

Exploration of Phytochemicals Found in *Terminalia* sp. and their Antiretroviral Activities

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ABSTRACT

The human immunodeficiency virus (HIV) infects cells of the immune system and destroys their function. Approximately, 2 million people die every year from HIV as reported by the World Health Organization. HIV/AIDS is difficult to treat as the virus continuously develops resistance to drugs being developed. Approach is now turning toward natural products for the development of anti-HIV drugs. Although HIV/AIDS is not a new disease, but research based on plant-derived products is still under clinical trials. Experimentally, it has been proven that plants have the potential for HIV treatment. The process involves identification of the active ingredients responsible for the reported anti-HIV activities, testing of the extract, and development of appropriate bioassays. Further development would require optimization of the formulation and manufacturing in compliance with preclinical safety and efficacy testing. The most challenging task for the natural product scientists is to separate these highly complex extracts containing several compounds into its individual components that are biologically active. Recently developed direct binding assay with mass spectrometry (MS) technology (viz., real-time time-of-flight-MS) is helpful in this respect but needs extensive optimization. At present, we have compiled all the information for the various phytochemicals present in *Terminalia catappa* having anti-HIV properties. These include tannins, gallotannins, ellagitannins, cyanidin, and flavonoids. Further, we have also discussed their pharmacological as well as pharmacokinetics studies.

Key words: AIDS, flavonoids, human immunodeficiency virus, pharmacokinetics, pharmacology, tannins

INTRODUCTION

Plants are the important source of several medicines for various incurable deadly diseases as known from literature (several thousand years old). Natural products obtained from the plants are known to be potential source for pharmaceutical applications. It has been found that about 80% of world population still believes in traditional medicine as compared to modern days' drugs therapy (WHO). Generally, a number of current drugs either mimic the naturally occurring molecules or have structures that are fully or in part derived from natural moieties.^[1] From ancient times to modern research, plants have made important contribution to the field of science due to their large number of medicinal properties. The scientific reports on the family of *Combretaceae* concluded that this family is very rich in secondary metabolites. These metabolites have significant physiological as well as pharmacological effects (www.wikigenes.org/e/mesh/e/20357.html).^[2,3] This family has attracted keen attention due to its potential medicinal applications in diseases such as cancer, diabetes, cardiac arrest, hepatocarcinoma, human immunodeficiency virus (HIV), and hepatitis. Thus, they have solution toward various incurable diseases. *Terminalia catappa* is an important member of this family, which is also known as Indian Almond, Malabar

Almond, and Tropical Almond. The leaves from *Terminalia catappa* contains various phytochemicals known for curing rheumatic joint pain, leprosy and skin diseases. Further, bark extract from the same plant is diuretic as well as cardiotoxic.^[4,5] The extract of *T. catappa* leaves inhibits Lewis lung carcinoma cells that contribute to lung cancer.^[6] Ethanolic extract of *T. catappa* leaves has been shown to protect against acute liver injury produced by some hepatotoxicants.^[7] It has been found that *T. catappa* inhibits the occurrence of preneoplastic lesions in rat colon carcinogenesis.^[8] It has been demonstrated that hydrolysable ellagitannins and other tannin-related compounds from leaves and bark of *T. catappa* have shown to inhibit HIV replication in infected H9 lymphocytes with little cytotoxicity.^[9-11]

Antiretroviral drug therapy has been very useful in drastic reduction of morbidity and mortality associated with HIV infection. Further, the use of combination drug therapies has significantly improved HIV patients' chances for long-term survival. Unfortunately, the effectiveness of antiretroviral therapy has been markedly reduced by the emergence of drug resistance. It has been reported recently that ~76% of world population exhibited resistance to single or multiple antiretroviral drugs (www.who.int/drugresistance/hivaids/en/).^[12,13] The presence of antiretroviral drug resistance is an important cause of treatment failure in HIV patients. There are multiple classes of synthetic antiretroviral drugs present in the market. These drugs are not been so successful as predicted due to emergence of drug-resistant virus which has complicated the HIV/AIDS therapy. Therefore, it requires intensive

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studies on the mechanism of drug resistance to come out with suitable solutions towards the disease. Lots of efforts are being made to discover new agents and classes, particularly from natural source to find solution with emerging HIV drug resistance.^[14-16] The search for safe and effective therapies to treat infections caused by the HIV and related opportunistic infections is among the highest priorities of the National Institutes of Health. NIAID, NIH has provided the chemical structures of all the anti-retroviral drugs known till date and their efficacies. It would be helpful for researchers for background information related to role of various functional groups for developing effective anti-retroviral drug with no side effects. Information on compounds evaluated preclinically for HIV and the opportunistic infections is acquired by continuous surveillance of primary literature sources (UNAIDS: Joint United Nations Programme on HIV/AIDS).

This review will focus on the various phytochemicals present in *T. catappa* having potential anti-HIV activity and their pharmacokinetics studies. The overview of the present review is shown in Figure 1. These analyses would assist investigators to elucidate what has been done with each phytochemical and their exploitation in HIV-therapeutics.

Morphological Description and Scientific Classification (www.cabi.org/lsc/datasheet/53143)

T. catappa L is distributed across the tropical region of the world containing mesic and wet coastal climate. However, Malaysia is known to be the originator of *T. catappa L*. *T. catappa* has shown strong salt, drought, and wind tolerance due to which it has premier significance to plant molecular biologists. The almond tree (*T. catappa*) has heights of 15–25 m, characterized by horizontal branches in circles at different levels on the trunk, large leathery leaves broadest toward apex, turning reddish before failing, and the elliptic slightly flattened greenish or reddish fruits containing a large edible seed or nut inside the hard husk. Fruits on ripening turn from green to purplish yellow and contain a hard shell covering edible seed. The ripe mesocarp (favorite target of larval infestation) of the fruit is mostly consumed by children neglecting the seed, which contains oil. Fruits are light brown in color with 5-10 cm in length and 2.5-3.5 cm of width having winged edges and pointed ends. The thin outer layer is slightly sour which can also be eaten. Inside the hard fibrous husk, there is a light brown, thick hard stone containing



Figure 1: Herbal medicine versus synthetics drugs for antiretroviral therapy: *Terminalia catappa* is one of the medicinal plants having antiretroviral constituents present in its leaves, bark, and seeds

an oily seed or nut ~3 cm long and 1 cm broad, somewhat like the true almond. The major countries growing this plant include Italy, Spain, Morocco, France, Greece, and Iran. Its flowers appear between April and May and between September and October. The fruiting season is from October to April. Leaves are alternate but crowded near ends of twigs, with stout finely brown hairy leaf stalks (1–2 cm). It has been found that leaves are preferred shed for oriental fruit flies. Flower clusters (narrow racemes) are found at leaf base which is 5–15 cm long. Flowers are numerous, small greenish-white (5–6 mm) across, mostly short-stalked with slightly unpleasant odor, mostly male and a few bisexual flowers near base (polygamous). Bark is gray, smoothish, thin, becoming slightly fissured. Inner bark is pinkish-brown, slightly bitter, and astringent. The heartwood is reddish brown while sap wood is pale yellow with coloration changes with age of the wood. The wood of *T. catappa* is hard and heavy with coarse texture along with interlocked grain. It is very susceptible to attack by dry-wood termites.

