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In vitro EFFICACY OF SOME INDIAN MEDICINAL PLANTS AS MICROBICIDES AND THEIR BIOSAFETY ANALYSIS

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ABSTRACT

Women acquire HIV virus mostly by heterosexual exposure and the anti-ritroviral therapy is costly as well as have various side effects. The topical microbicide formulations applied vaginally or rectally were investigated as a strategy for HIV prevention. In present study three medicinal plants, *Acacia catechu* (L.f.) Willd, *Lagerstromia spaciosa* (L.) Pers. and *Terminalia chebula* Retz., were examined for their safety profile against four strains of vaginal microflora. *In vitro* production of pro-inflammatory cytokines and their antimicrobial activities against vaginal pathogens were assessed. Aqueous and 50% ethanolic leaf extracts of *L. speciosa* and *P. longifolia* did not inhibit the growth of vaginal microflora, whereas n-butanol fraction of *A. catechu* showed some inhibitory action. Similar observations were noted with Caco-2 cell line pro-inflammatory cytokines analysis. The test plant materials were nontoxic at low concentration but n-butanolic fraction of *A. catechu* demonstrated some toxicity. The extracts also inhibited the vaginal pathogens as compared to the standard chemicals Zidouvdine and Saquinavir. The study revealed the positive therapeutic potential of the studied plants as effective microbicide and can be explored for the development of topical anti HIV agents.

Keywords: *Acacia catechu*; Caco-2 cell lines; *Lagerstromia spaciosa*; microbicides; MTT assay; *Terminalia chebula*; vaginal microflora; vaginal pathogens

INTRODUCTION

Worldwide, about 38.4 million people suffer from HIV infection with both men and women being almost equally affected (UNAIDS, WHO, 2021). Each year, approximately 1.5 million new cases of HIV infection are reported worldwide and ~0.65 million die of AIDS (UNAIDS, WHO, 2021). In India, approximately 2.4 million people are seropositive for HIV; of which 1.05 million are female and 0.69 million are children (NACO, 2021). Reproductive tract infections (RTIs) and sexually transmitted infections (STIs) are also emerging as a big suffering for human beings. As per conservative estimates, 374 million new cases of STIs (syphilis, gonorrhoea, chlamydia and trichomoniasis) occur annually throughout the world in the adults aged between 15-49 years (WHO, 2022). Genital herpes caused by herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) is considered important STI (Chaudhary *et al.*, 2019). The use of 'condoms' has been proposed to prevent HIV and other STIs transmission. Male condoms greatly reduce STIs, HIV infection and provide protection against conception. However, the use of condoms by males is very low, as its use is perceived to reduce the sexual pleasure. Female condom has been developed to overcome this problem, but due to the objections from male partners and their higher cost, its use is manifold lower than the male condom (de Bruyn *et al.*, 2010). Anti-retroviral drug therapy has been found useful to combat blood-

to-blood transmission of HIV, reduce viral load and minimize the chances of mother-to-child HIV transmission. Long-term use of available anti-retroviral drugs leads to the issue of drug resistance, and severe side effects such as diarrhea, nausea, lipodistrophy, hyperglycemia, liver toxicity, pancreatitis and neuropathy (Grant *et al.,* 2010). To resolve these problems, the use of microbicides has recently been proposed (Karim and Baxter, 2012). Microbicides may be available either in the form of a cream, gel, lubricant or even in the form of a tablet and can be applied topically to the vaginal- or rectal-surface. An ideal microbicide should be applicable hours before sex, preserve the natural anatomy of female reproductive tract (i.e. should not lead to the lesion and aberration in epithelial layer), protect the natural vaginal micro-ecological system and should not generate any pro-inflammatory cytokines (Shen *et al.,* 2022). Bacterial infections are most common opportunistic infections in HIV. Limited drug availability, cost of treatment along with drug resistance can hinder viral suppression and predisposing patients to opportunistic infections (Atrouni *et al.,* 2006). Major bacterial opportunistic infections are *Mycobacterium tuberculosis*, *M. avium*, syphilis, bacterial enteric disease, *Toxoplasma gondii*, *Pneumocystis jirovecii*, *Candida albicans*, *Herpes simplex virus*, *Histoplasma capsulatum*, cytomegalovirus, etc. (Vaillant and Naik, 2023).

Currently, Praneem, a combination of extracts prepared from neem *(Azadirachta indica*), saponins from *Sapindus mukorossi*, and menthe citrate oil (Talwar *et al.,* 2000), and polyherbal cream "Basant" (a combination of curcumin, *Emblica officinalis*, *Sapindus mukorossi*, aloe vera and rose water) are used as microbicides (Garg *et al.,* 1993). These drugs are WHO approved and clinically inhibit the growth of *Neisseria gonorrhoeae*, *Candida albicans*, *C. glabrata*, *C. tropicalis* and *Chlamydia*, and display a high viricidal action against HIV (Talwar *et al.,* 2008; Bhengraj *et al.,* 2009). In present study three medicinal plants *viz*., *Acacia catechu* (L.f.) Willd. (family: Fabaceae), *Lagerstromia spaciosa* (L.) Pers. (family: Lythraceae) and *Terminalia chebula* Retz. (family: Combretaceae) were examined for their microbicide potential. These plants reportedly possess anti-HIV properties (Nutan *et al.,* 2013a,b; Theepireddy *et al.,* 2014). Also, these plants show protective action when used in the form of herbal gel formulation against HIV-1 and HIV-2 infection (Mishra *et al.,* 2018).

