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Herbo-Nanoparticles an Herbal Medicine for Retrovirus and Opportunistic Infections Tuberculosis and Candidiasis: Key for Future Drug Development - A Short Review

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ABSTRACT

India is one of the countries with rich source of medicinal plants, but the usage of plant parts or secondary metabolites is still unknown for the deadly retroviral Human immunodeficiency virus (HIV) disease. Many plant secondary metabolites have the tendency to fight against the virus and other opportunistic viruses. Usages of allopathic drugs produce mutation in the human system and high cost makes concern to the patients as well as to the health care communities. A medicinal plant with nanotechnology (nanoparticle) is the future alternative nanomedicine for retroviral disease with an affordable cost without side effects. Here in this review, we enlightened the potential nature of medicinal plants and their antiviral properties with nanoparticle for drug development for retroviral diseases to traditional medicinal practitioners, health care industries and research communities.

INTRODUCTION

Human Immunodeficiency Virus (HIV), a causative agent for the disease Acquired Immunodeficiency Syndrome (AIDS). Globally in 2019, 24.5 million people undergone antiretroviral therapy for HIV.

The uniqueness of HIV that makes it different from other viral infections is once acquired the body of host wont able to get rid of it. The virus causes impairment of immune system during the progression to disease through reduction in CD_4 and CD_8 T-lymphocyte counts, delayed cutaneous sensitivity, impaired serological response after immunizations and no or reduced bactericidal activity in host [1]. An impaired immune system becomes more susceptible to other infectious pathogenic microorganisms and/or infection related cancer. Epidemiology studies around the world reported some of the common opportunistic infections in HIV infected individuals. In 2016, a study in Ethiopia revealed that tuberculosis, oral candidiasis and herpes zoster are the common infections among HIV patients [2]. In 2014, a case study at Karnataka district, India showed tuberculosis accounts for 50% of the opportunistic infections, followed by candidiasis with 49% [3]. A Korean cohort study from 2006 to 2013 on 1086 HIV infected patients revealed that the most common opportunistic infection is candidiasis followed by tuberculosis, cytomegalovirus, pneumonia and herpes zoster [4]. Hepatitis B and hepatitis C virus are also commonly occurring co-infections and the leading cause of death among HIV patients [5]. These case studies are more relevant to one other.

Still, now no permanent cure for the AIDS disease was discovered. The current treatment for HIV/AIDS is antiretroviral therapy (ART) which uses a combination of highly efficient drugs to reduce the viral load by inhibition of reverse transcriptase and protease enzymes of the virus. Even though the ART has reduced the mortality rate among HIV individuals over the years, the high cost of the treatment regime and adverse side effects, development of drug resistance and rapid mutation of the virus made it difficult to completely eradicate the disease [6]. Thus the healthcare industry needs to develop a novel drug to cure HIV/AIDS.

During the ancient period, plants played a major role in human life. In the process of civilization, our ancestors began to identify the properties of plants for food, medicine and recreational purposes. By repeated trial and error they found the proper use, method and dose of medicinal plants and formed their own medicinal system. Several secondary metabolites present in plants such as alkaloids and flavonoids have potential antiviral properties to control

viral diseases [7]. Traditional medicinal system around the world had been gaining attention as an alternative for western medicine due to less cost and side effects. For example, in China around 40%, in Chile around 71%, in Colombia 40% and in India about 65% of the population depend on traditional medicine for their primary needs. It also addresses certain life threatening diseases including HIV/AIDS [8]. But it lacks scientific analysis and proof making it nonacceptable in the modern world. Hence, it is important to conduct phytochemical, *in vitro* and *in vivo* analysis in medicinal herbs and fractionating to isolate the active compounds. Use of medicinal plants can be the only alternative way to cure the viral disease than other medicines. Traditional medicine has always being an alternative for Western medicine holds the key for the future novel drug development for the disease.

Retrovirus

Retroviruses are a diverse group of viruses that causes many diseases in humans and animals and prevalent over centuries [9]. The first documented case of retrovirus caused disease is pulmonary adenocarcinoma in sheep in 1825, later in 1870 as bovine leukosis. In the beginning of 20th century, these viruses were associated with anaemia of horses and erythrocyte leukemia of chickens. With the knowledge gained through the analysis of animal retroviral diseases four pathogenic viruses found be the major cause of disease in humans in 20th century.