Phytochemistry, Pharmacology, and Pharmacokinetics of Anti-Human Immunodeficiency Virus Constituents from *Terminalia catappa*

Flavonoids, hydrolyzable ellagitannins, and other tannin-related compounds have been isolated from the leaves, bark, seeds, and fruits of *T. catappa*. Several of these phytochemicals were shown to inhibit HIV replication in infected H9 lymphocytes with little cytotoxicity. A lot of pharmacological studies have confirmed that the extract of leaves, bark, and fruits of *Terminalia sp.* has anti-HIV reverse transcriptase (RT) activities.^[15,17-19] Schematic representation of steps involved in isolation, identification, and characterization of phytochemicals having anti-HIV or other biological activities is shown in Figure 2. The pharmacological and pharmacokinetic investigations of these complex phytochemicals present significant analytical challenges. These challenges include lack of availability of standards and/or biomarkers for many molecules of interest, detection limitations of the analytical methods, and chemical complexity of a botanical extract requiring multiple sample preparation

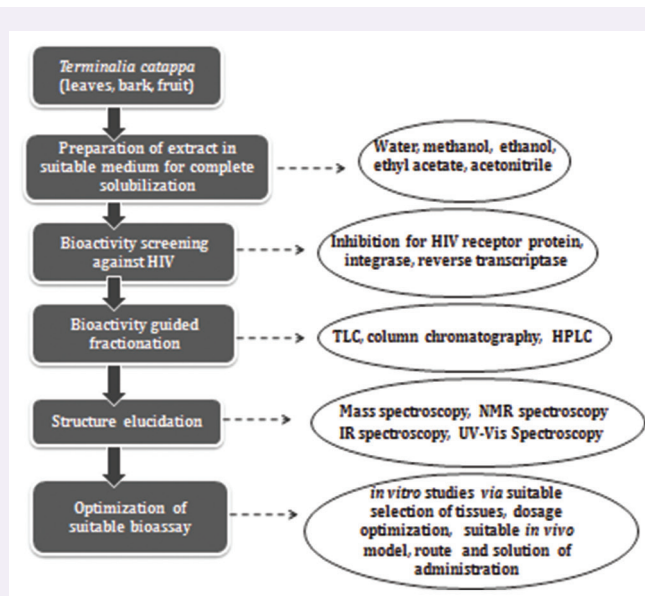


Figure 2: Schematic flowchart showing identification, isolation, and characterization of phytoconstituents present in various parts of *Terminalia catappa* having antiretroviral properties

steps. Recent techniques, such as liquid chromatography (LC) alone, or combined with mass spectrometry (MS), have been very sensitive in the detection and quantification of chemicals in phytochemical and pharmacokinetic studies.^[20] These techniques are helpful for analysis of samples taken from body fluids (serum, urine etc.) using authentic chemical standards. New mass spectrometric capabilities such as direct analysis in real time (DART)/time-of-flight MS (TOF-MS) can overcome these limitations.^[21] *In vitro* studies of phytochemicals found in *T. catappa* having anti-HIV properties are being an important target to use them as herbal-based dietary supplements to help AIDS patients. In the following sections, we have compiled the data elucidating pharmacology and pharmacokinetics of various anti-HIV phytochemicals from *T. catappa*.

Flavonoids are phenolic compounds which include: cyanidins, proanthocyanidin, flavonols and flavononols. Flavonoids may occur naturally in glycosylated or nonglycosylated (aglycone) forms, esterified, and/or methylated. Modified forms of flavonoids are bioavailable for both animals and humans.^[22] Methylated flavonoids can easily be metabolized by the kidneys as compared to unmethylated flavonoids. Further, glycosylated flavonoids are found to be easily absorbed by oral mucosa but could not be absorbed by stomach due to removal of glycan moiety. Gastrointestinal (GI) tract microflora has a major impact on flavonoid uptake as they actively transform and metabolize these chemicals. The precise mechanisms by which flavonoids are taken up by the GI tract are not fully known. It has been recently studied about significance of intestinal epithelial cell transmembrane and cytosolic β -glycosidases in the absorption of flavonoids. Two mechanisms have been suggested for their uptake:^[23]

1. Involvement of active transport by a glucose transporter of the hydrophilic glycosides into the cytosol of the epithelial cells of the intestinal lumen where they are deglycosylated to form the hydrophobic aglycones that passively diffuse across the membrane into the portal bloodstream
2. Deglycosylation of glycosides by a transmembrane β -glycosidase, followed by transportation of the aglycones to the cytosol and then into the bloodstream.

Tannins are found to decrease food intake, growth rate, feeding efficiency and protein digestibility using experimental animals.^[24] Tannins could be carcinogenic as found from pharmacokinetics studies of tannin rich foods like betel nuts, cranberries, blueberries, etc. However, other reports have indicated that the carcinogenic activity of tannins might be related to components that are associated with tannins, rather than the tannins themselves.^[25,26] Tannins have also been reported to exert other physiological effects, such as the acceleration of blood clotting, a reduction in blood pressure, a decrease in serum lipid levels, liver necrosis, and modulation of the immune response. Therefore, despite having medicinal implications, viz., anti-HIV from *T. catappa*, they have poor bioavailability. Further, geographical location has significant effect on the chemical composition of tannins that impair their standardization for medicinal purposes. Concise summary of role of various phytochemicals found in *T. catappa* having anti-HIV properties at various steps of viral infection is shown in Figure 3.