Acacia catechu, commonly known as catechu, cachou and black cutch, is widely used in Ayurveda against many diseases including skin problems (Sunil *et al.,* 2019). Ayurveda uses its bark and heartwood for skin tonic preparation and active ingredient in many medicines like Lavangadi Vati (Asolkar *et al.,* 1965). The gummy extract of its wood is used as an anodyne, astringent and bactericide. The heartwood extract is used to treat asthma, bronchitis, colic, diarrhoea, boils, skin afflictions, sores and stomatitis. The bark shows anti-helminthic, antipyretic and anti-inflammatory properties and is used to treat bronchitis, ulcers, psoriasis, anaemia and gum troubles (Shen *et al.,* 2006; Ismail and Asad, 2009). The roots of *Lagerstromia spaciosa,* known as "Jarul", have been used as astringent, stimulant, febrifuge and in stomach problems while its leaves in the form of tea are used to control diabetes mellitus and weight loss (Castro, 2006). The leaf decoction or infusion of *L. spaciosa* has been used for bladder, kidney inflammation, dysuria, urinary dysfunctions, cholesterol deduction, hypertension and diabetes (Kakuda, *et al.,* 1996). The bark decoction is used for gastrointestinal tract disturbance, stomach-aches, haematuria and depression while seeds were used as narcotic (Wei, 2003; Vardhana, 2008). *Terminalia chebula* (haritaki) is extensively used in ayurveda, siddha, unani and homeopathic medicines. 'Triphala', a herbal preparation of 'three fruits' *T. chebula*, *T. bellerica*, *Emblica officinalis*, is used as laxative in chronic constipation, detoxifying agent of colon, food digestive problems (poor digestion and assimilation) and rejuvenator of body (Prasad *et al.,* 2006). Many studies have shown that 'Triphala' stimulates appetite, is useful in cancer treatment and detoxification (Peterson *et al.,* 2017) and has been prescribed as a cardiotonic and for candid infection (Kaur *et al.,* 2005). As per anti-HIV studies, the selected Indian medicinal plant can be used as microbicides but further safety profiling against vaginal microflora, *in vitro* production of pro-inflammatory cytokines by human vaginal keratinocytes is needed. Thus, the present study was aimed to assess the antimicrobial potential of *A. catechu, L. spaciosa* and *T. chebula* against vaginal pathogens.

MATERIALS AND METHODS

Collection of plant material

The leaves of three medicinal plants namely *Acacia catechu, Lagerstromia spaciosa* and *Terminalia chebula* were collected from National Botanical Research Institute, Lucknow (India) in July to September 2021. The plant materials were shade-dried, grinded and strained through a 30 mesh (0.5 mm). The voucher specimen of each plant along with their Accession No. were submitted to Herbarium of NBRI, Lucknow.

Preparation of plant extracts

To prepare 50% ethanolic extract of *T. chebula* and *L. speciose,* the dried leaf materials [(100 g) were charged in percolator, treated with ethanol: water (500 mL, 1:1 v/v) and left overnight at 25-30°C. The percolate (300 mL) was drained and the marc extracted thrice by cold percolation, each time with 500 mL of ethanol: water (1:1 v/v) and the combined percolate (1200 mL) was evaporated at 40-45°C under vacuum to concentrate the extract up to 80 mL. The concentrated 50% ethanolic extract was lyophilized at -20 to -40°C to afford 8-10% dry extract.

For preparation of *T. chebula* and *L. speciosa* aqueous extract, the dried leaves (100 g) were treated with 500 mL MilliQ water at 65-75°C for 6-8 h. The hot water extract was filtered through Whatman filter paper No. 1. The marc was extracted thrice, each time with 500 mL water at 60-75ºC. The combined filtrate (1200 mL) was distilled at 45-50°C under vacuum to afford concentrated aqueous extract upt o 70 mL which was subsequently lyophilized at -20 to -40°C to afford 9-11% dry extract.

The dried 50% alcoholic extract (10 g) prepared from the stem bark of *A. catechu* was suspended in 100 mL petroleum ether (60- 80°C), by stirring at 10-20 rpm at 30-40°C for 30-60 min and evaporated. The clear petroleum ether soluble fraction filtered through Whatman filter paper No. The residual petroleum ether insoluble extract, was air dried for 20-30 h suspended in 100 mL chloroform followed by stirring at 10-20 rpm at 30-40°C for 30-60 min. The residual chloroform insoluble extract was concentrated, vacuum dried at -20°C and collected. The residual chloroform insoluble extract was treated in a similar way with n-butanol to prepare the n-butanol soluble fraction.

Antimicrobial activity against common vaginal pathogens

For antimicrobial screening the methods of Clancy and Nguyen (1997**)** and Malekinejad *et al.* (2011) were followed. Pure cultures of test bacteria, *Streptococcus pneumoniae* (MTCC 2672), *Staphylococcus aureus* (MTCC 3160) and *Candida albicans* (MTCC 183) were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The viability of microbial cultures against plant extracts/test compounds were assessed by MTT assay (Clancy and Nguyen; 1997; Malekinejad *et al.* 2011). In brief, microbial density was adjusted to an OD of 0.06 at a wavelength of 670 nm i.e. approximately 10^8 CFU mL^{-1}. Extracts were administered at concentrations ranging from 3.12-100 µg mL^{-1} into 96-well-round bottom plates along with 30 µL microbial suspension. Final volume was made up to 100 µL by using MRS broth (HiMedia, Mumbai, India). Negative control included cells treated with solvent/medium. After incubation for 24 h at 37ºC, 10 µL of methyl thaizolyl tetrazolium bromide reagent (MTT, 5 mg mL^{-1} in 50 mM PBS; Sigma-Aldrich Inc.) was added to each well containing microbial inocula and plant extracts. Plates were incubated for 3 h at 37°C, followed by centrifugation at 2500 g for 10 min. Supernatants were aspirated and 100 µL of acid-isopropanol (5 mL 1 N HCl in 95 mL isopropanol) was added to each well. Optical density was measured using microplate spectrophotometer (EL $_X$ 800MS**;** BioTek Instrument Inc., Vermont, USA) at 540 nm using reference filter at 690 nm. Percent viability was calculated by dividing the absorbance of treated cells to untreated cells multiplied by hundred.

