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Appraisal of antiretroviral herbal medicines with proven efficacy: modulation of drug-metabolizing enzymes and permeability glycoprotein activities

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ABSTRACT

Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) constitutes a source of great concern to health authorities worldwide. Herbal medicinal products are used as a significant treatment option for the highly active antiretroviral therapies, the efficacies of which are negatively impacted by the emergence of multidrug-resistant strains to the recommended treatment guidelines. This review provides an updated synopsis of available documents on herbal medicinal products with anti-HIV activities. Concurrent consumption of herbal products with conventional drugs, which is often necessitated by co-morbidity of HIV with other diseases, can potentially alter the pharmacokinetics of the co-administered orthodox drugs. Phytochemical constituents of the herbal medicinal products with antiretroviral activities were identified, and their potential to mediate pharmacokinetic changes through modulation of drug-metabolizing enzymes and P-glycoprotein was reported. Herb-drug interactions (HDIs) that can result in significant adverse effects were also discussed with documenting the information for the therapeutic utility of these clinically effective antiretroviral herbal medicinal products with potential for development into newer anti-HIV drugs.

Implication for health policy/practice/research/medical education:
Implication for health policy/practice/research/medical education: This study documents and evaluates the plants that are used traditionally in the treatment of HIV/AIDS. Specifically, the active phytochemicals such as andrographolide, lentinan, azadirachtin, thymoquinone, and pinocembrin have the potential and might be novel drugs for the treatment of HIV in the future.

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Introduction

Acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) is a notable global public health matter and constitutes a source of great concern to health authorities worldwide due to the high level of morbidity and mortality (1). However, it has become a controllable chronic health condition with the increasing availability of antiretroviral therapy (ART) occasioned from the global attention to the disease received in the form of significant funding and research. By the end of 2019, about 38.0 million people were globally living with HIV, out of which about 700 000 people died from AIDS-related illnesses in the same year (2,3). Africa bears a disproportionately higher burden of infection where over two-thirds of all people with HIV infection lived in the World Health Organization (WHO) African Region as of the end of 2019 (3).

The standard treatment of HIV/AIDS is highly active antiretroviral therapy (HAART). In spite of introducing ART, not more than 10% of HIV-infected people in

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developing countries, particularly in sub-Sahara Africa, have access to these therapies. Lack of curative agents, drug toxicities and resistance, high cost, and unavailability are among the shortcomings associated with ART consumption (3,4) resulting in more need to search for effective alternatives from natural sources (3). WHO has suggested testing ethnomedicines systematically against HIV to find more options for HIV/AIDS management (5). Thus, herbal medicines are regularly used as a complementary and alternative therapy by people living with HIV. About 26% of HIV-infected people in the USA and 25% in Europe use herbal medicines as a section of their treatments. However, in Africa, where HIV/AIDS is most prevalent, herbal medicines are used as a significant treatment option (6). Much literature on studies of herbal medicines investigated anti-HIV activity, and new compounds have been identified from plant extracts, some of which demonstrated activity against HIV. The mechanisms of anti-HIV activity of some of these isolated compounds have been discovered (4,7,8). Some synthetic derivatives of the anti-HIV natural products have been shown to have stronger *in vitro* inhibitory effects than their natural counterparts (4). These new compounds, when subjected to structure-activity relationship studies, can provide templates for the synthesis of new antiretroviral drugs. There are few reports on full-scale clinical trials using herbal medicines for the treatment of HIV/AIDS. However, there are several randomized studies that have investigated the beneficial effects of herbal medicinal products on patients with HIV/AIDS in which treatments were targeted at ameliorating the co-morbidities, compared with the control group (7-10). In scientific literature, it is estimated that more than 80% of the population, especially in developing countries, rely on complementary and herbal medicinal plants for their primary health care (11), but there are other reports indicating a considerably lower prevalence of alternative medicine use (12). Although the mechanism of action of most herbs is not yet well defined, herbal medicinal products are increasingly becoming popular due to the perception of their effectiveness, fewer incidence of side effects, and low costs compared to conventional medicines (4,8).

Several reports have shown a high prevalence in the use of medicinal plant products concurrently with orthodox medicines, and nearly all disease states are significantly associated with herbal use (13,14). The concurrent use of herbal medicinal products and conventional medicines may offer beneficial effects since it may necessitate the use of lower doses of orthodox medicines with a decrease in the observed adverse effects (15). Furthermore, the worldwide increase in the popularity of alternative medicines has prompted renewed interest and apprehensions about herb-drug interactions (HDIs). Herbal medicinal products contain a complex mixture of compounds of diverse chemical structures. Like any other active pharmacological agents, phytochemicals can modulate the activities of drug-metabolizing enzymes, especially cytochrome P450 (CYP) enzymes, as well as drug transporters such as permeability glycoprotein (P-gp) (16–19). This is in addition to the phytochemical compounds exerting their own intrinsic pharmacological effects. Therefore, the concurrent use of herbal medicinal products with conventional drugs can result in pharmacokinetic and/or pharmacodynamic interactions leading to beneficial effects, therapeutic failure, or drug toxicity.

