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Review Article **Open Access**

Phytotherapy as an alternative for the treatment of human papilloma virus infections in Nigeria: a review

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Abstract:

Human papillomavirus (HPV) has been incriminated as the causal agent of cervical cancer which has been rated as the second most common cancers among women in developing countries and seventh most common cancers in the developed world. In spite of the fact that HPV has been the major cause of cervical cancer, the dilemma lies in finding a cost-effective therapy. Approximately 291 million women are infected with HPV worldwide, 32% of whom are infected with HPV16 or HPV18. The estimated prevalence of HPV in sub-Saharan Africa is 24% and 11.7% globally. There have been studies reporting specific HPV prevalence rates in some part of Nigeria, with 37% in Abuja, 10% in Port Harcourt, and 26.3% in Ibadan. In the Nigeria population, awareness of HPV infections is low, HPV vaccines are inadequate, and the cost of HPV vaccination per person is beyond what an average citizen can afford. It has been suggested that herbal therapy such as *Echinacea* therapy reduces HPV replication and enhances the immune system. Although there is yet no scientific proof of the efficacy of *Echinacea* therapy against HPV infections, future emphasis should be placed on scientific research into this alternative therapy. There is need for more studies on development of antiviral agents against HPV, with a prospect of easy accessibility and affordability in Nigeria.

Keywords: Phytotherapy; HPV; Cervical cancer; Nigeria

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La phytothérapie comme alternative au traitement des infections à papilloma virus humain au Nigéria: une revue

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Abstrait:

Le papillomavirus humain (HPV) a été incriminé en tant qu'agent causal du cancer du col de l'utérus, classé comme le deuxième cancer le plus fréquent chez les femmes dans les pays en développement et le septième cancer le plus fréquent dans le monde développé. Malgré le fait que le VPH ait été la principale cause de cancer du col de l'utérus, le dilemme réside dans la recherche d'une thérapie rentable. Environ 291 millions de femmes sont infectées par le VPH dans le monde, dont 32% sont infectées par le VPH16 ou le VPH18. La prévalence estimée du VPH en Afrique subsaharienne est de 24% et 11,7% dans le monde. Des études ont signalé des taux de prévalence spécifiques du VPH dans une partie du Nigéria, avec 37% à Abuja, 10% à Port Harcourt et 26,3% à Ibadan. Dans la population nigériane, la sensibilisation aux infections au VPH est faible, les vaccins contre le

VPH sont inadéquats et le coût de la vaccination contre le VPH par personne dépasse ce qu'un citoyen moyen peut se permettre. Il a été suggéré que la thérapie à base de plantes telle que la thérapie à l'échinacée réduit la réplication du VPH et renforce le système immunitaire. Bien qu'il n'y ait encore aucune preuve scientifique de l'efficacité de la thérapie à l'échinacée contre les infections au VPH, l'accent devrait être mis à l'avenir sur la recherche scientifique sur cette thérapie alternative. Il est nécessaire de poursuivre les études sur le développement d'agents antiviraux contre le VPH, avec une perspective d'accessibilité et de prix abordable au Nigéria.

Mots-clés: Phytothérapie; HPV; Cancer du col utérin; Nigeria

Introduction:

Human papillomavirus (HPV) is now recognised as the major aetiological factor of cervical cancer (CC), with more than 99.7% of the cases associated with prior oncogenic/high risk human papillomavirus (HrHPV). Infection with HrHPV is therefore, the primary risk factor for cervical cancer and the pre-cancerous, cervical intraepithelial neoplasia (1). As HPV infection is a sexually transmitted infection, cervical cancer is now known as sexually transmitted cancer by origin (2). While infection with HrHPV is prevalent among sexually active women, it is usually transient and neutralised within 2 years of infection (3). A prospective study of 3,282 women in the Netherlands found that about 34% of young women failed to clear HPV in 2 years (4). The reported persistent rate of infection among women in Mainland China (age group 16-69 years) was about 23% (5).