Effect of plant extracts on the viability of lactobacilli

Various *Lactobacillus* strains such as *L. casie* (MTCC 1423), *L. fermentum* (MTCC 903), *L. plantarum* (MTCC 4462) and *L. rhamnosus* (MTCC 1408) were procured from IMTECH, India and

cultured in MRS broth. The effect of various plant extracts/test compounds on the viability of lactobacilli was determined by MTT assay (Clancy and Nguyen, 1997; Malekinejad *et al.*, 2011).

Transepithelial resistance (TER) measurement

The transepithelial resistance (TER) measurement was done as per Clancy and Nguyen (1997). For the measurement of transepithelial resistance Caco-2 model is used because it express cytochrome P450 enzymes, transporters, microvilli and enterocytes of identical to human small intestine. Biopharmaceutics Classification System (BCS) recommends Caco-2 cell line as model to screen solubility, bioavailability, drug-drug or herb-drug interaction in the gut lumen (Awortwe *et al.*, 2015). In this experiments Caco-2 cell lines were proposed to find out if the selected plant extracts showed and irritation effect on normal vaginal or gut cellular environment.

Cell line culture medium- Caco-2 cells, procured from American Type Culture Collection (ATCC), were maintained in DMEM supplemented with 10% FBS, 100 units mL^{-1} penicillin, 100 µg mL^{-1} streptomycin sulphate and 250 ng mL^{-1} amphotericin B at 37°C in a humidified atmosphere of 5% CO_2.

Cytotoxicity assay: MTT test will be used to assess cell viability based on the capacity for viable cells to metabolize a tetrazolium colourless salt to a blue formazan in mitochondria. MTT test was used to assess the cell viability **(**Alghoraibi *et al.*, 2020). In brief, the Caco cells (6 x 10^3 0.2 mL^{-1} culture medium $well^{-1}$) were added in 96 well culture plates and incubated in humidified atmosphere of 5% CO_2 at 37ºC for 24 h. After incubation, the cells were treated with varying concentration of plant extracts/fractions/isolated compounds for 24 h at 37ºC. Appropriate solvents were included as negative controls. After incubation, 20 µL MTT reagent [5 mg mL^{-1} in 50 mM PBS, pH 7.4] was added to the cells and culture plates were further incubated at 37ºC for 4 h. After this 200 µL MTT solvent [20% SDS and 50% dimethyl formamide (DMF) in 50 mM PBS] were added and the culture plates left as such for overnight incubation at 37ºC. The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan. The insoluble formazan crystals are dissolved using a solubilization solution and the resulting coloured solution is quantified by measuring optical density. The darker the solution, the greater the number of viable, metabolically active cells (Berridge and Tan, 1993). The absorbance was taken at 570 nm with 690 nm reference filter using ELISA reader ((BioTek Instruments Inc., USA). Percent viability was calculated as follows:

$$\% \text{ Viability} = \frac{\text{OD of extract/compound−treated culture}}{\text{OD of untreated cell control culture}} \text{ x } 100$$

TER measurement: The integrity of epithelial cell linings of reproductive tract is critical for the prevention of sexual transmission of HIV. The effect of plant extracts/compounds on epithelial cell integrity was measured by using voltmeter as per Felix *et al.* (2021**)**. Caco-2 cells (1 x 10^5 cell $well^{-1}$) were grown in transwells and medium was dispensed in the basolateral compartment of each well. The cells were allowed to grow for 36-48 h in 5% CO_2 at 37ºC. Resistance was measured using Millicell–ERS voltmeter (EMD Millipore Corporation, USA) each day until the resistance reached a plateau. After formation of monolayer, the extract (25 µg mL^{-1}) was added to the culture medium and the cells incubated in humidified atmosphere of 5% CO_2 at 37ºC. Resistance was measured at intervals of 0.5, 1, 2, 4, 8 and 24 h (cells were incubated at 5% CO_2 and 37ºC in between each reading.

Statistical analysis

Analyses of concentration-response data were performed by using nonlinear curve-fitting program Prism to determine CC_{50} and IC_{50} values (Volpe *et al.*, 2014). The results were presented as average of three independent experiments.

RESULTS AND DISCUSSION

Topical microbicidal application appears to have excellent potential for female-controlled preventive option, which would not require negotiation, consent or even the knowledge of partner. Both women and men would benefit, as these can be bidirectional (Klebanoff and Coombs, 1991; Zewdie and Holschneider, 2001). An ideal microbicide must effectively inhibit STD pathogen transmission while causing limited disruption to the structural integrity and function of the healthy cervico-vaginal epithelium without inhibiting the vaginal lactobacillus, the most prevalent component of the reproductive tract's dynamic ecosystem.

Lactobacillus induces inhibition by a number of mechanisms which include hydrogen peroxide, lactic acid and ribosomal-produced antimicrobial peptide (bacteriocins) production (Turovskiy, 2010). The antimicrobial activity of bacteriocins includes the cell membrane permeabilization with ATR amino acids and ion efflux, and transmembrane potential and pH gradient depletion. Lactic acid produced by *Lactobacillus* potentially inhibit *Neisseria gonorrhoeae*; while hydrogen peroxide suppresses the growth of Gram-negative bacteria, Gram-positive facultative and obligate anaerobe bacteria, including *Escherichia coli, Gardnerella vaginalis* and *Mobiluncus* species. Moreover, a recently identified bacteriocin, lactocin 160A, specifically targets the cytoplasmic membrane of *G. vaginalis*. Further, certain bacteriocins seem to be protective against HIV infection, while others appear to induce membrane permeabilization on Gram-negative bacteria (Turovskiy, 2010). Natural barriers of HIV are low pH, presence of lactobacilli, intact epithelial surface and the mucosal immune system of genital tract (Klebanoff and Coombs, 2010). Therefore, *in vitro* toxicity on lactobacilli may provide an effective and inexpensive method to evaluate the safety of candidate microbicides in a preclinical setting. The test plant extracts have high levels of flavonoids and, growth of lactobacilli are stimulated by catechins and polyphenol compounds (Alberto *et al.*, 2001; Lee *et. al.*, 2006).