The CYP enzymes are a superfamily of enzymes containing heme as a cofactor and mediate the metabolism of various endogenous and exogenous compounds (20). Permeability of glycoprotein is an important protein of the cell membrane, which functions as an ATP-activated pump, and is involved in pumping molecules from intracellular to extracellular spaces, particularly in the gastrointestinal tract (GIT), and also found in other tissues, including the cells of the liver, the kidney, as well as the blood-brain barrier. This activity results in decreased drug absorption and/or drug clearance increase (21). Due to the fact that conventional drugs usually contain single chemical entities, while each herbal medicinal product contains numerous phytochemicals, it is reasonable to suggest a higher possibility of HDIs compared to drug-drug interactions. The literature abounds with information on HDIs resulting in either beneficial or adverse effects with serious clinical consequences or lack of interaction (22-24). The knowledge of drug-herb interactions can help to prevent adverse drug effects, and such evaluations are essential to ensure the safety of the concomitant consumption of herbal medicinal products with orthodox medicines.

This paper reported herbal medicinal products that have been found to demonstrate efficacy in the management of HIV/AIDs. We also presented the phytochemical compounds in the herbal medicinal products that mediate the antiretroviral activities and used information from the literature to verify the possibility of modulation of activities of drug-metabolizing enzymes and drug transport proteins by the phytochemicals, which can potentially alter the pharmacokinetics of concurrently administered orthodox medicines. The safety of concurrent use of the herbal products with drugs that are the substrates of the enzymes and/or transporters, which the herbal medicinal products interact with was also highlighted.

Medicinal plants with proven antiretroviral efficacy

Herbal medicinal products have been used for the treatment or management of various disease conditions in developing countries, and there has been a growing increase in the popularity of herbal medicines in developed countries (5,11,25). Due to the constraints of accessibility, cost, drug resistance development, and side effects of HAART, the WHO has recommended that herbal

medicinal products and other natural products should be scientifically investigated for activity against HIV as they may serve as viable alternatives (5). Surveys conducted in different countries indicated a high prevalence (up to 60% in some countries) in the use of anti-HIV herbal medicinal products by HIV-infected patients (7,26-30). In most cases, the mechanism actions of herbal medicines on HIV infections or the associated conditions are not clear, necessitating further research (31).

Kurapati et al (32) extensively reviewed promising natural products for anti-HIV activity. These include ursolic and betulinic acids (triterpenes), calanolides (coumarins), baicalin (flavonoids), lithospermic acid (phenolics), and polycitone A (alkaloid) (32). Since the 1990s, a substantial amount of work has been done on exploring herbal medicinal products and natural products with anti-HIV activity. This suggests a significant potential for natural products for the treatment of HIV infections. However, *in vivo* and human studies are sparse. Traditional Chinese medicines have been subjected to more clinical tests, and a review reported that 12 randomized clinical trials have been carried out (7,33). The traditional Chinese medicines used as anti-HIV therapy all contain numerous herbs, which could be as many as 35 different herbs (34). More than 700 plant species belonging to 151 families have been reported to demonstrate varying degrees of HIV-inhibition activities *in vitro* (4). In this review, in addition to using the limited human clinical trials and epidemiological studies, *in vitro* studies, which could support the observations made in clinical trials to support the molecular mechanisms, were also included.

Medicinal plants that have been shown to be beneficial in the management of HIV/AIDS include:

1. *Andrographis paniculata*

This plan, belonging to Acanthaceae family, is an annual, branched, erect herb between half to one meter in height. It is used for the treatment of various diseases across Asia, America, and Africa, and it has been reportedly cultivated and grown well in most soil types (35). Previous studies revealed that *Andrographis paniculata* has a wide range of pharmacological activities, which include antihepatitis, anticancer, anti-HIV, antidiarrheal, anti-inflammatory, antioxidant, antihyperglycemic, immunostimulatory, and antimalarial activities (36). The juice and fresh or dried leaves are used in various Asian traditional medicines (37).

The compositions of phytochemicals in *A. paniculata* widely differ depending on the harvesting time, the part used, geography, and the season. Phytochemical studies of *A. paniculata* have reported the isolation of several plant metabolites, which include more than 55 diterpenoids, 30 flavonoids, 8 quinic acids, 4 xanthones, and andrographolides A, B, C, D, and E. Andrographolides are the most prominent in occurrence and quantity and reported to be the most important contributor in pharmacological activities of *A. paniculata* (36,38,39). Andrographolides and their derivatives have significant antiviral activities against influenza A, HIV, and Herpes Simplex Virus 1 with no cytotoxic effects. They have also shown a remarkable utility in the treatment of HIV infection (40-42). The chemical structures of andrographolide and some derivatives are presented in Figure 1.

1.1. In vitro anti-HIV activity

Andrographis paniculata is a promising plant for the management of HIV/AIDS. The andrographolide esters were reported as an inhibitor of HIV, inhibiting HIV-1 and HIV-2 (40). Dehydroandrographolide succinic acid monoester was reported to inhibit HIV-1 at the minimal concentration range (average) of 1.6-3.1 (2.0) mg/mL (41), while andrographolide and 14-deoxy-11,12-didehydroandrographolide proven to have anti-HIV activities with EC_{50} of 49 and 57 μg/mL, respectively, in a study where the activities of other derivatives and compounds were compared. These compounds included 14-deoxyandrographolide, andrograpanin, bis-andrographolide, 5-hydroxy-7,8-dimethoxyflavone, 14-deoxy-11,12-didehydroandrographolide, and (±) -5-hydroxy-7,8-dimethoxyflavanone (41).

