In-vitro anti HIV activity of plants of nine-planet forest

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ABSTRACT: One of the most emotionally and economically destructive burdens on the face of globe is HIV infection. Mankind is still helpless due to non availability of a safe vaccine and non toxic therapy. There is an urgent need for a non toxic, non expensive, eco friendly possibly a plant derived drug which will block any one or many or all the stages of life cycle of HIV. Traditional knowledge is valid and necessary and awaits its currently relevant wider application for human benefit. According to Indian Traditional Knowledge, nine plants represent nine astronomical entities. Nine plants were identified and authenticated. Powdered explants of nine plants were macerated individually, separately using ethanol and chloroform & evaporated. From our other experiments GCMS analysis of individual extracts was done to identify compounds and anti-HIVactivity of the individual compounds if any recorded, was known from chemdb NIAID database. The crude extracts of certain selected individual plants were mixed together forming biherabal, triherbal, tetraherbal and pentaherbal extract samples. Also, all nine different powdered explants were mixed together forming a unique polyherbal sample which was also macerated separately with ethanol and chloroform and evaporated to yield ethanol derived crude polyherbal extract and chloroform derived crude polyherbal extract which were mixed together to form an unique double polyherbal extract sample. Extract samples were tested against HIV-1-RTp66 using assay kit (Immunodiagnostics, USA). All the three biherbal, one tri, one tetra and one penta herbal extract sample showed more than 50% of inhibition at different concentration of HIV-1-RT rp66. A biherbal extract sample made of stem of *Ficus religiosa* (Linn.) and leaves of *Desmostachya bipinnata* (Linn.) Stapf. exhibited maximum of 88.88 % of inhibition. Double polyherbal extract sample showed maximum of 70.58% of inhibition. Further *in-vitro* research on these extract samples will be beneficial.

I. INTRODUCTION

20.9 million People were accessing antiretroviral therapy in June 2017. In 2016, 36.7 million people were living with HIV and 1.8 million were newly infected with HIV. (www.unaids.org). As the threat due to HIV/AIDS persists to rise, effective drug treatments are required to treat the infected people. The present therapy finds its limitations in the emergence of multidrug resistance and accordingly finding new drugs is the need of the hour. Natural products such as plant-originated compounds and plant extracts have enormous potential to become drug leads with anti-HIV activity. Accordingly many research groups are exploring the biodiversity of the plant kingdom to find new and better anti-HIV drugs. World Health Organization (WHO) has suggested the need to evaluate ethno-medicines for management of HIV/AIDS. There is need to evaluate traditional medicine, particularly medicinal plants. HIV/AIDS being an exceptional epidemic, demands an exceptional approach.(Keshava rao VK, 2016) . Every nation has its own set of sacred plants (Pandey D, 2016). Stars, Planets, and Zodiac are considered to be made manifest in specific plant and tree species.

From the Vedas the plant species governing the planets are referred in the following Sanskrit verse: (Chandrakanth *et* al., *1990*)
Arkasam idam Adithyaya (Calotropis gigantea to represent Sun)
Palashagam Somaya (Butea monosperma to represent Moon)
Khadiram Angarakaya (Acacia catechu to represent Mars)

Apamargam Bhudhaya (Achyranthus aspera to represent Mercury)
Ashwatham Brihaspathaye (Ficus religiosa to represent Jupiter)
Audumbarag Shukraya (Ficus glomerata to represent Venus)
Shamigam Shanaischaraya (Acacia catechu to represent Saturn)
Rahuve Doorvaya (Cynodon dactylon to represent Rahu)
Kethuve Kushaya (Saccharum spontaneum to represent Kethu)

The Sun is represented by *Calotropis gigantea*, Moon by *Butea monosperma*, Mars by *Acacia catechu*, Jupiter by *Ficus religiosa*, Venus by *Ficus racemosa*, Saturn by *Prosopis cineraria*, Dragons head by *Cynodon dactylon* and Dragons tail by *Desmostachya bipinnata.*

Out of 09 medicinal plants, flowers of *Calotropis gigantea* (Linn.) (Estari M,2014), wood of *Fiucs glomerata*(Kingkan B, 2009) and stem bark of *Acacia catechu* (Mimosa Family)(Nutan et al., 2013) were successfully reported against HIV-RT, HIV-Integrase, and HIV-Protease respectively.