Antimicrobial activity

The n-butanol fraction of *A. catechu* does not show any toxicity towards *L. casei* and *L. fermentum;* whereas, it reduced the viability of *L. plantarum* and *L. rhamnosus* by 15 to 30%, when tested at a concentration of 100 µg mL^{-1}. On the other hand, standard drug zidovudine at 100 µg mL^{-1} showed toxicity against *L. rhamnosus* and *L. casei* (viability of 60 and 80%, respectively) [Fig. 1]. The n-butanol fraction also reduced the growth of *S. aureus* and *S. pneumoniae* by ~ 25%, whereas it doesn't show any inhibition towards the viability of *C. albicans.* Zidouvdine, demonstrated 20-30% inhibition in the viability of *S. aureus* and *C. albicans*.

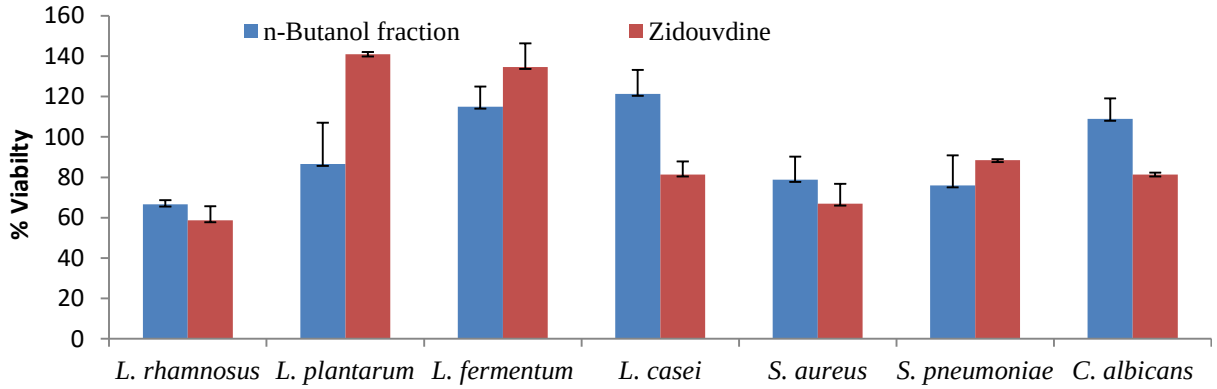

Fig. 1: Effect of n-butanol fraction of *A. catechu* stem bark on different *Lactobacillus* strains, and selected opportunistic pathogens

The 50% ethanolic extract of *L. speciosa* did not affect the viability of *L. fermentum* and *L. plantarum;* whereas the viability of *L. rhamnosus* and *L. casei* was reduced by 50 and 20%, repsectively (Fig. 2). The aqueous extract showed no significant decrease in the viability of *L.*

plantarum and *L. casei* but reduced the viability of *L. rhamnosus* and *L. fermentum* by 30-50%. Zidouvdine, an anti-HIV drug, which inhibits HIV infection by inhibiting reverse transcriptase enzyme, did not show any effect on the viability *of L. plantarum* and *L. fermentum*.

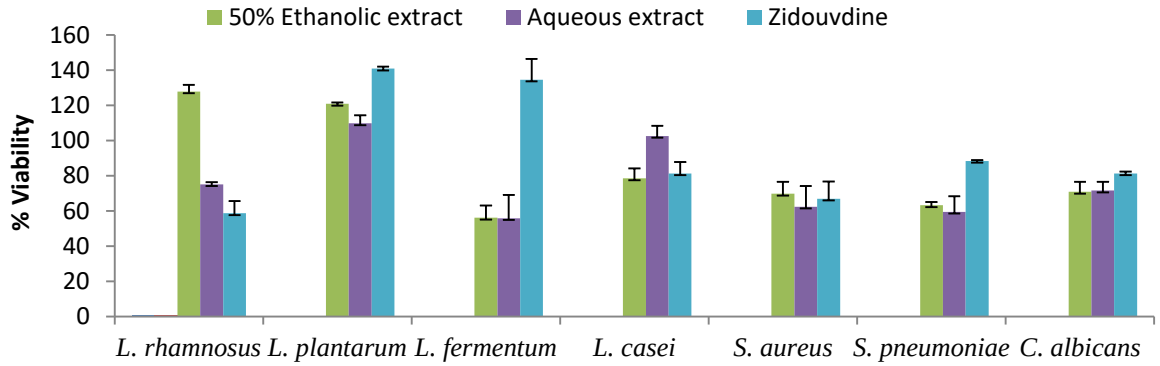

Fig. 2: Effect of leaf extracts derived from *L. speciosa* on different *Lactobacillus* strains and selected opportunistic pathogens

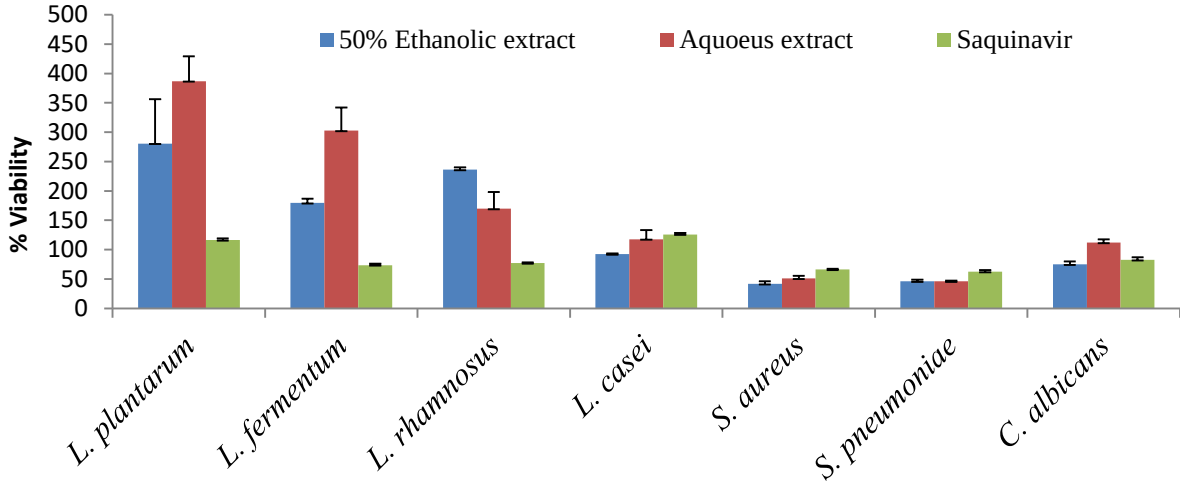

Fig. 3: Effect of *T. chebula* leaves extracts derived from on different *Lactobacillus* strains and selected opportunistic pathogens