Human papilloma virus is one of the 6 human viruses identified as Group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) (6,7). Worldwide, cervical cancer takes the lives of nearly 300,000 women a year, with more than 80% from developing countries (8). Global HPV data show that Africa has the highest prevalence of 22.1% (9), with the lowest prevalence of 2.2% in Sudan (10). Cervical cancer is the most common gynaecological cancers in Nigeria, and the leading cause of cancer deaths among Nigerian women (11), where it is responsible for the death of one woman every hour and more than 9,000 female patients annually (12).

Treatments for cervical HPV infections are restricted. The recommendations of the American Society for Colposcopy and Cervical Pathology (ASCCP) suggest that women with lesser cervical abnormalities such as HrHPV positive but cytologically negative, atypical Squamous Cell of Undetermined Significance (ASCUS), or Low Grade Squamous Intra-epithelial Lesion (LGSIL) should be diagnosed through genotyping and testing of HrHPV within twelve months (13). Nevertheless, according to recent studies, the risk of developing malignant lesions will be increased with chronic carcinogenic infection (14). If infection with HrHPV persists for more than a year, 21% of women progressed to CIN2 (15).

There has been debate over the treatment of HrHPV positive people. Several researchers in Taiwan have proposed cryotherapy as one of the LGSIL's treatment options, and a cohort study found that cryotherapy for women with LGSIL could minimize the incidence rate of CIN3+ by improving the clearance of HPV infections (16). In Nigeria some of the natural (herbal) remedies that have been used for the treatment of HPV infections include garlic, banana peel, apple cider vinegar, orange peel, potato, aloe vera, and pineapple. Although these have been used to treat warty HPV infections, they have not been used for HrHPV infections.

Methodology:

Online databases were searched for available relevant documents written in English language up till January 15, 2019. These included African Biomedical databases, Nigerian Scientific Journals database, HPV Information Centre Registry, and PubMed Central (NCBI). The keywords for search were 'HPV', 'traditional treatment of HPV in Africa', 'treatment of HPV in Nigeria', 'phytochemistry' 'HPV awareness and vaccine in Nigeria', and 'alternative medicine'.

We included original articles (cross sectional, prospective and randomized control trials), and review articles that provided information on current trends and meta-analysis of HPV infection and treatment in Nigeria and globally. Independent reviewers evaluated the eligibility of each document for consideration and eliminated the likelihood of bias. A total of 58 citations and 18 articles were found eligible for the review.

Prevalence of HrHPV infections in Nigeria

The prevalence of cervical HPV infection worldwide varies greatly, with some of the highest rates observed among African women. Approximately, two hundred and ninety-one million women are infected with HPV worldwide, 32% of whom are infected with HPV16 or HPV18 (9). The estimated prevalence of HPV infection in sub-Saharan Africa is 24%, and 11.7% globally (17). There have been studies reporting specific HPV prevalence rates in some parts of Nigeria, with 37% in Abuja, 10% in Port Harcourt, and

26.3% in Ibadan (18). In a population-based study conducted on women who have had sexual relations in their lifetime and their cervices examined by PCR and liquid based cytology, a 14.7% rate of high-risk HPV was detected in two third of the women (19). In another study conducted in north-eastern Nigeria, 48.7% of the women participants were positive for HPV type 18, 13.2% for HPV16, and 18.5% for combined HPV 31, 33 and 35 (20).

In a similar prevalence study conducted in Ibadan, southwest Nigeria on 932 sexually active women aged 15 years and above, 19.7% had HrHPV types 16, 31, 35, and 58 as the main HPV types, while 3.2% each had HPV types 16 and 35 (11). In Kano, northwest Nigeria, a hospital-based study conducted on 50 women aged 18 years and above to determine the prevalence of HPV infection using PCR for HPV DNA detection, 76% of the study participants were positive for either HPV 16, 18 or both while 60.5% were co-infected with both HPV 16 and 18 (21). In Nigeria, HPV infection tends to be predominantly reported in patients with high grade intraepithelial lesion as shown in Fig 1, although about 8% of the patients who have normal cytology had HPV infection, with higher prevalence among younger age group. Some biological mechanisms, such as immaturity of the cervix, insufficient cervical mucus production and increased cervical ectopia in younger women and adolescents, have been postulated to make them more susceptible to HPV infections (22).