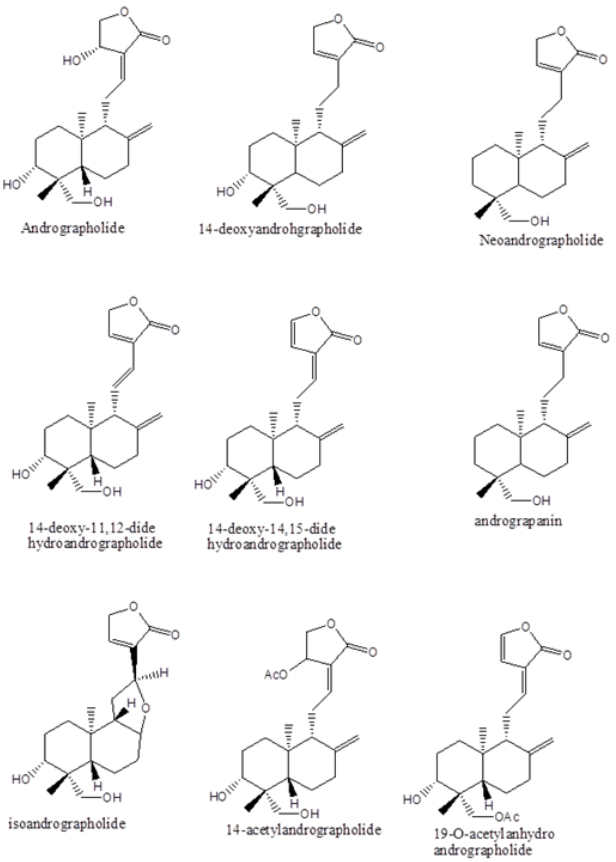

Figure 1. Chemical structures of anti-HIV bioactive phytoconstituents of *Andrographis paniculata*.

1.2. In vivo anti-HIV activity

In a phase I study on dose-escalation, andrographolide from *A. paniculata* was safe, well-tolerated with improved CD4+ T lymphocyte and reduced plasma virion HIV-1 RNA levels (43). The study participants were only given the andrographolide without any orthodox antiretroviral medications throughout the period of the trial. The initial dose of 5 mg/kg was administered for 3 weeks and later escalated to 10 mg/kg for 3 weeks and to 20 mg/kg for a final 3 weeks. The mean CD4+ T lymphocyte level of HIV subjects was enhanced following 10 mg/kg andrographolide administration. Hence, andrographolide can inhibit HIV-induced cell cycle dysregulation, which may lead to the enhancement of CD4+ T lymphocyte levels in HIV-1 infected patients (43).

2. *Lentinus edodes*

Lentinus edodes is a popular edible mushroom in the global market and has therapeutic potential, especially for flu and colds, fungal infection and other infectious diseases, cancer, depressed immunity, environmental allergies, heart disease, hyperlipidemia, bronchial inflammation, hypertension, hepatitis, diabetes and urinary inconsistencies (44). Numerous useful compounds with therapeutic applications have been isolated from this mushroom, which include lentinan, lectins, lenthionine, and eritadenine, which have been found to have differential efficacies as antiviral, antifungal, antioxidants, immunomodulators, hepatoprotective, cardiovascular, and hypolipidemic agents (44). The major compound with anti-HIV activity in this macrofungus has been identified to be lentinan (45,46) (Figure 2). Lentinan is a known immunostimulant polysaccharide, which is made up of β-(1→6) branched β-(1→3)-glucan.

2.1. In vitro anti-HIV activity

The laccase enzyme, produced by *L. edodes*, inhibits the reverse transcriptase of the HIV-1 *in vitro*. From the study conducted on the U373-MAGI cell line infected with recombinant viruses, the enzymatic extract inhibited the replication of HIV-1, suggesting the potential anti-HIV-1 activity (47). The study was conducted using an extract from the culture medium of *L. edodes* mycelia, fractionated by ethanol precipitation. The product blocked both the cytopathic effects of giant cell formation and specific antigen expression due to HIV. Administration of the mycelia without ethanol precipitation was also suggested to block the expression of HIV antigen in MT-4 cells, particularly at a high concentration (48).

2.2. In vivo anti-HIV activity

Similarly, *in vivo* studies revealed that lentinan possessed anti-HIV activity. Gordon et al study demonstrated a trend toward an increase in CD4 cells and in some patients' neutrophil activity in a two-phase placebo-controlled trial on HIV-infected patients (49). Moreover, a multi-site trial

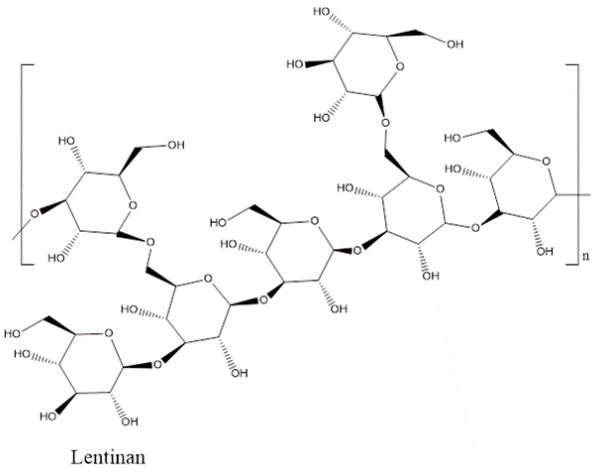

Figure 2. Chemical structure of anti-HIV bioactive phytoconstituent of *Lentinus edodes*.

on lentinan, in combination with didanosine (ddI), was evaluated in a controlled study in HIV-infected patients with low CD4+ T lymphocyte levels with a test and a control group where the control received ddI only. The ddI/lentinan combination caused a significant increase in CD4+ T lymphocyte levels, whereas ddI alone was not significant compared with the baseline values (49).

