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Isolation, Characterization and Purification of Bio-Active Compounds from *Andrographispaniculata* and *Cinnamon verum* for Anti-Hiv Activity

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ABSTRACT: Extracts of two ethnobotanically selected medicinal plants used in the Isolation and characterization of bioactive compounds which are investigated against anti-HIV properties. Enzymes and proteins that play a role in the HIV life cycle in which antiviral activity was studied through the pepsin-based assay targeting the HIV promoter activation induced by either the HIV-1 Tat protein. Assay methods to determine antiviral activity include multiple-arm trials, randomized crossover studies. These plants are used to investigate the bioactive compounds like tannins, saponins, flavanoids, coumarins, alkaloids, terpenoids and phenolic compounds against anti-HIV activity.

KEY WORDS: Anti-HIV, Bio-active compounds, Ethnobotanical, Pepsin assay, Antiviral.

I. INTRODUCTION

Andrographis paniculata is an annual herbaceous plant in the family *Acanthaceae,* native to India and Sri Lanka. It is widely cultivated in Southern and Southeastern Asia, where it has been traditionally used to treat infections and some diseases. Mostly the leaves and roots were used for medicinal purposes. *A.peniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications.The herb has a number of purported medicinal uses, although research has found evidence of its effectiveness is limited to treatment of upper respiratory infection, ulcerative colitis and rheumatic symptoms; in particular, there is no evidence of its effectiveness in cancer treatment[1]. The aerial parts of the plant (Leaves and stems) are used to extract the active phytochemicals. The leaves contain the highest amount of the andrographide, the most medicinally active phytochemicals in the plant, while the seeds contain the lowest. The primary medicinal compound of Andrographis is andrographide[2].

*Andrographispaniculata*commonlyknownasKingofbittersbelongstothefamilyAcanthaceae.It hasbeenusedforcenturiesasamedicinalherbforthetreatmentofuppergastrointestinaltractandupperrespiratoryinfections,fever,herpesandotherchronic diseases.Ithasabroadrangeofpharmacologicaleffects.Theprimarymedicinalcomponentof*A.paniculata*is andrographolide,whichisaditerpenelactone.Andrographolidehasbeenreportedforitsanti-cancer,anti-HIV,cardioprotectiveandhepatoprotectivepropertiesamongothers[3].*Cinnamomumverum* also called as true cinnamon, Ceylon cinnamon or Sri Lanka cinnamon, it is a small evergreen tree belonging to the family Lauraceae, native to Sri Lanka.

Among other spices, its inner bark is used to make cinnamon.*Cinnamon verum* gives numerous beneficial health effects

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against the HIV activity, and this plant exhibits anti-inflammatory property, anti-microbial activity, reducing cardiovascular disease, boosting cognitive function and reducing risk of colonic cancer.Recent studies suggest that consumption of cinnamon on a daily basis could significantly lower blood sugar and cholesterol levels and making it extremely helpful for patients Suffering from Type 2 diabetes. Cinnamon essential oil has significant antioxidant and antimicrobial properties as well [4].

Plants and other natural products present a large repertoire from which to isolate novel anti-HIV active compounds. HIV-1 is the cause of the world epidemic and is most commonly referred as HIV. It is a highly variable virus, which mutates readily. There are many different strains of HIV-1, which can be classified according to groups and subtypes; there are two groups, M and O. Within group M, there are currently known to be at least ten genetically distinct subtypes of HIV-1. These are subtypes A to J. In addition, Group O contains another distinct group of heterogeneous viruses [5].Treatment of HIV infected patients with currently available highly active anti-retroviral (HAART) drugs, though successful in reducing the burden of the disease but is associated with various side effects, including the emergence of drug resistant HIV strains. Hence, it is imperative to discover novel anti-HIV agents from natural sources that may have lesser side effects. Various studies have shown anti- HIV properties of the extracts prepared from a variety of plants. The plant extracts or purified phytochemicals may exhibit anti-HIV activity by inhibiting virus entry/fusion, HIV-1 reverse transcriptase (RT), protease or its integrase activity [6].