Preventive measures against cervical lesion in Nigeria

One of the most effective measures to prevent cervical cancer is to prevent HrHPV infection, and this can be achieved by administering vaccines such as Gardasil which target HPV 16, 18, 6 and 11 (23), Cervarix targeting HPV 16 and 18 (24), and Gardasil 9 targeting HPV 31, 33, 45, 52 and 58. These vaccines reduces the chances of being infected with HPV when given to women before exposure to sexual activities. Nevertheless, these vaccines still have limitations in their failure to protect other HPV types not present in the vaccines. However, it is the most favoured method of prevention.

The vaccinations were approved and launched in Nigeria in 2009, with only a few affluent people taking up between 0 to 49% (25). In the Nigeria population, awareness of HPV infections and uptake of HPV vaccines is low, and the cost of HPV vaccination per person is beyond what an average Nigerian can afford (26). In the parts of Nigeria where the level of education is high, the female children caregivers showed very low knowledge on the risk factors associated with HPV infections and cervical cancer (27). While HrHPV infections are being treated by these vaccines, warts caused by low risk HPVs often regress without treatment, although these viruses are difficult to manage, as infection can reappear in the same site or in other places. Some of the current therapy for HPV infections are shown in Table 1. Although these medications are effective, they require multiple applications on the lesions.

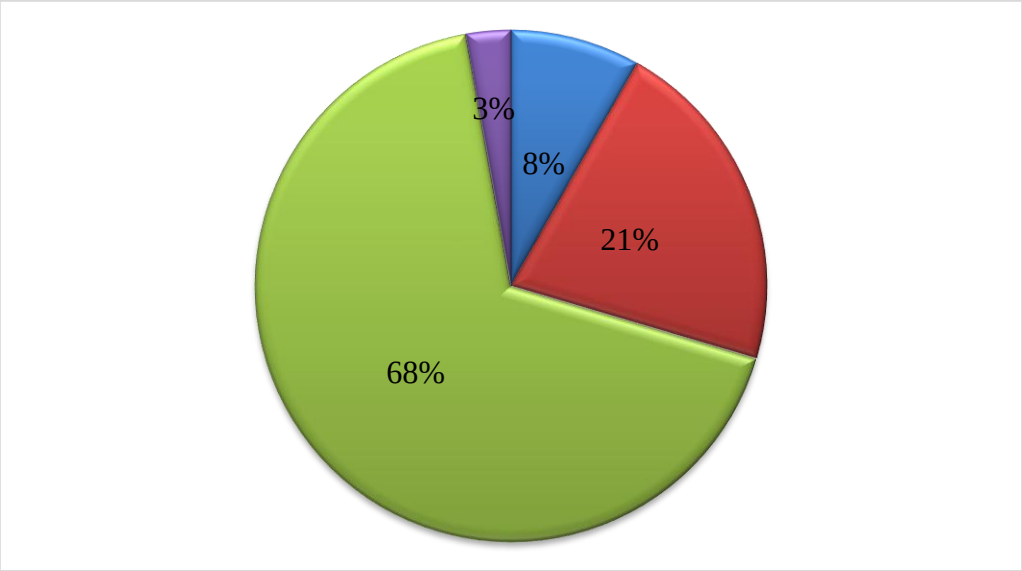

Fig 1.0: A pie chart showing Nigeria HPV prevalence in relation to Pap smear test

Blue colour represents the HPV prevalence in people with normal cytology, red colour shows prevalence of HPV in people with low grade intraepithelial lesion, and green colour depicts HPV prevalence in High grade intraepithelial lesion, while the purple colour is portraying the prevalence of HPV in cervical cancer patients.