In case of antimicrobial effects on opportunistic pathogens, both 50% ethanolic and aqueous extracts prepared from *L. speciosa* leaves inhibited the viability of *S. aureus,* and *S. pneumoniae* by 30-40%. No significant decrease in the viability of *C. albicans* was observed at 100 µg mL^{-1} by both 50% ethanolic and aqueous extracts. Zidouvdine also showed similar profile as observed in both the extracts with respect to *S. aureus, S. pneumoniae* and *C. albicans.*

The 50% ethanolic and aqueous extracts of *T. chebula* showed no cytotoxic effects up to 100 µg mL^{-1} concentration of *L. casei, L. fermentum, L. plantarum* and *L. rhamnosus* (Fig. 3). On comparison with Saquinavir, a commercially used protease inhibitor, less than 75% viable population of *L. fermentum* and *L. rhamnosus* was recorded. In case of opportunistic pathogens, both 50% ethanolic and aqueous extracts demonstrated potential antimicrobial effects as both reduced the viability of *S. aureus* and *S. pneumoniae* by approximately 50%. However, the viability of *C. albicans* was reduced by ~40% only. In comparison to the standard protease inhibitor Saquinavir, the 50% ethanolic and aqueous extracts showed slightly higher activity against *S. aureus* and *S. pneumoniae.*

Comparative viability analysis conducted by measuring CC_{50} values revealed that *L. speciosa* and *T. chebula* demonstrate high CC_{50} against most of the *Lactobacilli* strains (value range 108~322 µg mL^{-1}) [Table 1] i.e. non-toxic at higher concentration ranges. n-butanol fraction demonstrated

toxicity to the vaginal microflora with CC_{50} values ranging from 70~104 µg mL^{-1}. In case of opportunistic pathogens, the extracts effectively inhibited the growth of harmful vaginal pathogens with comparative low CC_{50}value ranging from 128 to 46 µg mL^{-1}.

Table 1: CC_{50} values of standard drugs and plant extracts (µg mL^{-1})

Bacterial species	*L. speciose*		*T. chebula*		*A. catechu*	Zidouvdine	Saquinavir
	Aqueous	50% ethanol	Aqueous	50% ethanol	Butanol		
L. rhamnosus	207.67	426.18	287.17	238.53	70.39	26.18	95.76
L. plantarum	313.34	322.31	388.81	216.71	85.48	1.92	82.22
L. fermentum	108.49	106.73	314.71	322.28	103.97	1.19	73.67
L. casei	235.78	202.48	100.54	91.87	104.40	2.09	100.07
S. aureus	100.10	100.20	88.11	66.86	89.79	5.24	51.76
S. pneumoniae	107.69	107.69	75.23	46.90	92.41	7.27	61.77
C. albicans	128.64	123.87	95.19	77.06	104.66	8.60	97.48

CC_{50}: The cytotoxic concentration of the extracts/compounds that caused the reduction of viable cells by 50%. All the data presented are average of duplicate experiment.

Effect of various plant extracts/fractions on TER of Caco-2 cells

The ability of mucosal epithelial cells to maintain an intact polarized monolayer in presence of a microbicide is a possible predictor of extract/compound safety on cervical and colorectal tissues (Gali *et al.*, 2010). The effect of plant extracts on their ability to maintain intact epithelium was determined by measuring TER. First, the selected plant extracts were screened for their cytotoxicity on Caco-2 cells, prior to their evaluation for the effect on cellular integrity. On the basis of MTT viability assay, TER was performed at non-toxic concentration of extracts. The extracts were added after the cells established a confluent monolayer, followed by TER measurement for 24 h. *A. catechu* butanol fraction showed some toxicity. Butanol fraction contains flavan-3-ol subclass of flavonoids (Sunil *et al.*, 2019), higher amount of ellagic acid, rutin, quercetin, gallic acid, catechin, chlorogenic acid (Lee *et al.*, 2006; Hong *et al.*, 2015), umbelliferone, kaempferol, epicatechin, coumaric acid and caffeic acid (Lee *et al.*, 2011). Besides, camphor, phytol, hexadecane and vitamin E

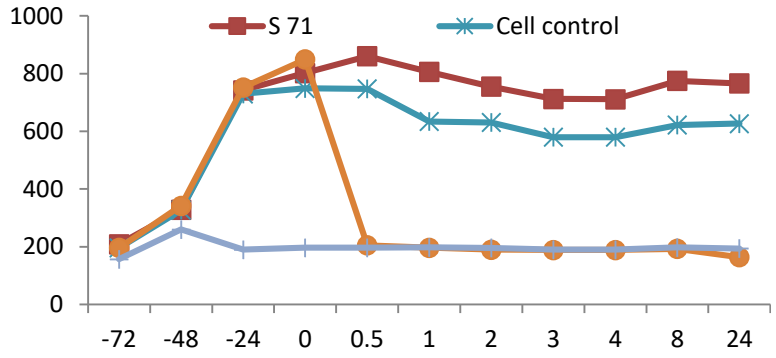

Fig. 4: Effect of extracts of *A. catechu* on epithelial monolayer integrity: S 71 used for butanol extract (µg mL^{-1})