3. *Azadirachta indica*

Azadirachta indica belongs to the Meliaceae family, occurring in semitropical and tropical regions of the world. It has various pharmacological attributes, including antipyretic, hypolipidemic, antidiabetic, microbicidal, anti-inflammatory, and hepatoprotective activities. Other pharmacological activities include antiulcer, cardioprotective, neuroprotective, antioxidant, insecticidal, and antileishmaniasis properties (50,51). It is a safe medicinal plant modulating the biological processes with no reported side effect (52).

The plant has various components, including carbohydrates, flavonoids, terpenoids, alkaloids, steroids, and fatty acids. About 135 novel compounds with various chemical structures have been extracted from different parts of this medicinal plant like seed, flower, leaf, root, and barks. About sixty different constituents have been isolated from neem leaf, most chemically characterized (53). Among them, 35 are biologically active compounds, and the most important active constituents with different biological activities include nimbolinin, azadirachtin, nimbin, nimbidol, nimbidin, gedunin, quercetin, and sodium nimbinatesalannin (50,51,53) The plant shows therapeutic roles in health management due to the various types of phytoconstituents (52,54,55). In a study to determine the phytoconstituents of *A. Indica* with antiretroviral activity, it was shown that the inhibition percentages greater than 50% were obtained for the extract and azadirachtin pure compound, suggesting that the compound has antiretroviral activity (56). In another

study, significant antiretroviral activity was obtained from neem leaf extract (57). Azadirachtin, nimbin, nimbolide, and gedunin have been identified as possessing antimalarial activities (53). Chemical structures of the phytoconstituents with possible anti-HIV activities are shown in Figure 3.

3.1. In vitro anti-HIV activity

Previous reports have shown that hydroacetone extract of *A. indica* leaves possesses both anti-retroviral and immunomodulatory potentials (58), as it was found to inhibit the activity of reverse transcriptase polymerase. The extract also could block the HIV-1 envelope-mediated membrane fusion. Besides, another *in vitro* study showed that HIV-1 replication was hindered in C8166 CD4+ T lymphocytes through the inhibition of the HIV-1 reverse transcriptase leading to a subsequent decrease in HIV p24 antigen concentration. Interestingly, no cytotoxicity was seen on uninfected target cells when the extract was used within the effective dose range. The extract also reduced the levels of phytohemagglutinin A and stimulated human peripheral blood mononuclear cells (PBMCs) and immune activation markers (CD38 and CD69), in a dose dependent manner. Hence, neem may offer health benefits to HIV/AIDS patients by acting as a reverse or fusion transcriptase inhibitor or by playing an important role in regulating hyperimmune activation (58).

3.2. In vivo anti-HIV activity

Udeinya et al have demonstrated that the leaf extract of neem might improve HIV conditions. The study established that the haematological parameters such as haemoglobin concentration, mean and CD4+ T lymphocyte count, erythrocyte sedimentation rate, and platelet count were significantly improved in the treated patients. Besides, the mean body weight was found to have significantly improved, and no serious adverse effects were noted among the study participants (57). Another study (59) monitored the efficacy of a neem leaf extract amongst 60 HIV/AIDS subjects with CD4+ T lymphocyte count < 300 cells/mm^3 who were ART naïve. Among the 60 participants, approximately 83% were compliant and a significant improvement in the CD4+ T lymphocyte count among those who adhered with the treatment (P<0.001) was observed at 12 weeks. Besides, a reduction in HIV/AIDS-related pathologies, an improvement in the erythrocyte sedimentation rate, and a significant enhancement in mean body weight, haemoglobin concentration, and lymphocyte differential count were observed (59).

4. α-Zam

α-Zam (*Nigella sativa*) contains black seeds in a ratio of 60:40 in freshly harvested pure honey (10). The seeds of *N. sativa* are used in different civilizations globally to treat various ailments. The seed and its main constituent,

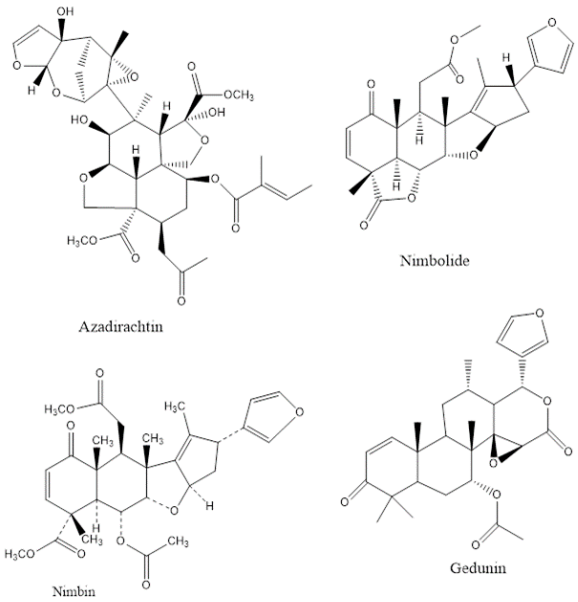

Figure 3. Chemical structures of possible anti-HIV bioactive phytoconstituents of *Azadirachta indica*.

thymoquinone, are useful against inflammatory conditions, mental illness, neurological disorders, cancer, diabetes, cardiovascular disorders, infertility, and various bacterial, viral, fungal, and parasitic infections (60-62).

Honey is mostly used as an antioxidant, antibacterial, antifungal, antimalarial, and antitumor remedy (63,64). Among several bioactive phytochemical compounds isolated from this herb, thymoquinone is reported to contribute most of the therapeutic properties. Other phytochemicals reported from different varieties of *N. sativa* include sterols, alkaloids, saponins, phenolics, novel lipid constituents and fatty acids, and volatile oil (65). The composition of essential oil (0.4-0.45%) includes limonene, p-cymene, trans-anethole, α-thujene, β-Pinene, carvone, thymoquinone, dithymoquinone, thymohydroquinone, and carvacrol (66,67). The fatty acid composition of the seeds (32%-40%) is mainly oleic, palmitoleic, linoleic, palmitic, stearic, arachidonic, eicosadienoic, and myristic acids (66,67).