II. REVIEW OF LITERATURE

Paniculata has been reported as having antibacterial, antimalarial, antifungal, antiviral, antiinflamatory, fertility effects and protection of the liver and gallbladder. And also in broad range of pharmacological effects. The main activity of this plant is that it willpufifiesa bloodwhen it is entered into the human body, so that it cures Leprosy, Gonorrhea, Seasonal fevers, and also helpsin the prevention and treatment of the common cold. And it is extremely used as a hepatoprotective agent in Indian system of medicine [7].

HIV begins its infection of a susceptible host cell by binding to the CD4 receptor on the host cell. CD4 is present on the surface of many lymphocytes, which are a critical part of the body's immune system. It is now known that a co-receptor is needed for HIV to enter the cell. Several reviews on the natural products for the chemotherapy of HIV infection have been published earlier [8]. As a part of our screening program to investigate antiviral activity from plants [9]. The importance of investing in the high growth sectors of biotechnology and phytomedicine was also articulated in the founding document of the New Partnership for Africa's Development (NEPAD), and adopted by the African Biosciences Initiative (NEPAD, 2001; African Biosciences Initiative, 2005) [10].

III. MATERIALS AND METHODS

Detection of anti-HIV activity of the extracted compound

Enzyme pepsin inhibition assay

Pepsin has a quite close resemblance in proteolytic activity with HIV-1 protease protease one key enzyme of HIV-1 life cycle as both of them belong to an aspartate enzyme family. This enzyme was used as a substitute of HIV-1 protease to check out anti-HIV activity of plant extracts in the present investigation.

Preparation of Hemoglobin

Hemoglobin was prepared as 2.5 gm hemoglobin powder (HiMedia) was dissolved in 100 ml distilled water. It was blended, at maximum speed for 5 min and then filtered through gauze. Eighty ml of filtrate was diluted with 20 ml of 0.3N HCL and stored in 4º C until further use.

Pepsin assay

Pepsin assay was carried out as described by Singh *et al.*, Briefly, 50 µg pepsin (HiMedia), 800 µg hemoglobin (HiMedia) and different concentrations of each extract were taken in 500 µl of reaction mixture. The mixture was allowed to incubate

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at 37º C for 20 min. After incubation, 700 µl of 5% trichloro acetic acid (TCA) (HiMedia) was added to stop the reaction. It was then centrifuged (Rotina 38R) at 14000 rpm for 5 min and the supernatant was collected. Optical density was recorded spectrophotometrically (Cary 50 Bio UV-Visible spectrophotometer) at 280nm. Pepstatin-A (Sigma) was included as a standard. Negative control without extract(s) was set up in parallel. Separate blanks were used for extracts. All the determinations were done in triplicate. Percent Inhibition was calculated as, Inhibition (%) = (A negative control-Atest) /A negative control X 100. Was Aabsorbent. The result is expressed as IC_{50}. IC_{50} values were calculated using a Microsoft Excel program.

Biophysical characterization of plant extract

Thin layer chromatography

TLC for the identification of anti-HIV activity in crude extracts

Thin layer chromatography (TLC) was employed in this study to analyze the presence of anti-HIV activity in the *A.paniculata* and *C.verum* crude plant extracts of the different solvents i.e., ethanol, methanol, water, petroleum ether, chloroform, n-hexane at different temperature for 5 min followed by one or two cycles.Normal phase silica gel GF precoated TLC (scored 10×20 cm) plates; 250 microns were used. The solvent extracts were applied as separate spots to a TLC plate about 1.3 cm from the edge (spotting line), using micro tips.

The mobile phase, chloroform/methanol=9: 0.6 (v/v), were used for each crude extract. All TLC separations were performed at room temperature, i.e. 18-23ºC. After sample application the plates were placed vertically into a solvent vapor saturated TLC chamber. The spotting line was about 0.5 cm from the developing solution. After the mobile phase had moved about 80% from the spotting line, the plate was removed from the developing chamber.Indeed, anti-HIV activity presence has been proven by several fluorescent green stains under UV to 366nm. The sheets were dried in hot air oven for 4-5 min, the colorization was developed by passing iodine fumes on dried sheets. RF values were tabulated and compared it with standard RF values.