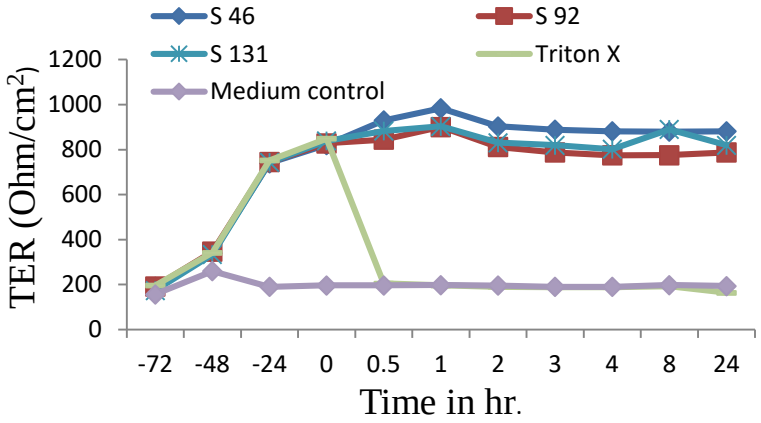

Fig. 5: Effect of extracts of *L. 203peciose* on epithelial monolayer integrity: S46 used for cell control; S 92 aqueous extract; S 131 for 50% ethanolic extract.

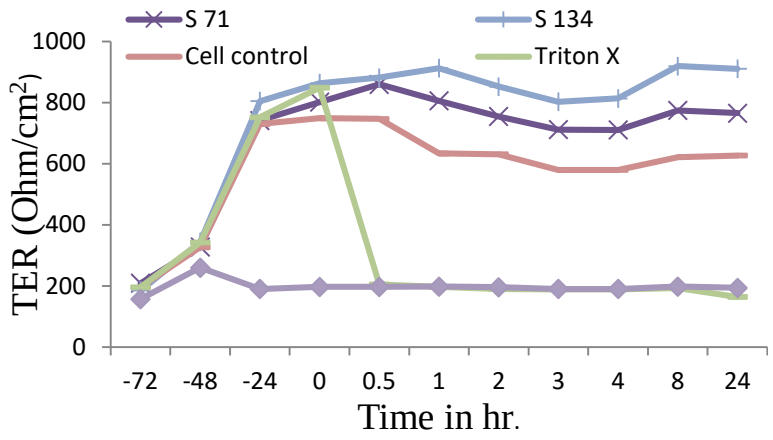

Fig. 6: Effect of extracts of *T. chebula* on epithelial monolayer integrity S71 used for aqueous extract; S 134 for 50% ethanolic extract

acetate (Kumar *et al.*, 2018) were identified from plant leaf extracts. These compounds may be responsible for cytokinin production in Caco-2 cell line and cytotoxicity (Fig. 4). The 50% ethanol and aqueous leaf extracts of *L. speciosa* showed similar resistance as their control (Fig. 5), Triton X disrupt the cell integrity within 30 min. of application whereas 50% ethanol and aqueous extract maintain cellular integrity equal to the control cells (Fig. 5).

After 24 h of application of both aqueous and 50% ethanolic leaf extracts of *T. chebula*, the TER values were same as those of untreated cells, except for Triton-X that led to the reduction of TER even after 30 min after application (Fig. 4). Overall, the non-toxic concentrations of extracts of *T. chebula* did not affect TER and hence may be suitable for topical application (Fig. 6).

Caco-2 cells were grown in transwells supports until they formed stable monolayer. Plant extracts or vehicle control were added to the apical chamber at t = 0 and resistance was measured at 0.5, 1, 2, 4, 8 and 24 h. As a toxicity control Triton-X (10%) was added to the indicated apical chambers whereas the test extracts were evaluated at 50 µg mL^{-1}. The data shown represent the mean ± SE of two independent experiments.

In present study, three Indian medicinal plants *viz.*, *A. catechu*, *L. speciosa* and *T. chebula* (which had shown anti-HIV activity in different *in vitro* assays) were screened for their microbicide properties. All the test plant extracts were safer to vaginal microflora as compared to the standard Zidovudine and Saquinavir. Butanol fraction of *A. catechu* showed some toxicity to lactobacilli strains. In case of opportunistic pathogens all the 5 extracts possessed lower CC_{50} value as compared to the *Lactobacillus* strains. The lower concentrations of plant extracts effectively inhibited the growth of *S. aureus*, *S. pneumonia* and *C. albicans* but were safer to the growth of *Lactobacillus casie*, *L. fermentum*, *L. plantarum* and *L. rhamnosus.* In TER measurement, the plant extracts had the ability to maintain an intact epithelium. When the selected plant extracts were screened for their viability on Caco-2 cells at a non-toxic concentration, Butanol fraction of *A. catechu* show some cellular toxicity. Whereas, extracts of *L. speciosa* and *T. chebula* the values of TER are same as cell control.

Conclusion: The test medicinal plant extracts showed effective anti-HIV activities. In present study, these plant extracts demonstrated antimicrobial activities against vaginal pathogens and exhibited no harm to the lactobacilli strains (higher CC_{50} values) as compared to the standard anti-HIV drug. Selected plant extracts did not disrupt transvaginal epithelial integrity, except *A. catechu*. The plant extracts appeared to contain bioactive constituents and has been used for centuries in Indian tradition medicines. As the plant extracts have all the properties that is needed to prepare potent microbicide, thus new scope of effective anti-HIV medication is generated. The study provides some insight for the use of medicinal plant extracts in the treatment of HIV. However, the isolation, characterization and utilization of bioactive templates in drug development for the management of the formulation of effective microbicide is desired.

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REFERENCES

Alberto, M.R., Farias, M.E., Manca, De and Nadra, M.C. 2001. Effect of gallic acid and catechin on *Lactobacillus hilgardii 5w* growth and metabolism of organic compounds. *Journal of Agriculture and Food Chemistry,* **49:** 4359-4363.

Asolkar, L.V. and Kakkar, K.K. 1992. *Second Supplement to Glossary of Indian Medicinal Plants with Active Principles: A-K (1965e1981)*. CSIR, New Delhi, India.