Honey is known to have high contents of both enzymatic and non-enzymatic antioxidants, including ascorbic acid, flavonoids, alkaloids, catalase, and. Thus, flavonoids and polyphenols, which act as antioxidants, are two main bioactive classes of molecules present in honey (63). A unique flavonoid, pinocembrin, is present in high quantities in honey, while other flavonoids found in honey are chrysin, galangin, gallic acid, myricetin, quercetin, luteolin, and kaempferol. Different kinds of honey are known to contain different flavonoid profiles depending on the floral source for the nectar (64). Chemical structures of the phytoconstituents of *N. sativa* and honey with possible anti-HIV activities are shown in Figures 4 and 5, respectively.

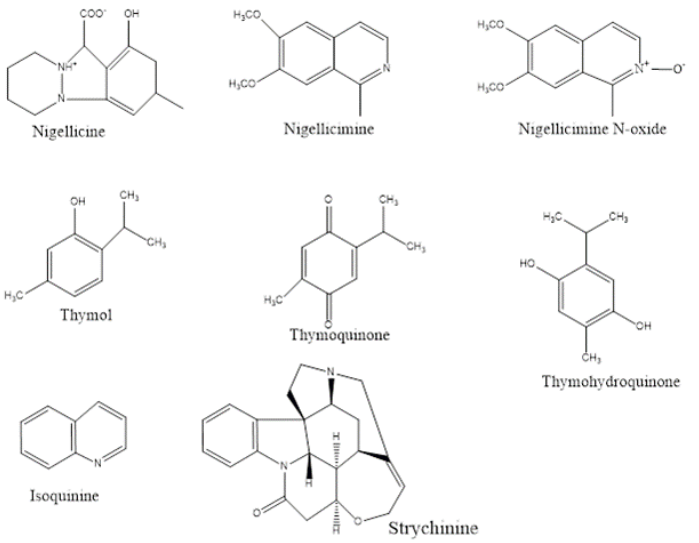

Figure 4. Chemical Structures of bioactive phytoconstituents of *Nigella sativa*.

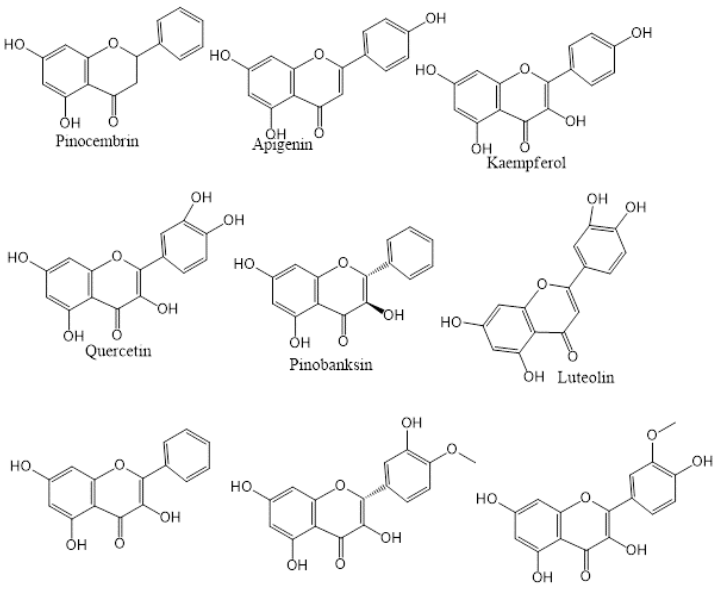

Figure 5. Chemical structure of some bioactive phytoconstituents of honey.

4.1. In vitro anti-HIV activity

In a study that analyzed monofloral Iranian honey from *N. sativa* to determine its anti-HIV-1 activities and T lymphocyte proliferation, PBMCs prepared from five healthy volunteers who were seronegative for hepatitis B virus, HIV, hepatitis C virus, and tuberculosis were used. Anti-HIV-1 activities of eight monofloral honey types and their fractions were evaluated through an HIV-1 P24 antigen kit. CD3, CD4, CD19, and CD45 T-lymphocyte subsets and their expression intensities were evaluated on PBMCs in the presence of the monofloral honey by flow-cytometry. Six types of honey (including that of *N. sativa* flower) suppressed viral replication dose-dependently. Also, the frequency and intensity of CD4 expressions in PBMCs were enhanced in the presence of all honey types, which increased the frequency of CD4+ T cells in PBMCs (68).

4.2 In vivo anti-HIV activity

Based on the observations from two case reports, *N. sativa* is considered a promising natural therapy for the management of HIV infection after validating its full therapeutic efficacy by clinical investigations.

A study conducted by Onifade et al (69) showed a complete recovery and seroreversion of a 46-year-old HIV-positive patient after treatment with 10 mL of black seed twice daily for 6 months (69). The patient visited the herbalist with symptoms of HIV, and Western blot

confirmed seropositivity to HIV infection with pre-treatment HIV-RNA load and CD4+ T lymphocyte count of 27 000 copies/mL and 250 cells/mm^3, respectively. Several repeats of the HIV tests remained seronegative, aviraemia, and normal CD4+ T lymphocyte count after two years without herbal therapy. Safety profile and efficacy were monitored during the treatment period. The signs and symptoms of HIV infection disappeared in spite of a reduction in CD4+ T lymphocyte count at early phase of treatment in the patients. The post-therapy CD4+ T lymphocyte count was 650 cells/mm^3 with undetectable viral load.