In present study certain polyherbal extracts made of Stems of *Calotropis gigantea* (Linn.), *Acacia catechu* (Rottler) Willd., *Achyranthes aspera* (Linn.), *Ficus religiosa* (Linn.) *and Prosopis cineraria* (Linn.); Bark of *Butea monosperma* (Linn.) *and Ficus racemosa* (Linn.); Leaves of *Cynodon dactylon* (Linn.) Pers. *and Desmosatchya bipinnata* (Linn.) were selected to investigate their *in-vitro* inhibitory activity against HIV-1 RT p^{66}

II MATERIALS AND METHODS

II 1 Collection of Plant material

Calotropis gigantea, Acacia catechu, Achyranthes aspera, Ficus religiosa, Prosopis cineraria, Ficus racemosa, Butea monosperma, Cynodon dactylon were identified in main campus of Osmania University, Hyderabad and *Desmostachya bipinnata* was identified in coastal Andhra Pradesh. Plants were authenticated using standard keys and descriptions.

II 2 Method of sample preparation, extraction and evaporation

II 2.1 Individual plant sample

10 gram of each coarse plant sample was added in a closed sterile, flat-bottom glass container containing whole 100 ml of analytical grade Ethanol and kept at room temperature, with vigorous, occasional shaking at regular intervals, for 7 days. At the end of 7th day solution was, filtered through whatman no. 1 filter paper and filtrate was extracted using Rotary evaporator. Thus nine ethanol extracts were preserved for further use. 10 gram of each coarse plant material was also extracted with chloroform separately, as stated above. Nine chloroform extracts were preserved for further use.

II 2.2.0 Polyherbal plant sample containing nine explants

1.1 gram of each coarse plant sample were mixed together to form a polyherbal plant sample which was added in a closed sterile, flat-bottom glass container containing whole 100 ml of analytical grade Ethanol and kept at room temperature, with vigorous, occasional shaking at regular intervals, for 7 days**.**At the end of 7th day, solution was, filtered through whatman no. 1 filter paper and filtrate was extracted using Rotary evaporator. Thus an ethanol extract of polyherbal sample was prepared.

II 2.2.1 Also, 1.1 gram of each coarse plant material were mixed together and extracted with chloroform separately, as stated above.

II 3 Mixing of above plant crude extracts

Above different individual plant extracts were mixed to form biherbal, triherbal, tetraherbal and pentaherbal extract samples. A double polyherbal extract sample was prepared by mixing ethanol and chloroform derived polyherbal extracts.

II 3.1 Biherbal extracts samples

Four different **biherbal extract samples** were prepared by adding

1) ethanol extract of bark of *Ficus racemosa*, Stem of *Acacia catechu*
2) chloroform extract of bark of *Butea monosperma* and stem of *Ficus religiosa*
3) chloroform extract of stem of *Ficus religiosa* and leaves of *Desmostachya bipinnata*
4) chloroform extract Stem of *Prosopis cineraria* and leaves of *Desmostachya bipinnata*
5) Each biherbal extract was prepared by adding 0.05 mg of each plant extract.

II 3.2 Triherbal **extract sample** was prepared by adding 0.03 mg of chloroform extract of bark of *Butea monosperma*, chloroform extract of stem of *Ficus religiosa* and chloroform extract of leaves of *Desmostachya bipinnata*

II 3.3 Tetraherbal extract sample was prepared by adding 0.025 mg each of ethanol extract of bark of *Butea monosperma*, chloroform extract of stems of *Calotropis gigantea*, *Achyranthes aspera* and *Acacia catechu*

II 3.4 Pentaherbal extract sample was prepared by adding 0.02 mg each of chloroform extract of bark of *Butea monosperma*, stem of *Ficus religiosa*, leaves of *Desmostachya bipinnata*, ethanol extracts of bark of *Butea monosperma* and stem of *Acacia catechu*;

II 3.5 Unique **double polyherbal extract sample** was prepared by adding 0.05 mg of chloroform extract of polyherbal sample and 0.05 mg of ethanol extract of polyherbal sample.