Tannins, Gallotannins, and Ellagitannins

Phytochemistry

Phytochemical properties of tannins, gallatannins, and ellagitannins are best characterized by flow injection analysis (FIA) by electrospray ionization ion-trap multiple stages (ESI-IT-MSⁿ) and high-performance LC with photodiode array detector (HPLC-PDA) [Figure 4].^[27-29] The mass spectra generated in negative mode by FIA are shown in Figure 5a. The precursor ions have $[M-H]^-$ m/z 1083.19, 781.46, 635.32, and 301.30 corresponding to punicalagin, punicalin, gallagic acid, and ellagic acid, respectively. The chromatographic profile by

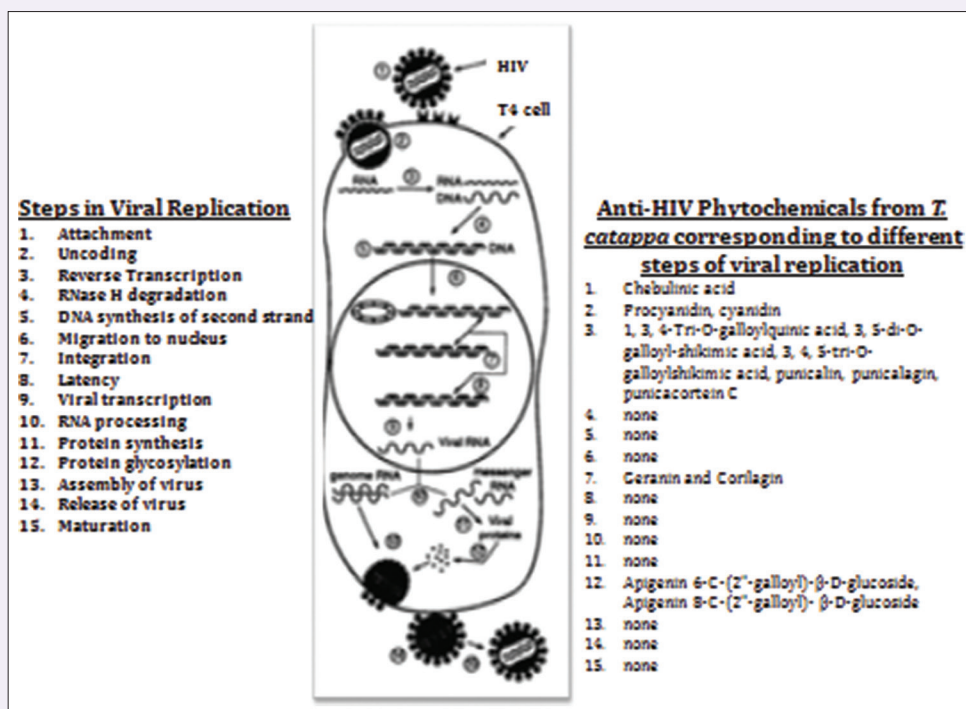


Figure 3: Various stages of human immunodeficiency virus (retrovirus) infecting human T4 cells, correspondingly shown phytoconstituents from *Terminalia catappa* active against particular stage of infection

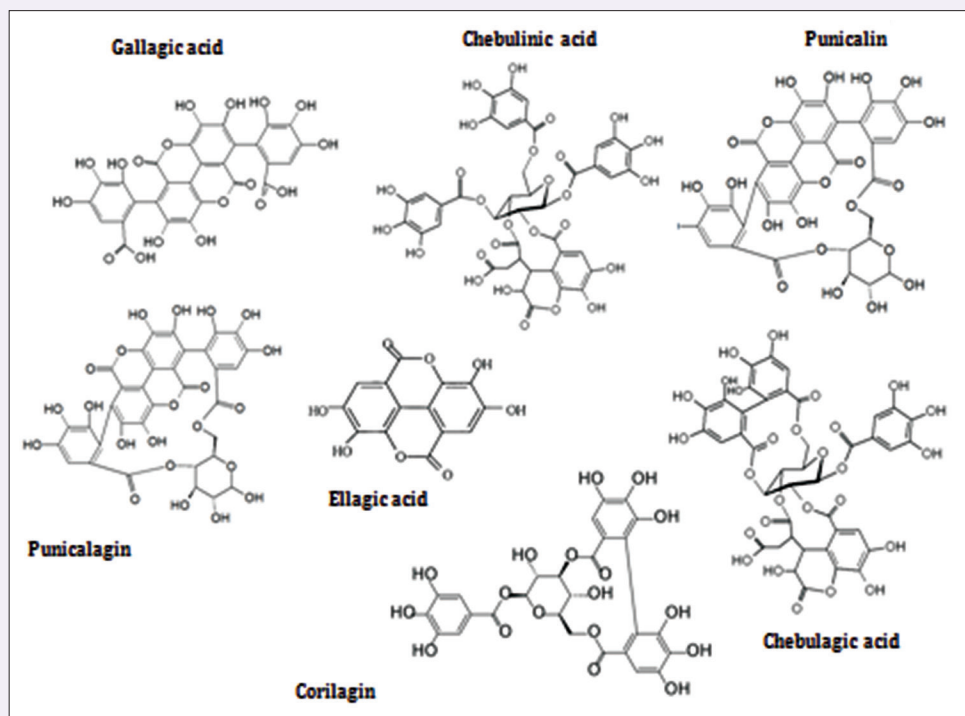


Figure 4: Examples of tannins, gallotannins, and ellagitannins from *Terminalia catappa* having antiretroviral activities (chemical structures are drawn using ChemDraw)

HPLC-PDA has found retention time (t_r) of 14.75 min, 18.80 min, and 47.30 min corresponding to punicalagin, gallagic acid, and ellagic acid, respectively. The ultraviolet (UV) absorption maxima (λ_{max}) of punicalagin were at 218, 260, and 379 nm while that of ellagic acid at 250, 306, and 368 nm. In solution, punicalagin exists as the mixture of anomers of α - and β -punicalagin having t_r of 15.29 min and 21.07 min as found from HPLC-PDA. Corilagin can be best analyzed using HPLC by Hanbon-Kromasil 5 μ m C₁₈ column at 30°C with the wavelength of 268 nm for detection having mobile phase of 0.5% phosphoric acid and methyl cyanide in a ratio of 76:24. Corilagin has t_r of 13.70 min with absorbance maximum at 267 nm by UV-Vis spectroscopy. Analysis of chebulagic acid and chebulinic acid by HPLC-PDA having high-speed counter-current chromatography column (HSCCC, viz., Alltima C18 column) has found their t_r of 15 min and 27 min, respectively [Figure 5b]. HSCCC is a very specific technique with specificity of almost < 90% toward chebulagic and chebulinic acid with respect to any other plant tannin. Analysis using fast-atom bombardment (FAB)-MS in negative mode has found that $[M-H]^-$ m/z was 953 and 955 for chebulagic and chebulinic acid, respectively. Absorbance maxima (λ_{max}) of gallic acid, ellagic acid, chebulinic acid, and chebulagic are: 215, 271, 275, and 278 nm (in water) and 253, 364, 297, and 305 nm (in methanol), respectively as found using UV-Vis spectroscopy.