Another interesting finding was found in a 27-year-old HIV-infected woman who was diagnosed during antenatal care. She was ART-naïve and initiated on 10 mL black cumin and honey mixture three times daily for a year. The results of serology assessments were negative while the viral load was reported to be undetectable and CD4+ T lymphocyte count was not less than 750 cells/mm^3 (70). A summary of herbal medicinal products with their main phytochemical constituents responsible for anti-HIV activities is presented in Table 1.

Herbal medicinal products containing more than one medicinal plants

There are reports of clinical trials of herbal medicinal products for the management of HIV/AIDS in which the herbal medicines contain two or more plant species. Examples can be found in the following instances:

- In an open-label study of a herbal medicinal product consisting of five herbs, namely: *Artemisia capillaris*, *Glycyrrhiza glabra*, *Astragalus membranaceus, Morus alba,* and *Carthamus tinctorius*, there was a reduction in plasma HIV-1 RNA levels and increase in CD4+ T lymphocyte count (71).
- In a long-term study with traditional Chinese medicine (Chan Bai Sa), consisting of 30 undefined herbs, there were improvements in quality of life, mortality rate, and CD4+ T lymphocyte counts, Treatment also revealed positive results after 1-3 years, and drug-resistant HIV strains did not emerge (72).
- In a single-blind placebo study (24 weeks) with a traditional Chinese medicine called iQian-kun-Nin capsules containing five medicinal herbs- *Poria cocos, Coptis chinensis*, *Gardenia jasminoides, Astragalus membranaceus*, and *Sparganium stoloniferu*m, there was a significant decrease in plasma viral load and increase in blood CD4+ T lymphocyte counts (73).

Modulation of drug-metabolizing enzymes and permeability glycoprotein

There is a high prevalence in concurrent use of anti-HIV herbal medicinal products with orthodox HAARTs (74). For example, a study in Western Uganda found that 38% of HIV-positive patients were concurrently using herbal medicines and ART and this finding follows the trend as reported in other countries (26,75). Apart from the concomitant use of herbal anti-HIV medicines with the standard antiretroviral drugs, these herbal products are also used concurrently with other orthodox medicines used in the treatment of other co-infections since HIV/AIDS patients are susceptible to fungal and bacterial opportunistic infections resulting from immunosuppression (25).

The safety of the practice of co-administration of antiretroviral herbal medicinal products with orthodox medicines requires comprehensive investigation because of the high possibility of HDI. The potential for HDI is amplified by the high number of medicines used in HAART along with concurrent administration of drugs used in the management of other comorbidities. The mechanisms of drug-drug interactions are similar to those of HDI since herbal medicinal products are complex mixtures of phytochemical compounds just like conventional drugs that are chemical entities. The major mechanisms responsible for pharmacokinetic interactions are the modulation of drug-metabolizing enzymes activities, particularly the membrane transporter activity, the efflux transporter P-gp, and the CYP isozymes (18,19,23). The displacement of one drug from protein-binding sites by another agent usually increases the concentration of unbound drug available to target tissues. Pharmacodynamic interactions or interactions at receptor sites can occur; these can be agonistic or antagonistic. HDIs can also occur through the effect of the herbal medicinal products on GIT motility and through the formation of insoluble herb-drug complexes in the GIT, which can reduce the bioavailability of co-administered drugs (23). CYP enzymes are categorized into families and subfamilies with isoforms (76). About seven CYP isoforms belonging to 3 families are known to play important roles in human drug metabolism and these include CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. It is known that CYP3A4 mediates the metabolism of more than 50% of drugs in the market, while the CYP2C subfamilies are responsible for the metabolism of approximately 16%–20% of clinically relevant drugs (77,78). Mechanism of pharmacokinetic HDI, just like in drug–drug interaction, is either the induction or inhibition of the drug-metabolizing enzymes and transporters/efflux proteins, especially the P-gps, although the major reason for the interactions is overlapping substrate specificity (79). The increase in herb–drug interactions affects the treatment safety and efficacy, mainly through pharmacodynamic and/or pharmacokinetic modulations.

1. Modulation by Andrographis paniculata

The ability of *A. paniculata* extract and andrographolide compound to modulate CYP activities was examined *in vitro* and *in vivo* using rat and human liver microsomes

Table 1. Major phytochemical constituents of medicinal plants with proven anti-HIV activities