Human T- lymphotropic virus type-I (HTLV-I) was the first identified in humans in 1980, associated with cancer and/or neurological disorders. HTLV-II has been found shortly after the HTLV-I discovery in a patient infected with hairy cell leukemia but the association of virus with the disease is not known. Later in 1983 and 1986 HIV type 1 and 2 were found respectively, both cause AIDS [10]. The formation of the HIV 1 particles with host genomes was through ENV glycoprotein and GAG protein fusion with cell membrane [11] (Fig. 1).

Retroviruses contain a single stranded RNA genome that undergoes reverse transcription and integrates itself in to the chromosomal DNA of the cell after its insertion into the host cell. This unique mechanism of replication is because of viral enzymes, reverse transcriptase and integrase which play a major role in the pathogenesis [12].

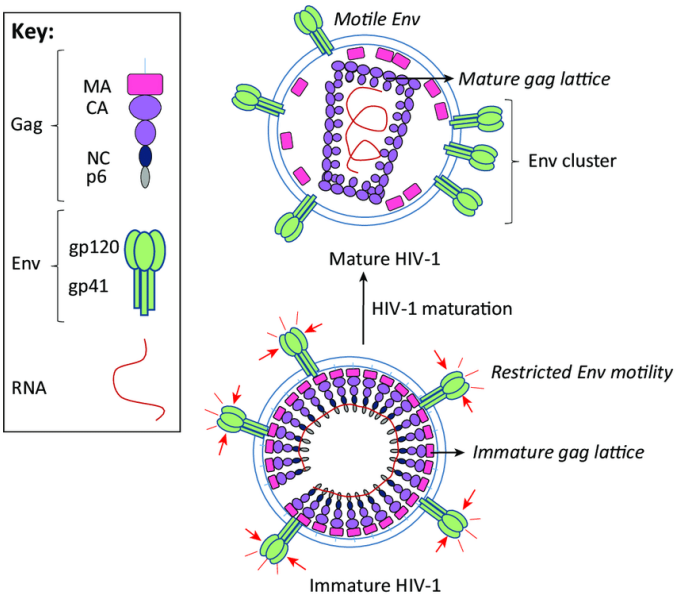


Figure No. 1: Formation of HIV-1 (Courtesy: Jakobsdottir *et al.*, 2017)

Medicinal Plants and their Metabolites against HIV and Opportunistic Infection

The emergence of drug resistance among the HIV infected patients had become a major barrier for the current anti-retroviral therapy. Due to this concern, WHO in need of new drugs to fight the infection suggested the testing of potential anti-HIV natural products that will provide effective therapeutic agents for the treatment [13]. But finding a perfect plant from an overall of 300,000 species is difficult. In this case, Traditional medicinal system can be used as the tool for finding the plants with anti-HIV properties.

Followed by the suggestion of WHO in 1989, a significant amount of research had been done in plants for anti-HIV potential and certain secondary metabolites found to possess activities against reverse transcriptase of HIV 1, proteases and integrases of HIV 1 and HIV 2, the metabolites such as calanolides (coumarins), ursolic, betulinic acids (triterpenes), baicalin (flavonoid), polycitone A (alkaloid) and lithospermic acid (phenolic compound) [14]. Researches focused on the individual plant species against HIV/AIDS and the associated diseases based on the knowledge gained from the herbal medicinal practitioners and/or people of different cultures results, emphasises the necessity for further testing and developing to discover a novel drug candidate for HIV. The researches on individual plants conducted around the world.

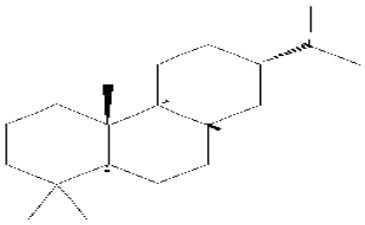
The fruit rind of *Terminalia bellerica* and rhizome of *Zingiber officinale* (Fig. 2) used in Ayurveda for treating herpes had been proven to inhibit the HIV virus infectivity. TZM-bl cell lines infected with the pseudoviruses ZM53 M.PB12, ZM109F.PB4 and RHPA 4259.7 of HIV-1 envelope and inhibitory effect of the plant extracts was tested in the 96 well plates. *Terminalia bellerica* exhibited inhibition at a concentration less than 2 µg/ml against all three pseudovirus while *Zingiber officinale* at 4.1 µg/mL concentration against ZM53 and RHPA and at 98 µg/mL against ZM109. The NMR analysis of *Terminalia bellerica* showed the presence of ferulic and caffeic acids [15].