Table 1: Current treatment for HPV infection and its associated problem

Current anti-HPV agents	Problem associated with the anti-HPV agents
Gardasil	Effective for only those infected with the virus
Cervarix	Inability to clear existing HPV infection
Imiquimod	Severe itching, hives, and long treatment time (4 months).
Condylox	Causes serious allergic reaction
Cidofovir	Expensive, side effects such as ocular disturbances
Interferon	Have limited efficacy for high grade HPV associated lesions
Podophyllotoxin	Not HPV-specific with up to 65% recurrence rate
Heparin	Has an anticoagulant effect
5-fluorouracil	Generates strong inflammatory response

HPV therapeutic vaccines

Human papillomavirus therapeutic vaccines are administered to treat pre-malignant and malignant lesions associated HPV, as opposed to Gardasil, Cervarix and Gardasil 9 vaccines, which are used to stimulate the development of L1 capsid protein neutralizing antibodies. HPV therapeutic vaccines are administered in order to target onco-proteins such as E6 and E7 proteins, which are expressed by HPV throughout their life cycle, although these therapeutic vaccines are still in phase II and III clinical trial stages. An example of HPV therapeutic vaccine that has passed the clinical phase III evaluation is the MVA E2 which targets HPV16, and contains the bovine papilloma virus E2 protein. Since the E2 protein regulates the expression of E6 and E7 onco-proteins, truncated E2 has negative regulatory gene to E6 and E7 promoter sequence. Hindering the E6 and E7 onco-proteins transcription in HPV infected cells thereby reduce progression to malignancy. Other HPV therapeutic vaccines are reported to have local mild to moderate side effects (28).

Development of new anti-HPV agents from natural products

The recent major developments in medical treatment and biomedical technology includes various antiviral agents developed and used to treat infectious diseases, yet such progress has also led to the development of viral resistance strain. Hence it is necessary to develop new antiviral agents with various kinds of antiviral actions. The quest for novel antiviral agents is based not only on synthetic substances, but also on natural compounds such as herbal pharmaceutical products (29).

Herbal products possess their own specific metabolites, which may identify variations between host and viral metabolism, contributing to antiviral activity. Most herbal pharmaceutical products can be easily obtained at a cheaper cost, and can be important for development of new antiviral agents with different antiviral activities than the current antivirals. The need to thoroughly investigate the causal agent associated with cases of cervical cancer is highly imperative, thus several diagnostic approaches are needed including the routine virological culture involving tissue culture screening (30-32). Viral isolation could be carried out on either embroynated chicken egg (33,34) as well as cultivation on a continuous cell culture systems (35-39). Often, these situations were synonymous with an approach to establish cultures that improved biological products such as insulin for diabetic remedy employing the goats' islets for xenotransplantation (40-43). Similar scenario stands in the selection of suitable cells for study of apoptosis (44-46).

The function of protein-receptor inhibitors used in *in silico* research is very important in drug design. Fig 2.0 summarises the steps to be taken to ensure herbal remedies are not only identified, but the mechanisms of actions can be properly understood. Recently countries like China have used detoxification therapy in the treatment of HPV, which tends to have positive effects on increasing HPV clearance rate, increasing the rate of CIN regression and influencing the proportion of certain immune cells, and also the level of cytokines in the genitalia following treatment (47).

