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Research Article

EVALUATION OF *RUMEX VESICARIUS* LINN AND *SYMPLOCOS RACEMOSA* ROXB AS PROBABLE HIV-PROTEASE INHIBITORS

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ABSTRACT

The objective of the present study to evaluate in-vitro antiviral activity of different extracts of leaves of *Rumex vesicarius* Linn and *Symplocos racemosa* Roxb. In this study in-vitro antiviral activity was assayed by pepsin. Pepsin was used as a substitute for HIV-protease to evaluate inhibitory activity of these plants, as pepsin has close resemblance with HIV-protease in proteolytic activity. We have evaluated antiviral activity of different extracts of leaves of *Rumex vesicarius* Linn and *Symplocos racemosa* Roxb. But neither different extract of leaves of *Rumex vesicarius* Linn shows antiviral activity or protease inhibitory activity nor different extract of leaves of *Symplocos racemosa* Roxb. We have used pepstatin A as a standard (Positive control) which is a natural inhibitor of pepsin enzyme. The study demonstrated that the different extracts of *Rumex vesicarius* Linn and *Symplocos racemosa* Roxb did not show antiviral activity or protease inhibitory activity.

Keywords: *Rumex vesicarius* Linn, *Symplocos racemosa* Roxb, HIV-protease inhibitor.

INTRODUCTION

The advances in the knowledge regarding the human immunodeficiency virus (HIV) biology, pathogenesis and treatment, and their consequences on the HIV-related incidence of disease and death are quite unique in the past history of medicine. Today, antiretroviral (ARV) therapy is potent, convenient/simple or easy and usually well tolerated, and causes capable of reducing HIV blood concentration to undetectable values within a few weeks from therapy initiation and of inducing a robust and sustained CD4 T-cell gain.[1, 2]

The introduction of HIV protease inhibitors (PIs) in 1995 and the application of highly active anti-retroviral therapy (HAART), i.e. combination of PI with other antiretrovirals, mainly inhibitors of the HIV reverse transcriptase, increased the life expectancy of of HIV-positive patients.[3, 4]

The mechanism of HIV protease, it's drug-resistant and their interactions with different inhibitors have been studied for nearly 20 years to combat the challenges of AIDS & drug resistance.[5]

HIV protease plays a crucial role in viral maturation, multiplication and producing infectious virus particles. The protease breakdown the precursor Gag and Gag-Pol polyproteins at a minimum of 9 distinct sites. The breakdown release the structural proteins matrix, capsid, and nucleocapsid, spacer peptides p1, p2, and p6, and functional enzymes reverse transcriptase, protease, and integrase. Alteration of protease activity leads to defective viral particles and reduced level of infectivity.[6, 7]

The plant has been a prime source of highly effective conventionally drug for the treatment. *Rumex vesicarius* Linn (Chooka) belongs to perennial herbs to the family Polygonaceae. The plant is an erect usually with a long tap root. Traditionally the plant is used as stomachic, Diuretic, used for the disorders of the lymphatic and glandular system, for bronchitis, asthma, constipation, dyspepsia and the diseases of the liver. Plant leaves are rich in ascorbic acid, citric acid, and tartaric acid, it also contains glycoside, alkaloid, flavonoids, tannins and phenolic compounds.[8, 9]

Symplocos racemosa Roxb.(Lodhra) belongs to the family Symplocaceae, is a small evergreen tree up to 6 m tall. The traditional system it is mainly used as cardiotonic, antipyretic, anthelmintic and laxative properties. It is beneficial in billow fever, urinary discharge; pharmacologically it is used as antimicrobial, antidiarrhoeal, spasmogenic and heart depressant. The plant mainly contains monomethyl pelargonidin glucosides, loturidine also contain oxalic acid, phytosterol, ellagic acids and oleanolic acid.[10, 11, 12]

Pepsin has a close structurally resemblance with HIV-protease in proteolytic activity and as both of them belong to same family i.e. aspartate enzyme.[13] Hence in present study, pepsin was used as a substitute for HIV-protease.[14]

The objectives of present study were to prepare different extracts of selected plants *Rumex vesicarius* Linn and *Symplocos racemosa* Roxb and to evaluate as a probable HIV-protease inhibitory activity.

MATERIALS AND METHODS

Plant material: The fresh leaves of *Rumex vesicarius* Linn and *Symplocos racemosa* Roxb used in this study, collected at the flowering stage (Month: August -November) from the local area of Sangli and Satara, Maharashtra state, India respectively and authenticated by Botanical Survey of India, Pune, Maharashtra.

Extraction: The leaves were separated from fresh stems and dried under shade at room temperature until it becomes completely dry. After drying leaves were subjected to size reduction. The shade-dried coarsely powdered leaves (500 g) were subjected to Soxhlet extraction. A) *Rumex vesicarius* Linn. leaves: (500 g) were subjected to Soxhlet extraction with 95% ethanol and ethyl acetate to obtain ethanolic and ethyl acetate extract respectively. B) *Symplocos racemosa* Roxb. Leaves: (500 g) were subjected to Soxhlet extraction with 95% ethanol and N-hexane to obtain ethanolic and N- hexane extract respectively. The extracts obtained were subjected to the Rotary flash evaporator to remove excess of solvent, and dried extracts were stored in a cool place in tight pack container for further use.