II 4 Anti *HIV assay*

Above samples were evaluated for their *in-vitro* anti HIV-1 RT p^{66} efficacy using RT p^{66} capture ELISA kit from Immuno Diagnositcs (USA). Wells of microtitre plate supplied in kit were coated with anti-body for HIV-1-RT rp^{66} antigen. RT p^{66} protein was supplied in kit. Different dilutions of RT p^{66} viz. 50ng, 12.5ng, 3.12ng, 0.78ng and 0.19ng were prepared using a diluent buffer supplied in kit and 100 ul of each dilution was added into designated wells of microtitre plate. 0.1mg of above each extract sample was added to 100ml of diluent buffer. 100ul of each extract sample was added into their respective labeled wells in microtitre plate. Zidovudine (ZDV/AZT) was used as positive control. 0.1mg of AZT was mixed with 100ml diluent buffer and 100ul of AZT was added in respective wells. Percentage of inhibition was calculated as -

%Inhibition = OD of Control – OD of plant sample/ OD of control × 100

III RESULTS

III. 1 Anti HIV-1 RT p^{66} activity of biherbal samples

To the well containing 50ng/100ul of rp^{66} when 100ul of AZT (0.1mg/100ml) added 0.027 OD was recorded and to an another well containing same concentration of rp66, when -

a. 100ul of a biherbal sample (0.1mg/100ml of biherbal sample made of 0.05mg of chloroform extracts of leaves of *Desmostachya bipinnata* and 0.05mg chloroform extract of stems of *Ficus religiosa* in diluent buffer) was added 0.003 OD was recorded. By applying the above formula, % of inhibition of this biherbal sample was 88.88. Lupeol acetate and n-hexadeconoic acid were reported from GCMS analysis of chloroform extracts of leaves of *Desmostachya bipinnata* and stems of *Ficus religiosa* respectively. Both were reported to be potent anti-HIV compounds in chemdb NIAID database. They may have contributed to 88.88 percentage of inhibition which was the maximum among samples experimented.

b. 100ul of a biherbal formulation (0.1mg/100ml of biherbal sample made of 0.05mg of chloroform extract of Bark of *Butea monosperma* and 0.05mg of chloroform extract of stems of *Ficus religiosa* in diluent buffer) was added, the OD recorded was 0.007. % of inhibition was 74.07. Betulin and n-hexadeconoic acid were reported from GCMS analysis of chloroform extracts of bark of *Butea monosperma* and stems of *Ficus religiosa* respectively. Both were reported to be potent anti-HIV compounds in chemdb NIAID database. They may have contributed to above percentage of inhibition.

c. 100ul of a biherbal formulation (0.1mg/100ml of biherbal sample made of 0.05mg of chloroform extract of leaves of *Desmostachya bipinnata* and 0.05mg of chloroform extract of stems of *Prosopis cineraria* in 100ml diluent buffer) was added the OD recorded was 0.011. % of inhibition was 59.25. Lupeol acetate was reported from GCMS analysis of chloroform extracts of leaves of *Desmostachya bipinnata.* 9-Hexadecenoic acid, 9- octadecenyl ester (Z, Z)- (Oleyl palmitoleate),1-Nonadecene, 6-(Diethylamino)benzofuran-3(2H)-one ,Cyclotridecane ,4-Octadecenal ,1, 2, 5-Azoniadiboratole, 2, 2, 3, 4, 5-pentaethyl, 2, 5-dihydro-1-trimethylsilyl ,5-Octadecenal were reported from stems of *Ficus religiosa.* Lupeol acetate was reported to be potent anti-HIV compounds in chemdb NIAID database.

At 12.5ng/100ul concentration of rp66, 100ul of AZT (0.1mg/100ml) showed 0.034 OD.

a. 0.05mg of chloroform extract of leaves of *Desmostachya bipinnata* and 0.05mg of chloroform extract of stems of *Prosopis cineraria* was mixed in 100ml diluent buffer. 100ul of this biherbal sample when added to the well containing 12.5 ng/100ul of rp66, the OD recorded was 0.012. % of inhibition was 64.70

b. 0.05 mg of chloroform extract of Bark of *Butea monosperma* and 0.05mg of chloroform extract of stems of *Ficus religiosa* were mixed in 100ml of diluent buffer. 100ul of this biherbal formulation when added to the well containing 12.5 ng/100ul of rp66, the OD recorded was 0.009. % of inhibition 73.52. Betulin and n-hexadeconoic acid were reported from GCMS analysis of chloroform extracts of bark of *Butea monosperma* and stems of *Ficus religiosa* respectively. Both were reported to be potent anti-HIV compounds in chemdb NIAID database. They may have contributed to above percentage of inhibition.