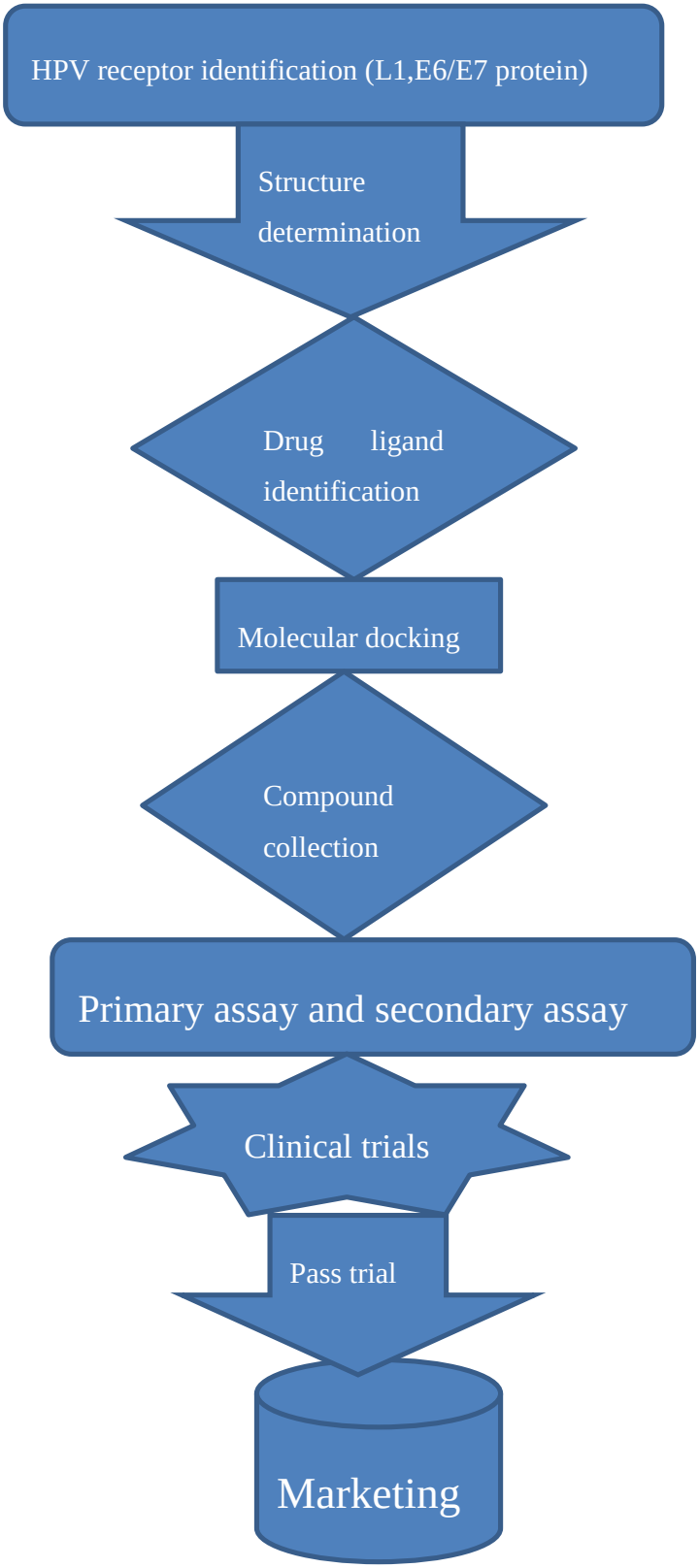

Fig 2.0: New anti-HPV agent drug testing algorithm

Potential herbal compounds as alternative for HPV infection therapy

Silymarin:

Silymarin is an herbal antioxidant that is believed to have anti-cancer and anti-HPV properties (48). It is contained in milk thistle as an active agent and, possesses antioxidant properties. It was reported to induce apoptosis by modulating Bcl-2 family proteins and also activating caspase 3 (48).

Curcumin (diferuloylmethane):

Curcumin is a polyphenol produced in the *Curcuma longa* plant, with a common name known as turmeric. For more than five decades, researches have shown that curcumin has onco-therapeutic effect with its antioxidative properties. Curcumin is a perennial herb which also possess anti-inflammatory properties and was reported to down-regulates AP-1 and nuclear factor kappa light-chain enhancer of activated B cells both of which plays major role in HPV transcription (49). One research showed the therapeutic potential of curcumin in HrHPV oral cancer cells, with reported ability to selectively inhibit E6 oncogene-mediated P53 degradation in HPV 16 positive cells in oral cancer throughout carcinogenesis (50). It also down-regulates the expression of uPA, COX2, NOS, LOX, MMP-9, and TNF chemokines (50).

Epigallocatechin gallate (EGCG):

Tea is one the world most consumed non-alcoholic beverages. The major tea cathecins are epigallocatechingallate (EGCG), epicathechingallate (ECG), epicatechin (EC) and epigallocatechin (EGC) (51). ECGC represents over 40% of all green tea catechins and it plays a key role in the prevention of cancer, obesity, neurodegenerative disorders, and stroke (52). The cancer prevention capability of EGCG is largely supported by epidemiological, *invivo* and *invitro* studies (53). Despite adequate evidence of EGCG effects against liver, breast and prostatic cancers, its effects on cervical cancer prevention is however controversial and still inconclusive (54).