Atrouni, W., Berbari, E. and Temesgen, Z. 2006. HIV-associated opportunistic infections. Bacterial infections. *Lebanese Medical Journal*, **54**(2): 80-83.

Awortwe, C., Fasinu and Rosenkranz, B. 2014. Application of Caco-2 cell line in herb-drug interaction studies: current approaches and challenges. *Journal of Pharmaceutical Sciences*, **17**(1): 1-19.

Berridgem, M. and Tan, A. 1993. Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. *Archives of Biochemistry and Biophysics*, **303**(2): 474-482.

Bhengraj, A.R., Goyal, A., Talwar, G.P. and Mittal, A. 2009. Assessment of antichlamydial effects of a novel polyherbal tablet Basant. *Sexually Transmitted Infections*, **85:** 561-562.

Castro, I.R. 2006. *A Guide to Families of Common Flowering Plants in the Philippines*. The University of the Philippines Press. Quezon city, Philippines.

Chaudhary, N., Kalyan, R., Singh, M., Agarwal, J. and Qureshi, S. 2019. Prevalence of reproductive tract infections in women attending a tertiary care center in Northern India with special focus on associated risk factors. *Indian Journal of Transmitted Diseases*, **40**(2): 113-119.

Clancy, C.J. and Nguyen, M.H. 1997. Comparison of a photometric method with standardized methods of antifungal susceptibility testing of yeasts. *Journal of Clinical Microbiology,* **35**: 2878-2882.

de Bruyn, G., Martinson, N.A. and Gray, G.E. 2010. Male circumcision for HIV prevention: Developments from sub-Saharan Africa. *Expert Review Anti-Infect Therapy,* **8:** 23-31.

Felix, K., Tobias, S., Jan, H., Nicolas, S. and Michael, M. 2021. Measurements of trans-epithelial electrical resistance (TEER) are affected by junctional length in immature epithelial monolayers. *Histochemistry and Cell Biology*, **156**(6): 609-616.

Gali, Y., Ariën, K.K., Praet, M., Van den Bergh, R., Temmerman, M., Delezay, O. and Vanham, G. 2010. Development of an *in vitro* dual-chamber model of the female genital tract as a screening tool for epithelial toxicity. *Journal of Virological Methods*, **165**: 186-197.

Garg, S., Taluja, V., Upadhyay S.N. and Talwar G.P. 1993. Studies on the contraceptive efficacy of Praneem polyherbal cream. *Contraceptive,* **48**(6): 591-596.

Grant, R.M., Lama, J.R., Anderson, P.L., McMahan. V., Liu. A.Y. and Vargas, L. 2010. Pre-exposure chemoprophylaxis for HIV prevention in men who have sex with men. *The New England Journal of Medicine,* **363**: 2587-2599.

Hong, S.S., Choi, Y.H., Suh, H.J., Kang, M.J., Shin, J.H., Kwon, O.O. and Oh, J.S. 2015. Flavonoid constituents of *Acacia catechu. Journal of Applied Biology and Chemistry*, **58**(2): 189-194.

Ismail, S. and Asad, M. 2009**.** Immunomodulatory activity of *Acacia catechu*. *Indian Journal of Physiology* and *Pharmacology,* **53**(1): 25-33.

Kakuda, T., Sakane I., Takihara T., Ozaki, Y., Takeuchi H. and Kuroyanagi M. 1996. Hypoglycemic effect of extracts from *Lagerstomia speciosa* L. leaves in genetically diabetic KK-AY mice. *Bioscience, Biotechnology and Biochemistry*, **60**(2): 204-208.

Karim, S.S.A. and Baxter C. 2012. Overview of microbicides for the prevention of human immuno-deficiency virus. *Best Practical Obstetrics and Gynaecology*, **26**(4): 427-439.

Kaur, S., Michael, H., Arora, S., Harkonen, P.L. and Kumar, S. 2005. The *in vitro* cytotoxic and apoptotic activity of Triphala - An Indian herbal drug. *Journal of Ethnopharmacology,* **97**: 15-20.

Klebanoff, S.J. and Coombs, R.W. 1991. Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: Possible role in heterosexual transmission. *Journal of Experimental Medicine*, **174**(1): 289-292.

Kumar, R., Arora, R., Mahajan, J., Mahey, S. and Arora, S. 2018. Polyphenols from cutch tree (*Acacia catechu* Willd.) normalize *in vitro* oxidative stress and exerts antiproliferative activity. *Brazilian Archives of Biology and Technology*, **61:** e18160717. [*DOI:10.1590/1678-4324-2017160728*].

Lee, C.H., Jenner, A.M., Low, S.C. and Lee, Y.K. 2006. Effect of tea phenolics and their aromatic fecal bacteria metabolites on intestinal microbiota. *Research in Microbiology*, **157**: 876-884.

Li, X.C., Liu, C., Yang, L.X. and Chen, R.Y. 2011. Phenolic compounds from the aqueous extract of *Acacia catechu. Journal of Asian Natural Product Research*, **13**(9): 826-830.

Malekinejad, H., Tukmechi, A., Ebrahimi, H. and Bazargani-Gilani, B. 2011. One step forward to improve the latest method of antibacterial susceptibility testing of *in vitro*-cultured bacteria: An implication for antibacterial efficacy of enrofloxacine on *Aeromonas hydrophila. World Journal Microbiology and Biotechnology*, **27**: 147-151.

Mishra, N.N., Kesharwani, A., Agarwal, A., Polachira S.K., Nair R. and Gupta, S.K. 2018. Herbal gel formulation developed for anti-human immunodeficiency virus (HIV)-1 activity also inhibits *in vitro* HSV-2 infection. *Viruses*, **10**(11): 580-598.

National AIDS Control Organization. 2022. *Indian Council of Medical Research-National Institute of Medical Statistics (ICMR-NIMS)*. Ministry of Health and Family Welfare, Government of India, New Delhi. p. 15 [*https://main.icmr.nic.in/*].