Gallic acid and its derivatives like ethyl and methyl gallate, chebulagic and chebulinic acid, tetra and penta-galloylglucose are characterized by reverse phase HPLC (phenomenex luna 10 μ m C₁₈ column with 0.2% formic acid in mobile phase). Gallic acid is a polar compound with respect to methyl gallate and ethyl gallate as found by its earlier elution by reverse phase HPLC. Ellagic acid is least polar with respect to gallatannins due to very long retention time (t_r). Chebulagic and chebulinic acid have almost similar structures, but they have different t_r s. It is due to the fact that chebulagic acid has biphenyl linkage between two

galloyl groups, which increases its polarity, thereby causing its elution earlier than chebulinic acid. The t_r of penta-O-galloyl- β -D-glucose and chebulinic acid is very close; therefore, they eluted out as mixture.

Pharmacology and pharmacokinetics

In recent years, more and more often been reported antiviral activity of various ellagitannins. Ellagitannins are known to be potential inhibitors of various enveloped viruses due to its capability to bind different proteins and altering their structure to inactivate them.^[30] However, their mechanism of inhibition is not known. There are several experiments showing effect of inhibition of enzyme reverse transcriptase (RT) and concluded them to be a potential target in the inhibition of replicative cycle of HIV.^[31,32] In case of ellagitannins such as geranin and corilagin, they reduce HIV replication by inhibiting HIV-1 protease and HIV-1 integrase enzyme. Other gallo- and ellagi-tannins such as 1,3,4-tri-O-galloylquinic acid, 3,5-di-O-galloyl-shikimic acid, 3,4,5-tri-O-galloylshikimic acid, punicalin, and punicalagin are evaluated as potential inhibitors of HIV replication. Punicalin and punicalortein C were found to inhibit purified HIV RT with ID_{50} of 8 μ M and 5 μ M, respectively. Tannins from *T. catappa* have shown to inhibit virus (HIV)-cell interactions by inhibiting RT, but mechanism of inhibition is not known till date.^[15,17-19]

A study on bioavailability of punicalagin in the rats reported that punicalagin and its metabolites were observed in feces, urine, as well as plasma, but the mechanism of intestinal uptake of punicalagin was unknown. Although the bioavailability of punicalagin was reported, the mechanism of its transportation through blood across the body system was not studied so far. The low cytotoxicity and high functional attribute at its low concentration, its bioavailability across the body have rendered the punicalagin as multifunctional bioactive molecule, which can be employed for human health benefits. In one of the report based on bioavailability of punicalagin, it has been found from analysis of

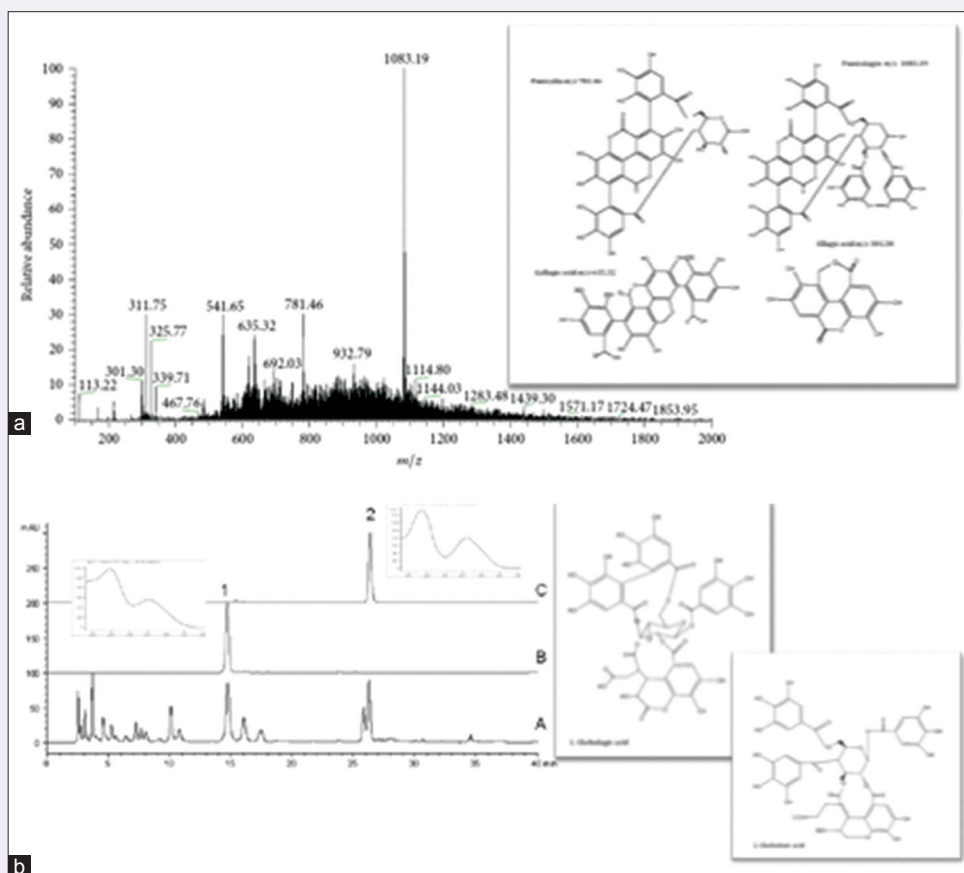


Figure 5: (a) First-order mass spectrum of the hydroalcoholic extract from the leaves of *T. catappa* in the negative mode using flow injection analysis-electrospray ionization ion-trap multiple stages, range of ions with m/z 100–2000 Da. Inset shows punicalin, punicalagin, ellagic acid, and gallagic acid with their obtained m/z values. (b) High performance liquid chromatography chromatogram of hydroalcoholic extract from the leaves of *Terminalia* sp. obtained by high-speed counter-current chromatography column using Alltima C_{18} column (250 mm \times 4.6 mm id, 5 μ m) with mobile phase (ACN-0.1% aqueous phosphoric acid) in a gradient mode (17.83–23.77) in 30 min. The flow rate was 1.0 ml/min, and the effluents were monitored at 216 nm by a photodiode array detector; (A) crude extract, (B) fraction "I" obtained by high-speed counter-current chromatography, (C) fraction "II" obtained by high-speed counter-current chromatography, peak 1: chebulagic acid, peak 2: chebulinic acid. Inset I: ultraviolet spectra of chebulagic and chebulinic acid (adapted from^[27-29]), Inset II: chemical structures of chebulagic and chebulinic acid

serum, urine and fecal samples obtained from rats ($n = 5$) fed with diet containing 6% punicalagin for 37 days using HPLC-MS-MS that only 3-6% of ingested punicalagin was excreted. In case of serum samples, approx. 30 μ g/mL of punicalagin was detected. Punicalagin has been hydrolyzed into 6H-dibenzopyran-6-one derivative in all cases (plasma, urine and feces). Therefore, it can be concluded that left over punicalagin has been either be accumulated in the non-analyzed tissues or it has been converted into undetectable metabolites.^[33]