EGCG has been reported to induce mitosis (G1, G2, S and M) arrest in squamous Me180 cervical carcinoma cell at low concentration (0-25µg/mL) (55). Another study showed that EGCG also mediated G1 phase arrest in CaSki cells linked to HPV 16 and regulated gene expressions (56). The activation of epidermal growth factor receptor (EGFR) and its downstream target, extracellular signal regulated protein kinases (ERK1/2), is important during cancer cell proliferation (57). Through the level of P53 and CKI, EGCG inactivate EGFR and ERK1/2 protein kinases causing G1 arrest and increased apoptosis in several cervical cell lines (57). Studies showed that EGCG inhibits the proliferation of cervical adenocarcinoma, due to its effects on PK-i67 and suppression of telomerase function, thereby inducing apoptosis (58). Current researches also reveal that EGCG through telomerase, a reverse transcriptase, telomerase which adds new DNA to the telomeres at the end of the chromosomes (58), and RNA polymerase III, could control the growth of cancer cells (58).

Carageenan

Carageenan prevents the binding of HPV virions to the basal cells of the cervix, thereby preventing viral pathogenesis.

Nordihydroguaiaretic acid

Nordihydroguaiaretic acid is an active compound from Lignan plant, which inhibits HPV SP1-mediated transcription.

Echinacea purpurae

Echinacea purpurae is one of the most favourite medicinal plants in the world, belonging to the family Astracea. It is commonly used as a chemo-preventive and chemotherapeutic plant especially in upper respiratory tract. It is also used in treatment of cancer such as cervical cancer and serves as immunomodulator. Although investigators have noted the isolation and structural elucidation of its main compound, there is no conclusion about its mechanism of action (59).

The active ingredients are alkamide, caffeic acid and polysaccharides. The alkamide is reported to be responsible for plant immunomodulatory property which uses T-cell activation and enhances the production of TNF and IFN-*y*. Its antiviral properties can also be linked to the fact that it inhibits hyaluronidases produced by viruses while the polysaccharide is responsible for its anti-inflammatory effects (28). Caffeic acid, however, is not present in all *Echinacea* species and could be used for plant extract authentication and quality control (59).

Immunomodulatory activity of Echinacea

The immunostimulant properties of the plant involve three mechanisms: phagocytosis activation, fibroblast stimulation, and increased respiratory activity resulting in increased leucocyte migration. There are different studies that have reported the *invivo* immunomodulatory and anti-inflammatory properties of *Echinacea purpurae* suggesting enhanced innate immunity when the plant is administered and increased immune response by stimulating macrophages, polymorphonuclear leucocytes (PMN), and natural killer (NK) cells (60). It is thus an effective

preventive measure for the treatment of various infectious diseases such as infections of the respiratory tract, wound and pelvis (61).

The complex chemical composition of *Echinacea* roots and herbs contain keto-alkenes, alkamides, caffeic acid derivatives, polysaccharides and glycoproteins which are allegedly responsible for noted immuno-stimulatory and anti-inflammatory activities (60). The alkamides have been tested and documented to be acting on type 2 cannabinoid receptors (CB2), and this is also hypothesized as possible mechanism for their immunomodulatory properties (62). One research has shown that N-alkamides obtained from root and herbal tincture induced synergistic properties on CB2 and eventually contribute to the immuno-modulatory effects along with interleukin 10 (63). They also inhibit both cyclo-oxygenase enzymes (COX-1 and COX-2) and 5-lipo-oxygenase (F-LO), enabling natural cell inhibition and anti-inflammatory activity (64). It is reported that the plant portion (polysaccharides) increases the production of interleukin-1 (IL-1), interleukin-6 (IL-6) and TNF-α by macrophages, and also increase their phagocytic and microbicide activity, and cerebrospinal fluid secretion (65).

