocimum sanctum, Propolis, Rheum palmatum, Ephedra sinica, Trollius chinensis etc. as a preventive medicine which definitely boost our immunity to fight against microbes and other infectious conditions, as I believe prevention is better than cure and have faith in our friends called Plants.

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