TLC for the identification of anti-HIV activity in fractions

Normal phase silica gel GF precoated TLC (scored 10×20 cm) plates; 250 microns (Analtech, Uniplate No. 02521) were used. The fractions collected were applied as separate spots to a TLC plate about 1.3 cm from the edge (spotting line), using micro tips.The mobile phase, chloroform/methanol=9: 0.6 (v/v), were used for each fraction. All TLC separations were performed at room temperature, i.e. 18-23ºC. After sample application the plates were placed vertically into a solvent vapor saturated TLC chamber. The spotting line was about 0.5 cm from the developing solution. After the mobile phase had moved about 80% from the spotting line, the plate was removed from the developing chamber.

The sheets were dried in hot air oven for 4-5 min, the colorization was developed by passing iodine fumes on dried sheets. RF values were tabulated and compared it with standard RF values.

IV.RESULTS AND DISCUSSION

The two plant extracts showed a very significant inhibition of pepsin enzymatic activity Table No 1 and 2 may be these extracts inhibit the activity of HIV protease. Many similar works have been made with plant extracts.

Graph 1 and Graph 2 Shows nonlinear regression dose response plot determining IC_{50} values of *A.paniculata* and *C.verum* plant extracts. Both the plant extracts showed potent inhibitory activity with IC_{50} values of 84.58 and 56.08 µg/ml, respectively as shown in Table No 2 and Table No 4. This shows the inhibitory activity of pepsin enzyme should also inhibit activity of HIV protease. The inhibitory activity could be attributed to high coumarins and flavonoids content. The inhibitory effect was confirmed by the IC_{50} values.

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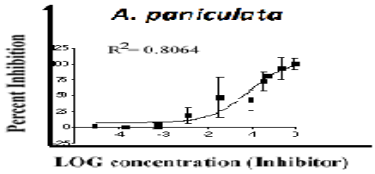
Sample	Absorbance at 280nm
Control (without any extract/inhibitor)	0.4211±0.0083
Control with Pepstatin A	0.0014±0.0083
Control with methanol plant extract	0.3632±0.0083
Control with ethanol plant extract	0.2310±0.0083
Control with water plant extract	0.1810±0.0083
Control with petroleum ether plant extract	0.3145±0.0083
Control with chloroform plant extract	0.1400±0.0083
Control with n-hexane plant extract	0.2100±0.0083

Table No 1: Effect of *A.paniculata* on HIV protease inhibition.

S.NO	Extract	Conc. (µg/ml)	% Inhibition (Mean±SD)	IC_{50} (µg/ml)
1	Methanol	1	13.74±0.1	84.58±5.01
2	Ethanol	20	45.14±1.2	
3	Water	40	57.01±0.4	
4	petroleum ether	60	25.31±2.3	
5	Chloroform	80	66.75±3.1	
6	n-hexane	100	50.13±1.0	

Table No 2: Effect of *A.paniculata*on pepsin assay



Graph 1: Nonlinear regression dose-response plot determining IC_{50} values of *A.paniculata*plant extracts; curve and IC_{50} were automatically determined by a computer program. Values were calculated using the following equation:

Y=bottom+ (top-bottom) / (1+10^ ((logEC50-X)),

Where X is the logarithm of concentration and Y is the response. Y increases in a sigmoid shape.

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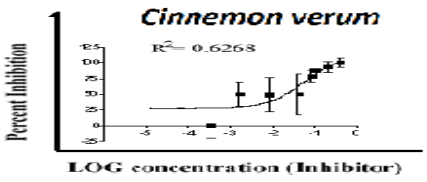
Sample	Absorbance at 280nm
Control (without any extract/inhibitor)	0.5101±0.0032
Control with Pepstatin A	0.0021±0.0032
Control with methanol plant extract	0.2310±0.0032
Control with ethanol plant extract	0.1426±0.0032
Control with water plant extract	0.3412±0.0032
Control with petroleum ether plant extract	0.4561±0.0032
Control with chloroform plant extract	0.2101±0.0032
Control with n-hexane plant extract	0.1232±0.0032

Table No 3: Effect of *Cinnamon verum* on HIV protease inhibition.