Medicinal plants	Phytochemical constituents	Pharmacological activities	Anti-HIV activities		References
			In vitro	*In vivo*	
Andrographis paniculata	Diterpenoids (andrographolide: $C_{20}H_{30}O_5$). Others are flavonoids and polyphenols	HIV, influenza A, and Herpes simplex virus 1	Inhibitor of HIV-1 & 2	Reduce plasma HIV-1 RNA levels and improve CD4+ T lymphocyte levels in HIV-infected patients and healthy volunteers	(40-43)
Lentinus edodes	Lentinan (a polysaccharide: $C_{42}H_{72}O_{36}$). Others are lectins, lenthionine and eritadenine	Antiviral, antifungal, antioxidants, immunomodulators, hepatoprotective, cardiovascular and hypolipidemic agents	Inhibitor of HIV-1 due to laccase enzyme produced by the plant	Lentinan alone or in combination with didanosine increase CD4+T lymphocyte levels in HIV-infected patients	(47-49)
Azadirachta indica	Azadirachtin (an isomeric mixture of seven or so tetra-triterpenoids: $C_{35}H_{44}O_{16}$). Also included are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin	hypolipidemic, microbicidal, antidiabetic, anti-inflammatory, hepatoprotective, antipyretic, and hypoglycemic. Other pharmacological activities include insecticidal, antiulcer, antioxidant, neuroprotective, cardioprotective, antimalarial and antileishmaniasis properties	anti-HIV-1 activity and immunomodulatory potentials by acting as fusion or reverse transcriptase inhibitors and also by down-regulating hyperimmune activation	Improved erythrocyte sedimentation rate, a decrease in HIV/AIDS-related pathologies, a significant increase in mean body weight, haemoglobin concentration and lymphocyte differential count	(57-59)
α-Zam (*Nigella sativa* + pure honey)	*Nigella sativa* include:mono-terpene **(thymoquinone: $C_{10}H_{12}O_2$,** thymohydroquinone: $C_{10}H_{14}O_2$, dithymoquinone: $C_{20}H_{24}O_4$ a carbonyl dimer of thymoquinone also known as nigellone), di-terpene, sesquiterpenes, monoterpenoid alcohols, and keton**e. Pinocembrin: $C_{15}H_{12}O_4$** is a unique flavonoid present in high quantities in honey. Others are chrysin, galangin, gallic acid, myricetin, quercetin, luteolin and kaempferol	*Nigella sativa*: anti-microbia, anti-oxidant, anti-inflammatory, anti-hyperlipidemic, immuno-protective, anti-cancer, anti-diabetic, neuro-protective, hepato and nephro-protective and wound healing activities while honey: possess activities such as antioxidant, anti-bacterial, antifungal, anti-malarial and anti-tumor properties	The frequency and intensity of CD4+ T lymphocyte expression on Peripheral Blood Mononuclear Cells increased in the presence of monofloral honey from *Nigella sativa*.	Undetectable viral (HIV-RNA) load along with increased body weight and CD4+ T lymphocyte count in participants.	(10,69,70)

(80). It was concluded from the study that the extract could cause HDIs in humans through CYP3A and 2C9 inhibition (80). This finding was further confirmed in another study in which the extract and pure compound caused significant decreases in CYP2C expression and activity in both *in vivo* and *in vitro* models. In addition, treatment with the extract and pure compound using human hepatocytes resulted in a decrease in CYP3A expression and activity (81). These studies suggest that *A. paniculata* extract can cause HDIs through modulation of CYP2C9 and CYP3A4 activities. Other *in vitro* inhibitory studies have corroborated the findings that *A. paniculata* may contribute to HDIs if they are co-administered with drugs metabolized by CYP2C9, CYP2D6, or CYP3A4 (82,83)

The potential of andrographolide to influence the pharmacokinetics of warfarin was investigated in an animal model. The study showed that andrographolide could increase the systemic exposure of warfarin in rats when andrographolide is administered concurrently with warfarin. Decreased hepatic metabolism of warfarin through the inhibition of the activities of CYP3A4 or CYP2C9 was responsible as warfarin is mainly metabolized by CYP3A4 and CYP2C9 in the liver (84). More studies have also demonstrated that co-administration of andrographolide with other drugs that are substrates of CYP3A4 and CYP2C, such as naproxen, meloxicam, nabumetone, and glyburide, resulted in an increase in the plasma levels of these drugs (85–88).

From the foregoing, it is apparent that concurrent administration of the extracts of *A. paniculata* with antiretroviral drugs or other orthodox drugs that are substrates of CYP3A4 or CYP2C has a high potential for HDI with a possible increase in the plasma levels of the co-administered drugs. It has been demonstrated that andrographolide is a substrate of P-gp and its poor oral bioavailability is attributable in part to its efflux by the intestinal P-gp. The bioavailability of andrographolide was significantly increased by verapamil, a P-gp inhibitor (89,90). Andrographolide produces a biphasic modulation of ATPase activity (stimulation at low concentration and inhibition at high concentration), and this is an indication of possible interaction of this phytoconstituent at the level of P-gp. Induction of P-gp can result in the reduction of the bioavailability of some other drugs, while inhibition of P-gp can increase the bioavailability of drugs that share substrate specificity.

2. Modulation by *Lentinus edodes*

Studies have demonstrated that lentinan suppresses CYP1As expression at both constitutive and inducible levels in the mouse liver (91). In an *in vivo* study in rat, a cocktail of probe drugs was used to determine the influence of lentinan on the activities of CYP3A4, CYP2D6, CYP1A2, CYP2C19, and CYP2C9. It was found that various concentrations of lentinan increased the activity of rat CYP1A2, CYP3A4, CYP2D6, and CYP2C19 but not CYP2C9 (92). The authors concluded that the findings suggest that lentinan has the potential to modulate the metabolism of concurrently administered drugs which are substrates of CYP3A4, CYP1A2, CYP2C19, or CYP2D6, which may lead to treatment failure due to a decrease in plasma drug concentrations (92). However, additional clinical investigations are warranted to fully assess the clinical implications of these HDIs. An extensive literature search did not show any studies on the influence of *L. edodes* extract or lentinan on P-gp activity.