Flavonoids

Cyanidin and procyanidin

Phytochemistry

Cyanidin and procyanidin are best solubilized in ethanol [Figure 6].^[34,35] They can be purified from the seeds of *Terminalia* sp. using Sephadex LH20 column [washing solution is methanol: water: formic acid (60:39.8:0.2) and elution solution is acetone: water: formic acid (60:39.8: 0.2)]. Their phytochemical properties are best characterized by MS (LC-MS/MS). In case of cyanidin and procyanidin, major signals were distributed in the m/z range of 250–1000 and 1000–2000, respectively [Figure 7]. Cyanidin is singly charged with m/z of 291.3 while procyanidin is doubly charged

with m/z of 1443.8. The procyanidin exists in dimer and trimer, giving parent peak (P) of (M+H)⁺ along with isotopic peaks (P+n) but no fragment peak of (P-2). It has suggested that there is polymerization of procyanidin but no fragmentation. Ultrafiltrate (3000 NMWCO filter) of procyanidin solution on being subjected to LC-ESI/MS analysis, major peaks were m/z 291 (P_1), 579 (P_2), 731 (P_2G_1), 867 (P_3), 1019 (P_3G_1), 1155 (P_4) and 1443 (P_5) using LC analysis (0 to 45 min). Further, supporting polymerization of procyanidin with no fragmentation.

Pharmacology and pharmacokinetics

CD4 molecule is the major cellular receptor for HIV along with other coreceptors (chemokine receptors such as CCR2b, CCR3, CCR5, and CXCR4). Recently, it has been found that the expression of CCR3 on Th-2 cells appears to be a prerequisite for the infection of the central nervous system. Further, receptors such as CCR5, CCR3, and CCR2b are present in the brain endothelial cells and facilitate HIV entry through blood-brain barrier. Procyanidin and cyanidin are known to exhibit significant anti-HIV effects, but no reports on the molecular mechanisms underlying this activity are known.^[36,37] It is believed that they might downregulate HIV-1 coreceptor gene expression and inhibit binding of virus to the cell containing these receptors and thus preventing their entry by inhibiting HIV attachment and uncoding.^[38,39]

Pharmacokinetic studies of cyanidin have found that it is readily taken up and detected in the blood within 20–30 min of ingestion when delivered as both a drink and in lozenge form. It has been found that maximum serum levels of cyanidin were reached between 30 and 75 min when delivered as a drink. The concentration of cyanidin in serum was found to be 3.1 nM and 5.4 nM using LC-MS and DART TOF-MS, respectively. The concentration of cyanidin in urine was 27.5 nM (LC-MS). Thus, it can be concluded that bioavailability of cyanidin taken as drink is approx. 0.2%. In case of cyaniding administration in the lozenge form, bioavailability was 20% as found from sample analysis using serum and urine. Therefore, lozenge form is more preferred for procyanidin administration. There is no literature related to bioavailability of procyanidin till date.^[21,40]

Flavonoid glycosides

Phytochemistry

Flavonoid glycosides can be easily purified by sequential usage of Diaion HP-20 and Sephadex LH-20 columns [Figure 8].^[41-43] There are various recent techniques which are used for analyses of various flavonoid glycosides. Particularly, apigenin, apigenin

6-C-(2"-galloyl)- β -D-glucoside, isoorientin, vitexin, isovitexin, and rutin are significantly important in their role of HIV inhibition. LC-MS/MS analyses using diode-array detector and Luna C₁₈ column have found their m/z values in the negative mode as 269, 271, 216, and 251 for apigenin, apigenin 6-C-(2"-galloyl)- β -D-glucoside, isoorientin, vitexin, and isovitexin, respectively. Figure 9 shows collective summary of apigenin, apigenin 6-C-(2"-galloyl)- β -D-glucoside, isoorientin, vitexin, and isovitexin using MS.

Summarized information on characterized properties of flavonoid glycosides has been given below:

Apigenin 6-C-(2"-galloyl)- β -D-glucoside

It is a yellow amorphous powder with m/z corresponding to 585 in the positive mode (FAB-MS) with infra-red absorbance lying in the range from 830 to 3350 nm. Analysis from UV-Vis spectroscopy has found that it has absorbance maximum at 279 nm in the solvent containing methanol and sodium acetate while minimum at 382 nm in the solvent containing methanol and aluminum chloride.

Apigenin 8-C-(2"-galloyl)- β -D-glucoside

Its optical and spectroscopic properties as found from MS and infra-red spectroscopy are exactly same as that of apigenin 6-C-(2"-galloyl)- β -D-glucoside. However, difference lies in the analysis from UV-Vis spectroscopy. It has absorbance maximum at 273 nm in the solvent containing methanol and sodium acetate and minimum at 313 nm in the solvent containing methanol and aluminum chloride. Therefore, UV-Vis spectroscopy is the best technique in distinguishing epimeric states of apigenin β -D-glucoside.

Isovitexin

It is yellow amorphous powder with m/z of 433 in the positive mode using FAB-MS having infra-red absorbance maximum lying at 1590 nm. Absorbance maximum is found to be sharp at 280 nm in methanol while absorbance minimum is not sharp rather blunt lying at 383 nm in methanol, aluminum chloride, and hydrochloride.