Terminalia bellerica

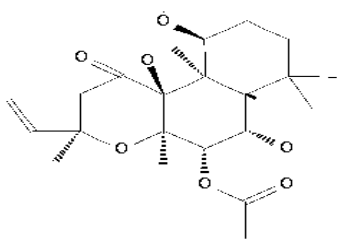
Zingiber officinale

Figure No. 2: Ayurveda Medicinal plant species used against HIV

The anti HIV-1 activity of *Plectranthus barbatus* used in traditional medicine of African countries exhibits a strong antioxidant activity and produces forskolin extract for Pharmaceutical/drug formulation [16, 17]. *In vivo* experiments in HIV infected individuals showed the reduction of viral load and disease related symptoms after treated with herbal extracts with major constituents of essential oil, abietane diterpenoids and 8, 13-epoxy-labd-14-en-11-one diterpenoids (Fig. 3).



Abietane



Forskolin

Figure No. 3: *Plectranthus barbatus* essential oil constituents against HIV

In South African traditional medicine, many plants in the use to treat AIDS related illnesses and one among them *Bridelia micrantha* (Hochst) Baill scientifically screened for its activity against HIV-1 reverse transcriptase enzyme. This plant belongs to the family Phylanthaceae and is native to Africa. From the crude methanol extract of roots, n-butanol fraction was obtained and it actively inhibited the RNA dependent DNA polymerization of the enzyme at a concentration of 7.3 g/ml [18].

Aspilia pluriseta and *Rumex bequaertii* are the plants used in Rwandan traditional medicine for treating infections and also for rheumatoid. Those plants were selected and their ethanolic extracts showed *in vitro* activity against HIV type 1 with best selectivity indices [19].

The plants of Lamiaceae family have many medicinal potential and used frequently in traditional medicine. The research on their anti-HIV properties showed the targets were the key enzymes of the virus and the *in vivo* testing aided in viral disease treatment [20].

A flowering plant species of family *Rubiaceae, Rubia cordifolia* also known as Common Madder or Indian Madder found to have anti HIV properities. *R. Cordifolia* contains Quinolizidine, alkaloids, lectins, non-protein amino acids, tannins and alkylsterols [21]. The extract showed potential HIV inhibition by suppressing LTR gene expression and p24 antigen production. Different plant parts such as leaves stem roots and whole plant extracts were prepared using isopropyl alcohol and water to test against HIV virus *in vitro*. Promising anti HIV activity was observed in the extracts [22] (Fig. 4).

Plectranthus barbatus

Bridelia micrantha

Aspilia pluriseta

Rumex bequaertii

Melissa officinalis

Rubia cordifolia

Figure No. 4: Efficient medicinal plant species against HIV

Hibiscus is one among the plants commonly used in different country's traditional medicine to treat heart, liver, nerve diseases and other symptoms. The herbal extract of hibiscus possessed effect against herpes simplex virus type 1 (HSV-1). Secondary metabolites such as polyphenols, flavonoids and anthocyanins were found in the phytochemical analysis. The aqueous and methanolic extracts of flower had best selective index of 7.7 and 8 [23]. When tested with the HSV-I infected vero cells in pre-treatment assay, the extracts exhibited tendency to prevent virus interaction with the cells and inhibition of virus replication with the prophylactic selectivity indices of 5.2 for aqueous and 6.1 for methanolic extracts.

Dunbaria bella Prain plant of Thai medicinal system exhibited anti-herpes (type I and II) activity at 1.21-1.25 µg/ml with 17 fold selectivity [24].