At 0.78ng/100ul concentration of rp66, 100ul of AZT (0.1mg/100ml) showed 0.015 OD. 0.05mg of chloroform extract of leaves of *Desmostachya bipinnata* and 0.05mg of chloroform extract of stems of *Prosopis cineraria* was mixed in 100ml diluent buffer. 100ul of this biherbal formulation when added to the well containing 0.7ng/100ul of rp66, the OD recorded was 0.007. % of inhibition was 53.33.

At 0.19ng/100ul concentration of rp66, 100ul of AZT (0.1mg/100ml) showed 0.006 OD. 0.05mg of chloroform extract of leaves of *Desmostachya bipinnata* and 0.05mg of chloroform extract of stems of *Prosopis cineraria* was mixed in 100ml diluent buffer. 100ul of this biherbal formulation when added to the well containing 0.1ng/100ul of rp66, the OD recorded was 0.002. % of inhibition was 66.66

III.2 Anti HIV-1 RT p^{66} activity of Triherbal sample

At 3.12ng/100ul concentration of rp66, 100ul of AZT (0.1mg/100ml) showed 0.007 OD. 0.033mg of chloroform extract of each of bark of *Butea monosperma, stem of Ficus religiosa and leaves of Desmostachya bipinnata* was mixed in 100ml diluent buffer. 100ul of this triherbal formulation when added to the well containing 3ng/100ul of rp66, the OD recorded was 0.002. % of inhibition was 71.42

Betulin, n-hexadeconoic acid and Lupeol acetate were reported from GCMS analysis of chloroform extracts of bark of *Butea monosperma*, stems of *Ficus religiosa* and leaves of *Desmostachya bipinnata*. These three were reported to be potent anti-HIV compounds in NIAID database. They may have contributed to above percentage of inhibition.

III.3 Anti HIV-1 RT p^{66} activity of Pentaherbal sample

At 50ng/100ul concentration of rp66, 100ul of AZT (0.1mg/100ml) showed 0.027 OD. 0.02mg of chloroform extract of bark of *Butea monosperma*, stems of *Ficus religiosa*, leaves of *Desmostachya bipinnata* and 0.002mg ethanolic extract of bark of *Ficus racemosa* and stem of *Acacia catechu* was mixed in 100ml of diluent buffer.100ul of this pentaherbal formulation when added to the well containing 50ng/100ul of rp66, the OD recorded was 0.007. % of inhibition was 74.07

Betulin, n-hexadeconoic acid and Lupeol acetate were reported from GCMS analysis of chloroform extracts of bark of *Butea monosperma*, stems of *Ficus religiosa* and leaves of *Desmostachya bipinnata.* Beta amyrin and Oleic acid were reported from GCMS analysis of ethanolic extracts of bark of *Fiucs racemosa* and stems of *Acacia catechu* respectively. These five compounds were reported to be potent anti-HIV compounds in chemdb NIAID database. They may have contributed to above percentage of inhibition.

III.4 Anti HIV-1 RT p^{66} activity of double polyherbal sample

A mixture of, same polyherbal sample, extracted with two different solvents namely Ethanol and Chloroform were experimented on two different concentrations of RT p^{66} viz. 50ng and 12ng. Results were as follows -

At 50ng/100ul concentration of rp66, 100ul of AZT (0.1mg/100ml) showed 0.027 OD. 0.05mg of Ethanolic extract of a Polyherbal formulation (made of stem of *calotropis gigantea, Acacia catechu, Achyranthes aspera, Ficus religiosa, Prosopis cineraria*, Leaves of *Desmostachya bipinnata, Cynodon dactylon* Bark of *Butea monosperma and Ficus racemosa* and Stem of *Ficus religiosa*) and 0.05mg of Chloroform extract of polyherbal formulation (made of same above explants) was mixed in 100ml diluent buffer.100ul of this double polyherbal formulation when added to the well containing 50ng/100ul of rp66, the OD recorded was 0.011. % of inhibition = was 59.25

At 12.5ng/100ul concentration of rp66, 100ul of AZT (0.1mg/100ml) showed 0.034 OD. 0.05mg of Ethanolic extract of a Polyherbal formulation (made of stem of *calotropis gigantea, Acacia catechu, Achyranthes aspera, Ficus religiosa, Prosopis cineraria*, Leaves of *Desmostachya bipinnata, Cynodon dactylon* Bark of *Butea monosperma and Ficus racemosa* and Stem of *Ficus religiosa*) and 0.05mg of Chloroform extract of polyherbal formulation (made of same above explants) was mixed in 100ml diluent buffer.100ul of this double polyherbal formulation when added to the well containing 12.5ng/100ul of rp66, the OD recorded was 0.010. % of inhibition was 70.58.