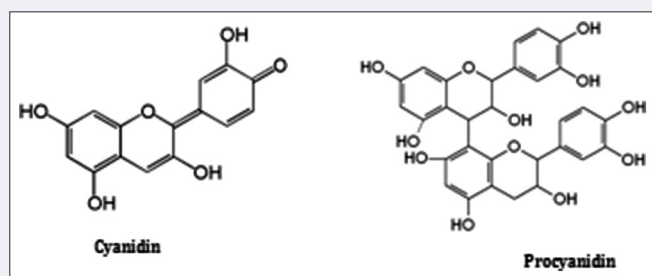


Figure 6: Chemical structures of cyanidin and procyanidin (structures are drawn using ChemDraw)

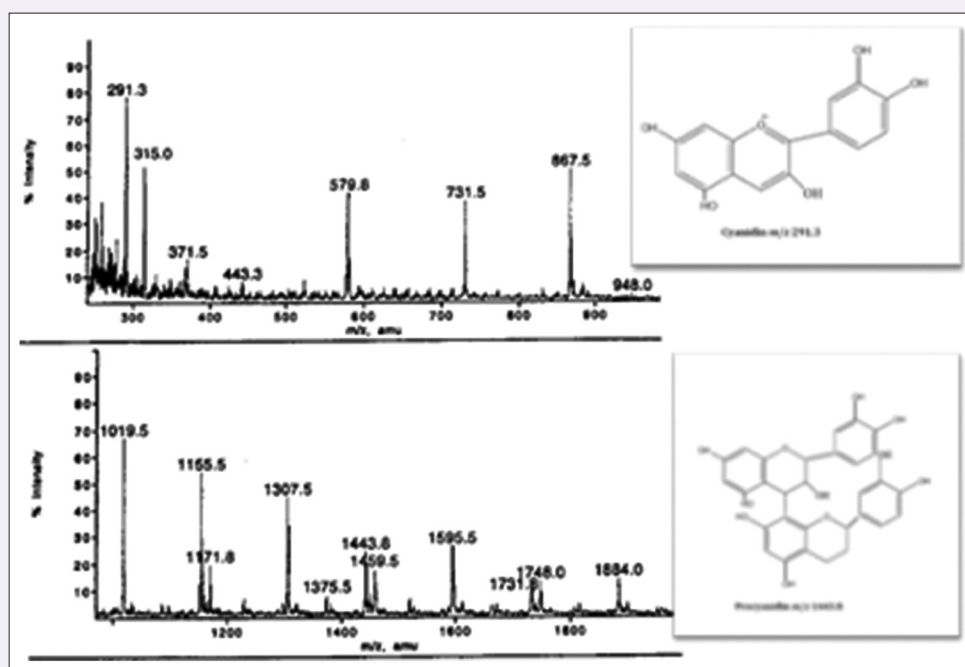


Figure 7: Spectral data of cyanidin and procyanidin polymer peak of *Terminalia sp.* seed extracts as determined by electrospray ionization/mass spectrometry. The mass spectrum of the cyanidin and procyanidin polymer peak was collected in the m/z ranges 250–1000 and 1000–2000, respectively (adapted from^[35])

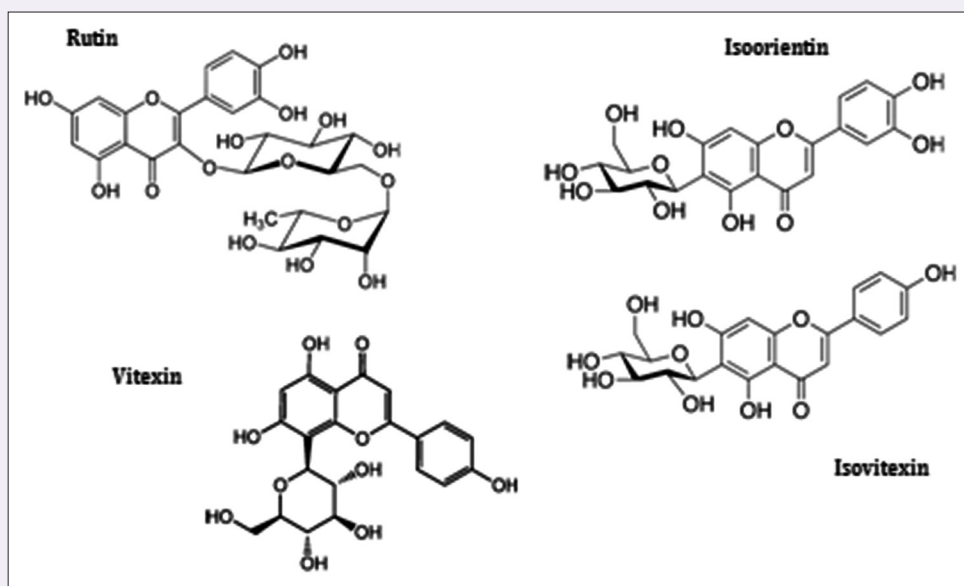


Figure 8: Examples of flavonoid glycosides from *Terminalia catappa* having antiretroviral activities (chemical structures are drawn using ChemDraw)

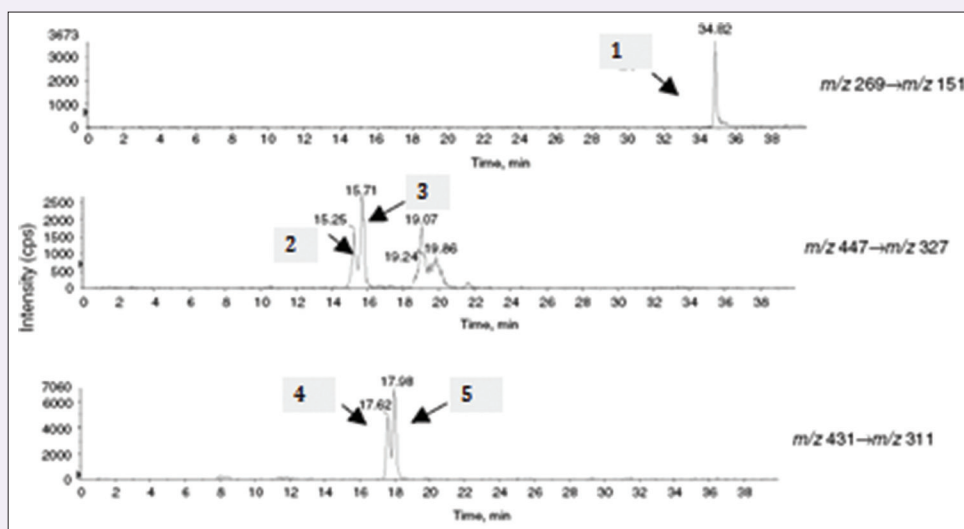


Figure 9: Prepared ethanolic extracts of *Terminalia catappa* dried leaves purified by Diaion HP-20 (H₂O-Methanol gradient) and Sephadex LH-20 column (80: 20 methanol: H₂O) were subjected to mass spectrometry by liquid chromatography MS/mass spectrometry using multiple reaction monitoring mode (adapted from ^[42,43])

Vitexin

It is similar in almost all respects of optical and spectroscopic properties as observed by infra-red spectroscopy and MS. However, it has blunt absorbance maximum at 270 nm as found from UV-Vis spectroscopy when methanol was used as solvent. Further, absorbance minimum is also not sharp rather a blunt peak at 384 nm in the solvent containing methanol, aluminum chloride, and hydrochloride.