Nutan, Modi, M., Dezzutti, C.S., Kulshreshtha, S., Rawat, A.K.S., Srivastava, S.K., Malhotra, S., Verma, A., Ranga, U. and Gupta, S.K. 2013a. Extracts from Acacia catechu suppress HIV-1 replication by inhibiting the activities of the viral protease and Tat. *Virology Journal*, **10**: 309-315.

Nutan, Modi, M., Goel, T., Das, T., Malik, S., Suri. S., Rawat, A.K.S., Srivastava, S.K., Tuli, R., Malhotra, S. and Gupta, S.K. 2013b. Ellagic acid and gallic acid from *Lagerstroemia speciosa* L. inhibit HIV-1 infection through inhibition of HIV-1 protease and reverse transcriptase activity. *Indian Journal of Medical Research*, **137**(3): 540-548.

Peterson, C.T., Denniston, B.S. and Chopra D. 2017. Therapeutic uses of Triphala in ayurvedic medicine. *Journal of Alternative and Complement Medicine*, **23**(8): 607-614.

Prasad, L., Khan, T.H., Jahangir, T. and Sultana, S. 2006. Chemomodulatory effects of *Terminalia chebula* against nickel chloride induced oxidative stress and tumor promotion response in male Wistar rats. *Journal of Trace Elements in Medicine Biology*, **20:** 233-239.

Shen, D., Wu, Q., Wang, M., Yang, Y., Lavoie, E.J. and Simon, J.E. 2006. Determination of the predominant catechins in *Acacia catechu* by liquid chromatography/electrospray ionization-mass spectrometry. *Journal of Agricultural and Food Chemistry*, **54**(9): 3219-3224.

Shen, L., Zhang, W., Yuan, Yi., Zhu, W. and Sheng, A. 2022. Vaginal Microecological characteristics of women in different physiological and pathological period. *Frontiers in Cellular and Infectious Microbiology*, **12**: 959793. [*DOI: 10.3389/fcimb.2022.959793*].

Sunil, M.A., Sunitha V.S., Radhekrishnan E.K. and Jyothis M. 2019. Immunomodulatory activities of *Acacia Catechu*, a traditional thirst quencher of South India. *Journal of Ayurveda and Integrative Medicine*, **10**(3): 185-191.

Sunil, M.A., Sunitha, V.S., Ashitha, A., Neethu, S., Sebastian, J., Midhun, E., Radhakrishnan, K. and Mathew, J. 2019. Catechin rich butanol fraction extracted from *Acacia catechu* L. (a thirst quencher) exhibits immunostimulatory potential. *Journal of Food Drug and Analysis*, **27**(1): 195-207.

Talwar, G.P., Dar, S.A., Rai, M.K., Reddy, K.V., Mitra, D., Kulkarni, S.V., Doncel, G.F., Buck, C.B., Schiller, J.T., Muralidhar, S., Bala, M., Agrawal, S.S., Bansal, K. and Verma, J.K. 2008. A novel polyherbal microbicide with inhibitory effect on bacterial, fungal and viral genital pathogens. *International Journal of Antimicrobial Agents*, **32:** 180-185.

Talwar, G.P., Raghuvanshi, P., Mishra, R., Banerjee, U., Rattan, A., Whaley, K.J., Zeitlin, L., Sharon,

L., Barré-Sinoussi, A. and David, G.F. 2000. Polyherbal formulations with wide spectrum antimicrobial activity against reproductive tract infections and sexually transmitted pathogens. *American Journal of Reproductive Immunology*, **43**(3): 144-151.

Theepireddy, S.K.R., Chinthala, R., Rao, L.V., Vishwakarma, S., Reddy, K.R. and Duvvada, M.R. 2014. Anti-HIV activity of *Terminalia chebula* crude extracts and gallic acid. *International Journal of Pharmacology and Technology*, **6**(1): 6346-6354.

Turovskiy, Y., Ludescher, R.D., Aroutcheva, A.A., Faro, S., Michael, L. and Chikindas, M.L. 2010. Lactocin 160, a bacteriocin produced by vaginal *Lactobacillus rhamnosus*, targets cytoplasmic membranes of the vaginal pathogen, *Gardnerella vaginalis. Probiotics and Antimicrobial Proteins*, **1**(1): 67-74.

UNAIDS/WHO. 2021. *AIDS Epidemic Update 2021*. UNAIDS/World Health Organization, Geneva, Switzerland. [*https://www.unaids.org/en/resources/fact-sheet*].

Vaillant, J.A.A. and Naik, R. 2023. *HIV-1 Associated Opportunistic Infections*. StatPearls Publishing, Treasure Island, Florida, USA. [*https://www.ncbi.nlm.nih.gov/books/NBK539787*].

Vardhana, R. 2008. *Direct Uses of Medicinal Plants and Their Identification*. Sarup and Sons, New Delhi, India.

Volpe D.A., Hamed S.S. and Zhang L.K. 2014. Use of different parameters and equations for calculation of IC 50 value in efflux assays: potential sources of variability in IC50 determination. *American Association of Pharmaceutical Scientists*, **16**(1): 172-180.

Wei, D. 2003. Philippine medicinal plants – Banaba, stuart exchange. [*http://www.stuartxchange.com/Banaba.html*] [Accessed in 10 April, 2019].

WHO. 2022. *Consolidated Guidelines on HIV, Viral Hepatitis and STI Prevention, Diagnosis, Treatment and Care for Key Populations.* WHO, Geneva, Switzerland.

Zein, R., Alghoraibi, I., Soukkarieh, Ch., Salman, A. and Alahmad, A. 2020. *In vitro* anticancer activity against Caco-2 cell line of colloidal nanosilver synthesized using aqueous extract of *Eucalyptus Camaldulensis* leaves. *Haliyon*, **6**(8): e04594. [*doi: 10.1016/j.heliyon.2020.e04594*].

Zewdie, D. and Holschneider, S. 2001. Expanding our prevention options: The role of the international community in providing an enabling environment for microbicides. *AIDS*, **15**: S5-S6.