GCMS analysis of Chloroform extract of polyherbal formulation yielded 4-Octadecenal; 6-(Diethylamino) benzofuran-3(2H)-one; 1-Eicosanol (**Anti-Fungal Drug);** 10-Heneicosene (c,t); 1-Nitro-beta—d-arabinofuranose,tetraacetate; 9-Hexadecenoic acid, 9- octadecenyl ester, (Z,Z)- (Oleyl palmitoleate). **GCMS analysis of ethanol extract of same polyherbal** formulation reported Naphthalene and Bicyclo (3.3.1) nonane-2, 4-dione, 9, 9-dimethoxy- . Any of above components or combination of some of above components or all the above components may have contributed to above inhibition (or) As Betulin, n-hexadeconoic acid and Lupeol acetate were reported in chloroform extracts of bark of *Butea monosperma*, stems of Ficus *religiosa* and leaves of *Desmostachya bipinnata* their presence or presence of Beta amyrin and Oleic acid in ethanolic extracts of bark of *Fiucs racemosa* and stems of *Acacia catechu* respectively may have contributed to above inhibition**(Table).**

IV.DISCUSSION

Further *in-vitro* and *in-vivo* research and application on polyherbal samples showed in table will be beneficial.

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REFERENCES

1. **Chandrakanth MG, Gilless JK, Gowramm V, Nagaraj MG** (1999). Temple forests in India's forest development, *Agroforestry system*, 11:199-211.
2. **Estari M *et al*** (2014), Calotropis gigantea flower extracts with HIV-1 reverse transcriptase (RT) inhibitory activity. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(9):1016-1022.

3. **Keshava rao VK** (2016), Natural products as Anti-HIV agents and Role in HIV-Associated Neruocognotive Disorders(HAND); A brief overview , *Frontiers in Microbiology*
4. **Kingkan B** (2010), Study of anti-HIV-1 integrase activity of Thai medicinal plants, Thesis of Master of Science in Herb Sciences, 2010, Prince of Songkia University.
5. **Nutan *et al*** (2013), Extracts of Acacia catechu suppress HIV-1 replication by inhibiting the activities of the viral protease and Tat, *Virology Journal*, 10:309

6. **Pandey D and Pandey VC** (2016), sacred plants from ancient to modern era: Traditional worshipping towards plants conservation. *Tropical Plant Research 3(1);136-141*
7. www.unaids.org

Table: Polyherbal extract samples and rp^{66} inhibition

Conc. of rp^{66}	Biherbal Sample	Inhibition percentage
50	*Ficus religiosa* *Desmostachya bipinnata*	**88.88**
	Ficus religiosa *Butea monosperma*	74.07
	Desmostachya bipinnata *Prosopis cineraria*	59.25
12.5	*Desmostachya bipinnata* *Prosopis cineraria*	64.70
	Ficus religiosa *Butea monosperma*	73.52
0.78	*Desmostachya bipinnata* *Prosopis cineraria*	53.33
0.19	*Desmostachya bipinnata* *Prosopis cineraria*	66.66
	Triherbal sample	
3.12	*Ficus religiosa* *Butea monosperma* *Desmostachya bipinnata*	71.42
	Pentaherbal sample	
50	*Ficus religiosa* *Ficus racemosa* *Butea monosperma* *Acacia catechu* *Desmostachya bipinnata*	74.07
	Double polyherbal sample	
50	***Ethanol extract of*** *Ficus religiosa* *Acacia catechu* *Ficus racemosa* *Cynodon dactylon* *Prosopis cineraria* *Calotropis gigantea* *Achyranthes aspera* *Butea monosperma* *Desmostachya bipinnata PLUS*	59.25

	Chloroform extract of *Ficus religiosa* *Acacia catechu* *Ficus racemosa* *Cynodon dactylon* *Prosopis cineraria* *Calotropis gigantea* *Achyranthes aspera* *Butea monosperma* *Desmostachya bipinnata*	
12.5	*As above*	70.58