Drugs and Chemicals: All the drugs and Chemical were used of analytical grade.Pepstatin-A (Sigma), Trichloro Acetic acid (HiMedia), Hemoglobin (HiMedia), Pepsin (HiMedia)

Instruments/Apparatus: Centrifuge (Remi R-8c), Digital Balance (Shimadzu ELB 300), UV-Visible Spectrophotometer (Jasco V-550), BOD incubator (BIO-TECHNICS India serial no BTI 06)

Assessment of pepsin enzyme inhibitory activity

a) Preparation of Hemoglobin: 2.5 gm of Hemoglobin powder was dissolved in 100 ml distilled water. Then it was blended at maximum speed for 5 min and filtered. From the filtrate , 80 ml was diluted with 20 ml of 0.3N HCl and stored at 4^0C.

b) Pepsin assay was carried out according to methods described by Singh et al.[15] 50 µg pepsin, 800 µg hemoglobin and different concentrations of each extract were taken in 500 µl of the reaction mixture. Then the reaction mixture was allowed to incubate at 37^0C for 20 min. After incubation, 700 µl of 5% trichloro acetic acid (TCA) was added to stop the reaction. Then it was centrifuged at 14000 rpm for 5 min and the supernatant was collected. And from collected supernatant absorbance was recorded by Uv at 280 nm. Pepstatin-A was taken as a standard. A negative control prepares without extract. All the determinations were done in triplicate and result is calculated as percent inhibition. Percent Inhibition was given by formula

% Inhibition = (A Negative control – A Test) / (A Negative control X 100,)

where A is absorbance.

RESULT AND DISCUSSION

The Plants has been used as traditional medicine and ethnobotanical literature has been described the use of the whole plant, plant extracts, infusions and powders for centuries.[16] There is an increasing need for search of new compounds which show antiviral activity because as the treatment of viral infections with the marketed available antiviral drugs is often not good enough due to the development of viral resistance & in recurrent infection in immunocompromised patients.[17,18]

Ethnopharmacology provides an alternative approach for the new discovery of antiviral agents, namely it includes the study of medicinal plants with a history of traditional use of plant as a potential source of substances which show significant pharmacological and biological activities.[19]

TABLE 1: EFFECT OF EXTRACTS ON PEPSIN ENZYME

Extracts	Concentration µg/ml	Percent Inhibition
EARV	20	2.695±0.2772****
	40	3.175±0.3909****
	60	3.479±0.3122****
	80	3.359±0.2151****
	100	3.791±0.1864****
ERV	20	1.623±0.2252****
	40	1.687±0.1693****
	60	2.031±0.0982****
	80	2.775±0.3011****
	100	3.519±0.3055****
ESR	20	1.703±0.1662****
	40	1.823±0.2151****
	60	2.015±0.3174****
	80	3.143±0.2199****
	100	3.511±0.1693****
NSR	20	1.831±0.1670****
	40	2.103±0.2209****
	60	2.217±0.1602****
	80	3.004±0.2304****
	100	3.327±0.2368****
Pepstatin-A(Standard)	0.2	89.880±0.2363

Value are expressed as (Mean ±SEM).n=3 ****P<0.0001 Statistically significant when compared with Standard group by ANOVA followed by Dunnett test.