Isoorientin

It looks similar to isovitexin and vitexin in coloration as well as analysis by infra-red spectroscopy. MS has found that it has m/z values corresponding to 449 in the positive mode using FAB-MS with absorbance maximum sharp at 276 nm in methanol and minimum at 300 nm in methanol, aluminum chloride, and hydrochloride using UV-Vis spectroscopy.

Rutin

It can be crystallized with peculiar yellow coloration having melting point at 242°C with m/z in the positive mode at 611 (FAB-MS) with infra-red absorbance maximum at 1660 nm.

Pharmacology and pharmacokinetics

There are a number of flavonoid glycosides known from *T. catappa* as reported above to exhibit anti-HIV activities, but their mode of action is not known yet. There are very few studies on apigenin and derived glycosides from *Terminalia sp.* as compared to other plants. It has been found that apigenin and derived glycosides have inhibited HIV expression in tumor necrosis factor alpha (TNF α)-treated OM-10.1 cultures with therapeutic index lying in between 5 and 10. Further, return to viral latency in OM-10.1 cells (exposed to TNF α) over a

shorter time interval when apigenin was added. However, the inhibition of HIV activation was independent on preincubation of apigenin along with TNF α . It was also found that nuclear factor-kappa B activation was not inhibited by apigenin. Thus, there is some novel mechanism adopted by apigenin. It could be a potential candidate for therapeutics aimed at maintaining a cellular state of HIV-1 latency.^[44,45]

Apigenin has been reported to interact with P-glycoprotein (P-gp) efflux pump present in Panc-1 cells and thus, helps in significant increase in cellular concentration of various drugs including antiretroviral drugs. Besides acting as anti-HIV, apigenin can also act as efficient drug delivery agent. Bioavailability and pharmacokinetics of apigenin have been studied by its oral and intravenous administration in rats. In one of the experiments, the plasma concentration-time profiles of drug A after an oral administration in the absence or presence of apigenin were recorded. The presence of apigenin significantly altered the pharmacokinetic parameters of oral drug A. Compared to the control group (given only drug A), the presence of apigenin has significantly increased by ~50% area under the plasma concentration-time curve (AUC) and the peak plasma concentration (C_{max}) of the oral drug A. Consequently, the absolute bioavailability of drug A was significantly increased by 30% by the concurrent use of apigenin. The presence of apigenin has prolonged the terminal plasma half-life ($t_{1/2}$) of oral drug A. In case of intravenous administration of drug A in the presence or absence of oral apigenin, there was significant increase in AUC with increasing concentration of apigenin. However, the $t_{1/2}$ of drug A was not altered significantly by concurrent use of apigenin with drug A in case of intravenous administration. The cell-based assay using rhodamine-123 indicated that apigenin significantly ($P < 0.01$) inhibited P-gp-mediated drug efflux. Those results suggest that apigenin might be effective to improve the bioavailability of drug A. The enhanced oral bioavailability of drug A in the presence of apigenin could be mainly due to enhanced absorption in the GI tract. Therefore, it has been suggested that apigenin can be used as antiretroviral drug delivery agent leading to improved chemotherapeutics via oral administration.^[46,47]

Isovitexin is known to have antiretroviral activities but there are no reports on its mode of action during viral infection. The pharmacokinetics studies of isovitexin were done using rat as model system. Solutions of isovitexin were prepared in propylene glycol-redistilled water (1:9, v/v), followed by filtration via 0.22 μ m syringe. Three groups of rats (each containing 6 rats with 3 male and 3 females) were taken and administered with isovitexin via tail vein injection at dose of 18.75, 3.75 and 0.75 mg/kg, respectively. After isovitexin injection, 0.3 mL of blood samples were drawn at 2, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min and followed by its plasma analysis via HPLC. The pharmacokinetics of the isovitexin in the three different concentrations (18.75, 3.75, 0.75 mg/kg) fit the two-compartment open model. A good linear correlation ($P < 0.01$) was obtained in the correlation and regression analysis of the AUC_{0-t} -dosage while $t_{1/2}$ was dose-independent. Isovitexin has displayed linear dynamics in a dosage range of 0.75–18.75 mg/kg. The pharmacokinetic of isovitexin after oral administration was also studied using dosage of up to 40 mg/kg. Here, it was found that the concentration of isovitexin in the plasma was very low. Therefore, the oral administration of isovitexin was of little practical significance. The tissue analysis based on isovitexin concentration has revealed the order from high to low as kidney > liver > lung > lung \approx ovary > heart \approx spleen > brain. Most importantly, it was found that the isovitexin concentration was highest in the ovary with respect to any other organ. The average concentrations of isovitexin in the ovaries of six rats after 10, 30, and 60 min were 2.81, 1.09, and 0.24 μ g/g, respectively. In case of lung, concentrations of isovitexin were 3.27, 1.23, and 0.91 μ g/g after 10, 30, and 60 min, respectively. Heart and spleen have the least amount of isovitexin with respect to

any organ. The blood flow or perfusion rate of the ovary is known to be lower than that of the above-mentioned organs. It can be concluded that isovitexin has strong affinity with the ovary. Therefore, female rats are the best model system to study role of isovitexin intravenously.^[48]

Rutin is one of the flavonoids, which is found abundantly in herbs as well in plant foods. Studies have found that rutin is not bioavailable when administered intravenously. Therefore, pharmacokinetic studies of rutin have been done by its oral administration. For oral administration rutin was orally administered in a solution prepared in glycofurool at 165 μ mol/kg and 328 μ mol/kg, respectively, via gastric gavage. Blood samples were withdrawn via cardiac puncture at 5, 15, 30, 60, 120, 240, 420, 600, 1440, 2040, 2880, 3480, 4440, and 4920 min postdosing of rutin. The parent forms of rutin were not detected in the blood plasma at any of the concentrations mentioned above. There were very low C_{max} and AUC_{0-t} , indicating that the absolute systemic bioavailability of rutin was essentially zero due to extensive conjugation metabolism during the first pass through gut and liver. One of the crucial reasons for poor absorption of rutin is that it is too hydrophilic to diffuse through cell plasma membrane and it was absorbable only after being hydrolyzed into quercetin which is absorbable.^[49] It should be also noted that quercetin is an effective anti-HIV flavonoid which blocked infection of healthy cells by the virus and inhibited reproduction of the virus from infected cells.^[50,51] Therefore, administration of rutin in the hydrolyzed form (i.e., quercetin) would be an effective therapy for HIV treatment than in its native state.