The expression of CD83, which is a marker of dendritic cell maturation, has been shown to be significantly enhanced by the plant's floral and root extracts, while the stem and leaf extracts can significantly decrease CD83 levels. In addition, studies have shown that the extracts from the plant's root and aerial section require up and down regulation of insulin-like growth factor 1 receptor (IGF-1R), respectively. In addition, the plant's root extract can control a variety of genes involved in immune cell activation or function including Chemokine (C-C motif) ligand 4 (CCL4), interleukin-7 receptor, nuclear factor of activated T-cells, cytoplasmic 2, T-box trans-cription factor, cytohesin-interacting protein (PSCDBP), integrin, alpha E (ITGAE), and intercellular adhesion molecule-1 (ICAM1), while CD34 and integrin beta-1 (ITG-B1) are down regulated by the aerial part of the plant extract in dendritic cells (DCs) (66).

Cytotoxic activity of Echinacea

It has been documented that the plant extract flower and cichoric acid inhibit both the human colon cancer cell lines, Caco-2 and HCT-16, in a dose dependent manner after 48 hours of exposure. It has also been reported that cichoric acid slows down the HCT-16 cell line telomerase activity, which could be presumed as the molecular mechanism of apoptosis indication (67). Nonetheless, an extract from the plant root known as n-hexane that can be obtained from three species of *Echinacea* has been shown to have potent anticancer activity (68).

Lethal dosage of Echinacea in animal studies

Animal studies of various preparations of *Echinacea* species showed generally low toxicity (69). In acute toxicity test, the LD50 value was estimated at 2500 mg/kg in an intraperitoneal injection of the plant's polysaccharides fraction into female mice (69). In other studies, oral and intravenous LD50 values for plant juice were estimated to exceed 30g/kg and 10g/kg in mice, and 15g/kg and 5g/kg in rats (70).

Antiviral activity of Echinacea

In an *invitro* study, aqueous solution from the plant extract of *Echinacea purpurae* was confirmed to be active against both acyclovir resistant and susceptible strains of herpes simplex virus 1 (HSV 1) and herpes simplex virus 2 (HSV 2) (71), whereby, the plant root hexane extract and cichoric acid exhibited HSV 1 inhibition (72). Furthermore, cichoric acid has been reported to inhibit integrase activity of human immunodeficiency virus type 1 (HIV-1) (73). Mouse embryonic fibroblasts incubated with the plant juice and alcoholic root extract was immuned to influenza A2, herpes, and 24-hour infection of the vesicular stomatitis virus (74).

Normal preparation of the plant extract showed strong inhibition of the influenza viruses A/Victoria/75 (H3N2) and A/Puerto Rico/8/1934 (H1N1), avian strains A/Thailand/1(KAN-1)/2004 (H5N1) and A/ FPV/Dutch/1927 (H7N7), and the pandemic novel influenza A (S-OIV) (H1N1) of swine origin in direct contact. Nonetheless, haem-agglutination (HA) assays showed that the preparation inhibited HA activity, and thus prevented the virus from entering the treated cells (75).

Conclusion:

This review highlighted the improve-ment in the design of anti-HPV, as well as potential therapy regimen for HPV infections in Nigeria and globally, using phytotherapeutic approaches. HPV being a major cause of cervical cancer, and cervical cancer being one of the leading causes of annual death in adult women, prevention of cervical cancer is possible by seeking a cost-effective treatment for the main causative agent, HrHPV. The main antiviral therapy against HPV is cidofovir (CDV), which in addition to being expensive, has side effects ranging from renal to ocular disturbances.

While alternative medicine approa-ches for therapy of cervical cancer ranges

from globally accepted homeopathic methods to the allopathic approach embraced by the Indians, Chinese, and Japanese to treat HPV-related diseases, their mechanisms of action are not established. Therefore, more studies on antiviral agents against HPV are required. It has been reported that herbal therapy such as *Echinacea* not only reduce viral replication but also enhance the immune system, with the prospect of easy accessibility and affordability in Nigeria. Although there is no scientific explanation for the efficacy of *Echinacea* therapy against HPV infections at present, there need for more researches in this area.

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