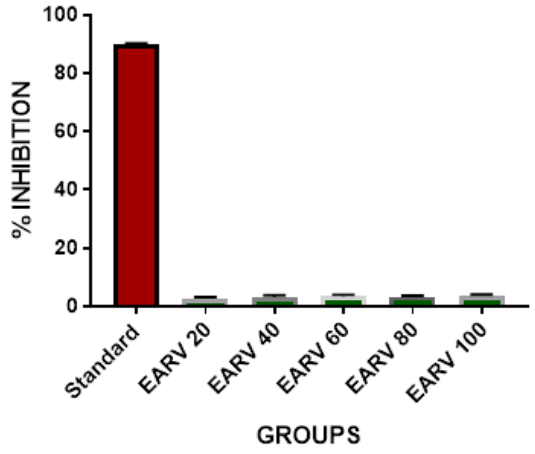

Figure 1: Graphical representation of % Inhibition of pepsin by EARV

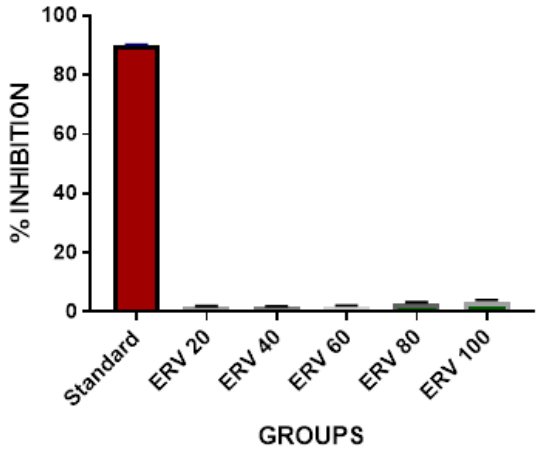

Figure 2: Graphical representation of % Inhibition of pepsin by ERV

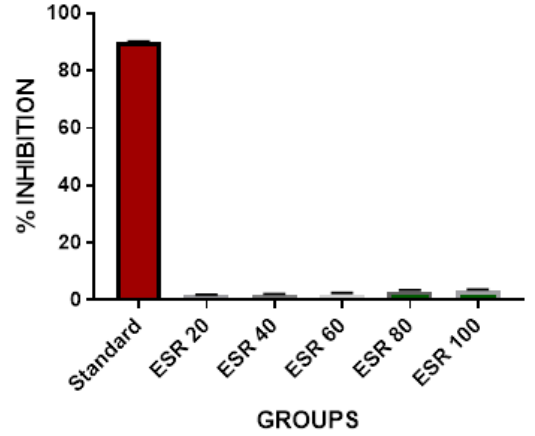

Figure 3: Graphical representation of % Inhibition of pepsin by ESR

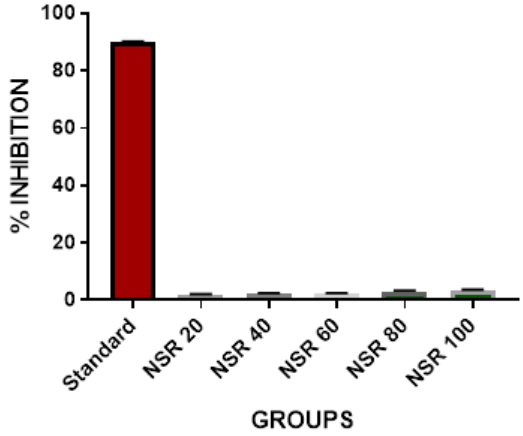

Figure 4: Graphical representation of % Inhibition of pepsin by NSR

HIV protease belongs to the family of aspartic proteases and has similar structural composition and mechanism resembles to aspartic protease enzymes.[20] Aspartic proteases include pepsin, cathepcin D, renin, chymosin and the proteases isolated from numerous fungi.[21] In the present study, pepsin was used as a substitute for HIV protease for screening possible HIV protease inhibitory activity of the selected plants. So we can use the cheapest substitute of HIV-protease. When we react enzyme and substrate in reaction vial left them for incubation for few minute enzyme pepsin claves substrate hemoglobin into small pieces after incubation. TCA added to stop the reaction and simultaneously the larger protein particle also get precipitated but the digested proteins or cleaved product of enzyme is still soluble in the reaction mixture and we can remove undigested protein parts in a reaction mixture by centrifugation at 14000 rpm and the absorbance is measured at 280 nm which is due to presence of tryptophan and tyrosine amino acid incorporated into the soluble peptide of digested hemoglobin.

Due to the high number of HIV infections and the rapid emergence of drug-resistant strains, the demand for new antiviral therapeutics against HIV-1 is increasing. Moreover, the standard antiviral therapies are too expensive. In order to manage the AIDS, alternative treatments are needed. One of the possible approaches is the screening of plants based on their ethnomedicinal data.[22]

Table 1 illustrate the comparision of inhibition of both standard and plant extract of both the plants. The percent inhibition of pepstatin is found to be 89.880. The percent inhibition of Ethyl acetate extract of *Rumex vesicarius* Linn (EARV) at various concentration 20, 40, 60, 80, 100 µg/ml obtained 2.695%, 3.175%, 3.479%, 3.359% and 3.791% respectively. The percent inhibition of Ethanol extract of *Rumex vesicarius* Linn (ERV) at various concentration 20, 40, 60, 80, 100 µg/ml obtained 1.623%, 1.687%, 2.031%, 2.775% and 3.519% respectively. The percent inhibition of Ethanol extract of *Symplocos racemosa* Roxb (ESR) at various concentration 20, 40, 60, 80, 100 µg/ml obtained 1.703%, 1.823%, 2.015%, 3.143% and 3.511% respectively. The percent inhibition of N-hexane extract of *Symplocos racemosa* Roxb (NSR) at various concentration 20, 40, 60, 80, 100 µg/ml obtained 1.831%, 2.103%, 2.217%, 3.004% and 3.327% respectively.

CONCLUSION

As there is no inhibition of pepsin enzyme by all extracts of *Rumex vesicarius* Linn and *Symplocos racemosa* Roxb so the present study revealed that *Rumex vesicarius* Linn and

Symplocos racemosa Roxb does not show antiviral activity or protease inhibitory activity.

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