Melissa officinalis L., a plant species of family Lamiaceae, commonly known as lemon balm, has shown inhibitory effect on replication of herpes simplex virus type 2 (HSV 2) on Hep-2 cells at a nontoxic concentration [25]. It was again confirmed by another research of the plant's oil extract on both type 1 and 2 virus infected monkey kidney cells. The study demonstrated the higher efficacy of the plant extract on virus before adsorption. Further,

monoterpene aldehydes citral a, citral b and citronellal were identified as the main constituents of the plant on GC-MS analysis [26].

A study in Nilgris, India reported the anti HSV-1 activity of *Hypericum mysorense*, *Hypericum hookerianum* and *Usnea complanta* used as medicinal plants [27].

One of the ancient medicinal systems in the world is Chinese Traditional Medicinal system. from it, the herbal extracts of *Agrimonia pilosa*, *Pithecellobium clypearia* and *Punica granatum* were tested against HSV-1 and resulted in the inhibition of the virus owing to the presence of polyphenolic compounds in the extracts [28].

Another commonly occurring opportunistic infection in HIV infected patients is Tuberculosis. The Madhya Pradesh tribal Society uses herbs in their traditional medicine such as *Alstonia scholaris*, *Glycyrrhiza glabra*, *Holarrhena antidysenterica*, *Mallotus philippensis*, *Eulophia nuda linda*, *Cocculus hirsutus Diels*, *Puerariatuberosa*, *Cyperus rotundus* had potential activity against 6 resistant strains of *Mycobacterium tuberculosis* from the sputum sample of patients [29] with minimum inhibitory concentration range of 500-31.25 µg/against Mtb H37Rv. *In vitro* cytotoxicity of plant extracts on human THP 1 macrophages does not exhibit any adverse effects (Fig. 5).

Similarly in South Africa, it was found that the water extract of Artemisia afra Jacq. and Carpobrotus edulis L Ex Willd and dichloromethane extract of Tulbaghia violacea Harv. plants species showed activity against *M. aurum* A+ strain, a strain related to *M. tuberculosis*, in two fold microdilution bioassay [30].

In South India, selected medicinal plants that are being used as spices were tested for their anti-TB activity. The results showed that *Allium sativum*, *Allium cepa*, *Syzygium aromaticum* and *Cinnamomum verum* had potential to inhibit *M. tuberculosis* H37Ra strain [31].


A. scholaris


G. glabra


H.antidysentrica


M. philippensis


E. nuda linda


C.hirsutus


P. tuberosa


C. rotundus

Figure No. 5: Traditional medicinal plants against opportunistic infection in HIV

Candidiasis, a commonly occurring fungal infection in HIV patients, also causes bloodstream infection around the world. It possesses a high risk of mortality in immune compromised people. *Cassia fistula* (aka Golden Shower), a flowering legume showed activity against the resistant strains of *C. albicans*, C. *glabrata*, *C. krusei*, C. *tropicalis*, C. *kefyr* and C. *Parapsilosis* from HIV patients [32].

The herbal leaves, seeds and barks showed potent inhibition of all species with the seed extracts exhibiting the most activity. Lanosterol 14 alpha demethylase, a precursor of fungal membrane enzymes ergosterol is mostly the target for antifungal drugs [33]. Gallic acid from the seed extract perfectly docked with the lanosterol 14- alpha demethylase enzyme, confirming it as a potent natural anti-fungal agent.

In an ethnomedical survey in Tanzania identified the use of 21 plants in the treatment for candidiasis, out of which 13 had proven activity against the fungus. Some plans also had activity against *Cryptococcus neoformans*, other fungi causing infections in HIV patients [34].

Earlier, four plants of South African traditional medicine found to have activity against oral candidiasis. The aqueous bulb extracts of *Allium sativum* L. and *Tulbaghia violacea* L.

inhibited the fungus at concentration of 0.56 and 3.25 mg/ml while the rhizome extract of *Glycyrrhiza glabra* L. and leaves extract of *Polygala myrtifolia* L. inhibited at 1.56 mg/ml [35]. Another study with water extracts of Cassia alata plant species used in West African traditional medicine showed promising antibacterial and antifungal activities for *E.coli* and *Candida albicans* comparable to that of the standard drugs chloramphenicol (antibacterial agent) and amphotericin B (antifungal agent) [36].