FUTURE PROSPECTS

WHO has given special attention on usage of multifaceted approach in prevention of HIV drug resistance. It has been strongly recommended for implementation of early warning signals for detection of the emergence of new HIV strain in the particular geographical region. There are various factors which are responsible for failures of introduced antiretroviral drug therapies as summarized below:^[52-54]

1. The HIV RT enzyme has low fidelity (the enzyme is nonselective during the copying from RNA to DNA) due to which prone to errors. It makes one error in each HIV genome per round of replication, i.e. ~0.1 mutation for every 2000 nucleosides
2. HIV has an exceptionally high rate of replication; ~ 10^6 copies/ml in acute infection. This high rate of replication coupled with the high rate of error for RT leads to generation of numerous HIV variants. These variants have different sensitivities to antiretroviral agents which significantly complicate the selection of drugs during therapy
3. Irregular usage of HIV medications and inappropriate choice of antiretroviral agent(s) also contribute to HIV drug resistance
4. Pharmacokinetic factors such as poor oral absorption and alteration of drug metabolizing enzymes by other agents and various drug-drug interactions are major contributor during antiretroviral drug resistance.

There are various reported cases of side effects by several drugs used for antiretroviral therapy. The effect varies from person to person, including diarrhea, nausea, vomiting, appetite loss, fatigue, insomnia, peripheral neuropathy, lipodystrophy, increased risk of heart attack, diabetes, nephrotoxicity, lactic acidosis, hepatotoxicity, and pancreatitis. Therefore, detailed knowledge of the pharmacology of antiretroviral drugs and mechanisms of HIV drug resistance would be required for effective pharmaceutical care for HIV-infected patients. Literature is continuously been updated with mechanism and prevalence of HIV drug resistance. However due to ever-changing nature of HIV, it is been suggested that more and more students, practitioners and faculty members should get involved for getting suitable solution to the problem. There are various strategies being used to prevent HIV drug resistance (www.who.int/drugresistance/hiv/aids/en):^[55]

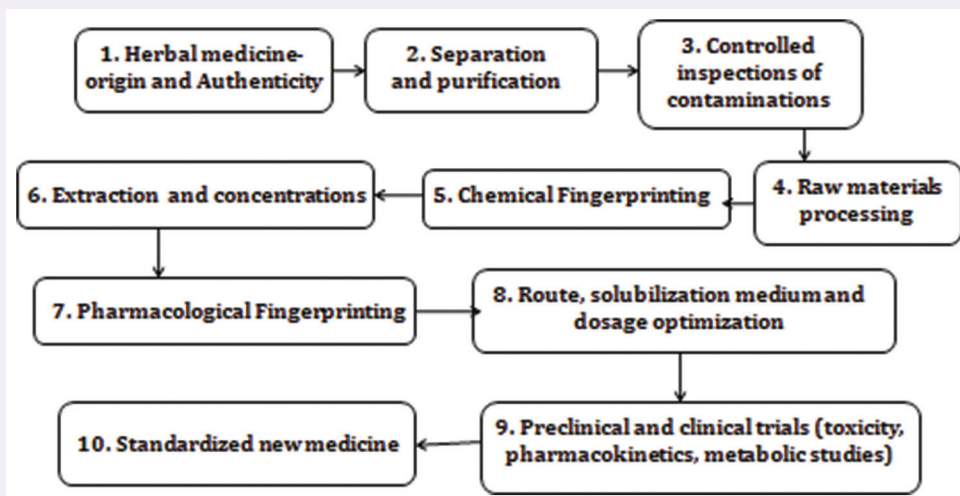


Figure 10: Overview of quality assessment of herbal medicines, requiring over more than 10 years to actually land into the market

1. Strict adherence to their drug regimen helps in obstructing viral proliferation
2. Resistance testing
3. Use of drug combinations
4. Simultaneous usage of antiretroviral drugs from various classes is a highly active antiretroviral therapy. The use of multiple drug classes in conjunction with resistance testing helps in effective check on proliferation of particular HIV strain.

Research across the world is actively involved in synthesizing new formulations of currently available antiretroviral agents allowing for less frequent dosing while maintaining their therapeutic blood levels. Efforts have also been directed in assessing the possible benefits of natural products in HIV treatment. Therapeutic benefits of natural products have been known since several decades ago. They have been found to be effective in several aspects; viz., in diseases epidemic due to strain switching. There are several products known being derived from natural products which are potential protease inhibitors. The use of herbal medicines is found to be more reliable and safe, especially in the developing countries with respect to synthetic drugs.^[56-58] Overview of quality assessment of herbal medicines has been shown in Figure 10, showing various steps being involved before being land in commercial market. The present review has suggested that there are various phytochemicals found in *T. catappa* having antiretroviral activities. The premier obstacles are their recipes for administration, particularly medium of solubilization and its other potential side effects. It would be an important issue for the scientists to investigate the safety and efficacy of pharmacologically active components against HIV derived from natural products. There are various components derived from natural products as mentioned above that they are not active against HIV but helps in efficient drug delivery to the targeted organ. Therefore, the pharmacokinetics of herbal medicine should be investigated which is helpful in preventing side effects from any drug-herb interaction. There is a need for analysis on herbal medicine claiming to have HIV treatment using pharmacokinetic and toxicological studies. Further, there should be systematic studies on the therapeutic efficacy of herbal medicine in addition to clinical trials. It has been found from *in vitro* studies that relatively high concentrations of herbals are being used. These results suggest that biologically active constituents (against HIV) of these herbal remedies have to overcome various inhibitory enzymes and efflux drug transporter systems for its efficacy. However, effective usage of herbal

medicines at such higher concentrations under *in vivo* conditions raises several queries as well as issues related to their possible toxicity to human patients. Thus, there is foremost requirement for precise clinical studies on various herbal drugs against HIV.^[59]

Philosophical views on life and health as well as cultural and personal beliefs have made herbal medicines more effective and successful, particularly in Asia and Africa. Therefore, efforts should be made by mainstream health professionals to provide validated information to those practicing traditional medicine and patients on the judicious use of herbal remedies. Further, initiative in finding herbal cure for HIV/AIDS would be cost-effective and easily accessible by patients. Synthetic antiretroviral drugs are unable to generate immunity and have the number of unpleasant side effects posing another set of health crises to patients. Efforts should be made in determining the safety, efficacy, and pharmacological profile of the several herbal compounds known to have anti-HIV activities.

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Conflicts of interest

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