S.NO	Extract	Conc. (µg/ml)	% Inhibition (Mean±SD)	IC_{50} (µg/ml)
1	Methanol	1	54.71± 01	56.08±0.87
2	Ethanol	20	72.04±46	
3	Water	40	33.11±11	
4	Petroleum ether	60	10.58±61	
5	Chloroform	80	58.81±19	
6	n-hexane	100	75.84±78	

Table No 4: Effect of *Cinnamon verum* on pepsin assay.



Graph 2: Nonlinear regression dose-response plot determining IC_{50} values of *Cinnamon verum* plant extracts; curve and IC_{50} were automatically determined by a computer program. Values were calculated using the following equation:

Y=bottom+ (top-bottom) / (1+10^ ((logEC50-X)),

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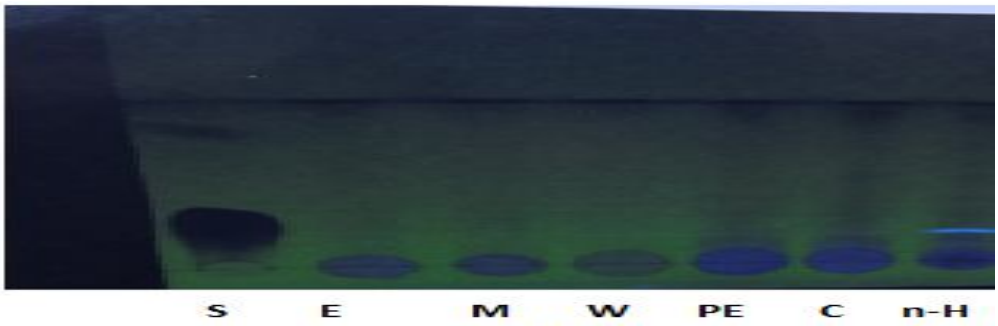
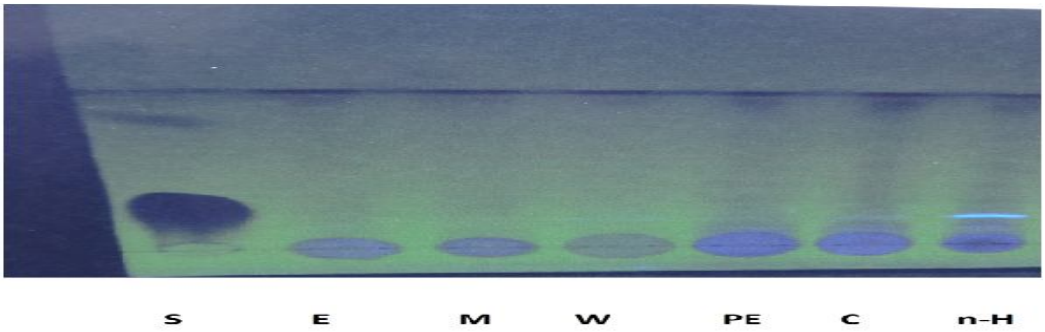
Where X is the logarithm of concentration and Y is the response. Y increases in a sigmoid shape.



S – Sample, E – Ethanol, M – Methanol, W – Water, PE – Petroleum ether, C – Chloroform, N-H – n-Hexane

Figure 1: Showing the presence of anti-HIV activity in *A.paniculata* plant extracts under UV.



S – Sample, E – Ethanol, M – Methanol, W – Water, PE – Petroleum ether, C – Chloroform, N-H – n-Hexane

Figure 2: Showing the presence of anti-HIV activity in *C.verum* plant extracts under UV.

IV. CONCLUSION

Medicinal plants have a long history of use of treating many diseases in many developing and developed countries. So herbal medicines can be developed as a safe, effective and economical alternative for AIDS. This work presents evidences that *A. paniculata* and *C. verum* plants contain anti-HIV bioactive compounds that could be developed into newer drugs to manage HIV/AIDS. This evidence should persuade further research.

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