Nanoparticles as Anti-Retroviral Agents

A nanoparticle, high surface to volume ratio, increases the reactivity when compared with the other material. This unique physiochemical property has been gaining importance in drug delivery system as their nano size enables them to directly interact with bacteria and viruses [37]. Gold, Zinc, copper, titanium and magnesium have ability of antimicrobial agents as nanoparticles. Also, the synthesis of nanoparticles using plant extracts is proving to be more effective than the other physical and chemical methods. The secondary metabolites such as terpenoids, flavonoids, alkaloids and phenolic acids acts as a reducing agent of bulk metals into nanoparticles and thus stabilizing it. The nanoparticles (NPs) produced by this method remained stable for a long time and environmental friendly [38, 39]. The size and shape of the nanoparticles differs with different plant species used and also based on the parts of the plant used in the reaction [40].

Of all the metals, mainly silver nanoparticle is mostly under research owing to the knowledge that silver has been in use in traditional medicine from ancient times to treat wounds and burns. Even many researchers conducted and validated the scientific proof for the silver nanoparticle's antimicrobial activity [41]. One such study synthesized silver nanoparticles (AgNPs) using *Alysicarpus monilifer* leaf extract that had potential to fight against two commonly occurring infection in HIV infected individuals i.e. Methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase negative *Staphylococci* (CoNS) which mostly opportunistic to tuberculosis disease [42]. *Alysicarpus monilifer*, native to Africa and Asia has been in use in Indian traditional medicine to cure various illnesses including diarrhoea, earache, fever, jaundice, stomach ache and inflammation [43].

Rhizophora lamarckii, commonly called Red Mangrove has been used extensively in folk medicine for treating asthma, backache, fever, lesions, sore throat, jaundice, leprosy. The aqueous plant extracts used to reduce the silver ions to nano size produced stable

nanoparticles at 12-28 nm size. Even at a low dose, the nanoparticles exhibited a strong anti HIV-1 activity by inhibiting reverse transcriptase enzyme. The anti HIV activity of the plant extract was tremendously increased due to the binding of nanoparticles [44]. The Rhizophora species have already proven to have diverse antiviral properties including HIV and hepatitis B virus [45].

An *in vitro* study showed the interaction of silver nanoparticles with HIV-1 virus. The nanoparticles interacted with virus through binding to GP 120 glycoprotein on the envelope. GP 120 is a glycoprotein in the surface of the virus which readily binds with the host cell's CD4 receptor during infection. By binding with GP120 knobs, the silver nanoparticles in the range of 1-10 nm showed the potential to inhibit the virus from infecting the host cell. High angle annular dark field (HAADF) scanning transmission electron microscopy used to study the interaction [46].

Other than silver nanoparticles, gold nanoparticles designed and synthesised with multivalent using SDC-1721 fragment of TAK-779 against CCR5 of HIV. TAK 779 inhibits binding of chemokine to receptor CCR2 and CCR5 for inhibition of HIV [47]. Gold nanoparticles conjugated the fragment to 2nm against HIV virus and produced significant inhibitory effect as comparable to TAK-779. The SDC-1721 alone does not show any inhibitory effect [48].This research proves the ability of nanoparticles to convert an inactive drug/fragment into an active drug component opening a new path in the drug development therapy (Fig. 6).