3. Modulation by *Azadirachta indica*

An *in vitro* study conducted to determine HDI with an aqueous extract of *A. indica* leaves showed the extract significantly potentiated CYP3A activity in rat liver microsomes (93). In the same study the effect of co-administration of the herbal product on the pharmacokinetics of glipizide was determined in a diabetes mellitus animal model. Pretreatment of the animals with *A. indica* extract significantly decreased the area under the curve (AUC) of glipizide and also shortened its elimination half-life, observations that are indicative of enzyme induction (93). Glipizide and other second-generation sulphonylureas like glibenclamide and glimepiride have been shown to be metabolised by different CYP enzymes, but mainly by CYP2C9 (94). These findings are in contrast to the results of a more recent report (95), which showed that *A. indica* methanol extract inhibited CYP3A4/5, 2C8, and 2C9 activity using pooled human liver microsomes. The reasons for the disparity are not clear, and further studies are required for clarifications to be made. However, a pharmacodynamics interaction study between *A. indica* and glibenclamide demonstrated an antagonistic HDI in spite of the antidiabetic effect of the extract when given alone (96). The authors reported enzyme induction by the extract, although a study by another group indicated that *A. indica* extracts potentiated the antidiabetic effect of gliclazide, a sulfonylurea when both were given in a combination. They also showed that the presence of *A. indica* extract did not alter the pharmacokinetics of gliclazide in rabbits (97). CYP2C9 is the major enzyme responsible for gliclazide metabolism (98).

Studies have also been carried out to evaluate the influence of *A. indica* extract on the pharmacokinetics of orthodox antimalarial drugs. The concurrent oral administration of aqueous leaf extract of *A. indica* on the pharmacokinetics of chloroquine sulphate in rabbits showed that the herbal product resulted in a significant decrease in the AUC along with a longer elimination half-life of the drug (99). Since the elimination of the drug was significantly prolonged, a decrease in AUC could not be attributable to enzyme induction but possibly a decreased absorption. A pronounced increase in the half-life of the drug (125.7%) is suggestive of the inhibition of metabolism of the drug. *In vivo* studies indicated that chloroquine and

its active metabolite, hydroxychloroquine, are metabolized by CYP2C8 and 3A4/5 (100).

Results of the *in vitro* and *in vivo* interaction studies with *A. indica* extracts on CYP enzymes, especially CYP2C8/9 and CYP3A4, have not been consistent. Therefore, caution should be exercised in the concurrent administration of *A. indica* extracts with anti-HIV or other drugs that are substrates of CYP2C8/9 and CYP3A4. Very limited studies have been done to determine whether *A. indica* extracts modulate P-gp activity. The effect of ethanol extract of *A. indica* on modulating P-gp activity was examined using multidrug-resistant cell lines. The extract had no P-gp-inducing effects but increased the accumulation of rhodamine 123, a P-gp substrate. The authors concluded that *A. indica* might be a source for a new class of P-gp modulators devoid of P-gp induction effects (101). Results of this study suggest that *A. indica* has the potential of increasing the oral bioavailability of P-gp substrates.

4. Modulation by α-Zam

In a study that investigated the potential effect of thymoquinone on the metabolic activity of drug-metabolizing enzymes (CYP1A2, CYP2C9, CYP2D6, and CYP3A4) in human liver microsomes, study results showed that the metabolic activities of all the investigated CYP enzymes were inhibited by thymoquinone in the following order: CYP2C9 (46.35%) > CYP2D6 (20.26%) > CYP1A2 (13.52%) > CYP3A4 (12.82%) (102). The authors concluded that the findings might suggest a high probability of HDIs following the concurrent administration of thymoquinone-containing herbs with orthodox drugs that are metabolized by CYP2C9. In an earlier *in vitro* study conducted using fluorescence-based high throughput assays and human c-DNA baculovirus expressed CYP enzymes, thymoquinone demonstrated a strong and a moderate inhibitory effect against CYP3A4 and CYP2C19, respectively (103). *N. sativa* (Black seed) extract on its own was shown to significantly inhibit CYP2D6 and CYP3A4 activities in human liver microsomes and in healthy human volunteers indicating that it has the potential to interact with CYP2D6 and CYP3A4 substrates (104,105). A similar *in vitro* study using a different probe drug, tolbutamide, as a substrate showed that the oil extract of *N. sativa* had significant inhibitory effects on CYP2C11 (106).

Literature search shows that very limited studies have been done to elucidate the effect of honey on P-glycoprotein activity. Multiple doses of honey administration did not significantly alter the plasma levels of digoxin (probe drug for P-gp), which suggests that honey may not significantly modulate P-glycoprotein activity (107). Thus, there is a need for exercising the concurrent use of honey with CYP3A4 substrates because the potential for the induction of the CYP isoform has been demonstrated by some honey sources.

Conclusion

Medicinal plants are an alternative source of anti-HIV candidate drugs that could be further investigated and developed into newer drugs to manage HIV/AIDS. As a result of the limitations associated with the use of the standard of treatment, which include side effects of the HAARTs and the emergence of multidrug-resistant strains of the organism, efforts are ongoing to explore complementary and alternative treatment modalities such as the use of herbal medicinal products to manage HIV/AIDS. Surveys conducted in different countries indicated a high prevalence of the use of anti-HIV herbal medicinal products by HIV-infected individuals. These herbal medicinal products have the potential to interrupt the life cycle of HIV, while some act as immunomodulators/immunostimulants to augment the immune system of infected patients indicating that herbal medicinal products provide potential for the development of therapeutics with significant anti-HIV activities.

Author's contributions

COO conceptualized the study. AJA prepared the manuscript. COO, SII, STA, and JOS edited the manuscript. All authors reviewed, confirmed, and approved the final version of the manuscript.

Conflict of interests

The authors declared no conflict of interest

Ethical considerations

Authors have carefully monitored ethical issues such as text plagiarism, duplicated publication, misconduct, data fabrication, and falsification.

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