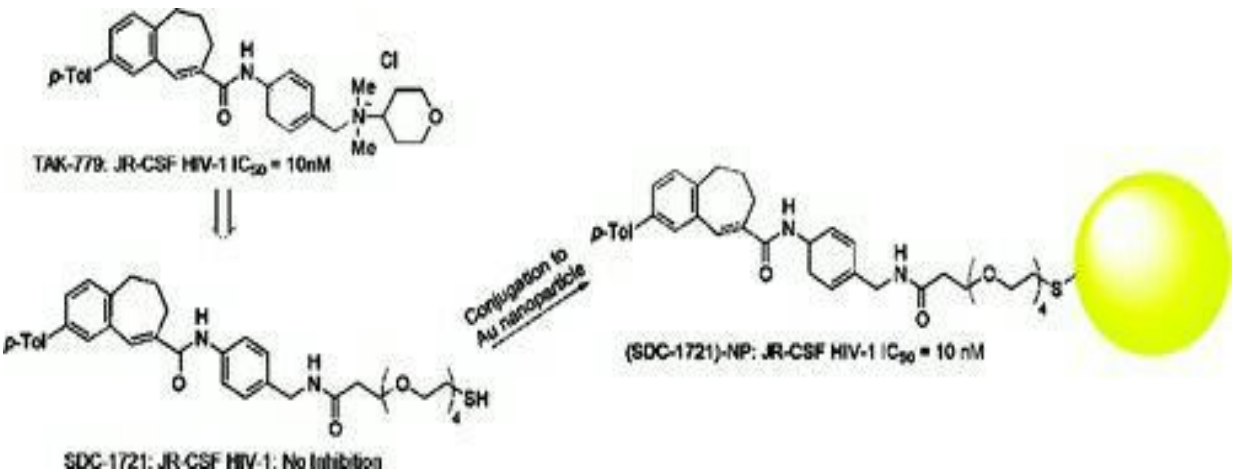


Figure No. 6: Method of synthesizing and conjugation of SDC-1721 with gold nanoparticle

Gold nanoparticles also have some ability on dendritic cells to inhibit HIV 1 infection of human T cells [49]. During HIV infection, the first immune cell that encounters with the virus is dendritic cells, often described as Nature's adjuvant [50] (Fig. 7). But the infection of HIV-1 virus subverts the dendritic cells and successfully reaches the lymph nodes where it replicates. Of all the receptors, dendric cell specific ICAM 3 grabbing non- integrin (DC SIGN) a major receptor on dendritic cells for HIV1, this receptor binds with the virus through multivalent and ca2+ dependent protein carbohydrate interaction with N linked high mannose glycan clusters on GP120 HIV. Thus with the size of 1.8 nm oligomannoside coated nanoparticle blocked the DC SIGN receptor in turn to inhibit the human T cell Trans infection of dendritic cells.

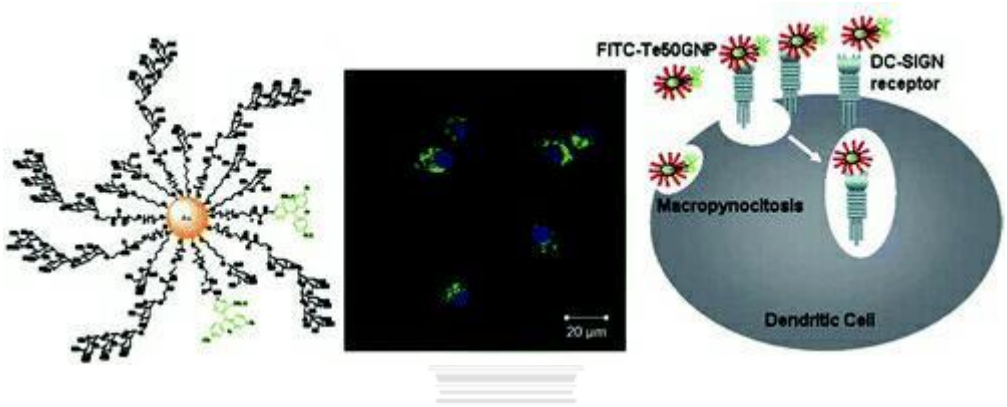


Figure No. 7: Inhibition of DC SIGN in dendritic cells

The development of an effective vaccine for HIV infection is still under research following many failures at the clinical trials [51]. In a way to achieve a higher efficacy against HIV-1 infection, the vaccine induced neutralizing antibodies (NABs) used together with the silver nanoparticles. The additive effect of nanoparticle's to four NABs HIV-1 gp41 126-7, HIV-1 gp120 Anti-serum PB1 Sub 2, HIV-1 gp120 Antiserum PB1, HIV-1 gp120 Monoclonal Antibody F425 B4e8 also tested. The overall combination, together AgNPs and NABs produced increased inhibitory effect [52].

CONCLUSION

In future, medicinal herbs secondary metabolite along with nanoparticles i.e. nanomedicine could be the only source of alternative medicine to control HIV and other opportunistic viral diseases. A limited research has only been performed using medicinal plants and nanoparticles, so more research is needed along with validated proof with single herb or multi herb formulation for controlling HIV viral disease. This review gives a path for medical

practitioners and scientists for further exploration on medicinal plant towards a generation of Nanomedicine in drug development.

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