

<https://doi.org/10.48047/AFJBS.7.1.2025.881-889>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

## Identification of Bioactive Compounds in *Phyllanthus amarus*

Schumach. & Thonn. Using GC-MS Method.

B.S.Hajgude and R. P. Patil

U.G, P.G. and Research Center, Department of Botany,  
Deogiri College, Chhatrapati Sambhajnagar-431005(M.S.)

Corresponding author's email: [bharathajgude@gmail.com](mailto:bharathajgude@gmail.com)

Volume 7, Issue 1, Jan 2025

Received: 15 Nov 2024

Accepted: 05 Jan 2025

Published: 29 Jan 2025

[doi:10.48047/AFJBS.7.1.2025.881-889](https://doi.org/10.48047/AFJBS.7.1.2025.881-889)

### ABSTRACT:

*Phyllanthus amarus* Schum.& Thonn., is globally distributed herbs known for its several therapeutic potentials. This species has a long history of use in the traditional system of medicine for over 2000 years owing to its wide array of secondary metabolites that confer significant medicinal attributes.(1) The present study was carried out to identify the phytochemicals present in the ethanol extract of *Phyllanthus amarus* Schum.& Thonn. Preliminary phytochemical screening of extracts of this species revealed the presence of proteins, alkaloids, phenolic compounds, saponins, amino acids, flavonoids and tannins. Extracts were subjected to GC-MS analysis which shows presence of phytochemicals viz palmitic acid, 2,6-dihexadecanoate, 1-methylethyl ester, methanosulfonic acid, ethyl ester, tetradecanoic acid, polyphenols, eupalitin, Epicatechin etc which has significant important in human life.

Keywords: Phyllanthus, *Phyllanthus amarus*, Phytochemical, Bioactive.

### INTRODUCTION:

For the first time genus *Phyllanthus* was described by Linnaeus in 1737.The genus *Phyllanthus* (phyllon = leaf, anthos = flower) consists of approximately 550–750 species that are further subdivided into 10–11 subgenera,including *Isocladius*, *Kirganelia*, *Cicca*, *Embllica*, *Conani*,

*Gomphidium*, *Phyllanthodendron*, *Xylophylla*, *Botryanthus*, *Ericococus*, and *Phyllanthus*. (Calixto *et al.*, 1998; Kathriarachchi *et al.*,2006) It is distributed throughout the tropical and subtropical regions of north and south hemispheres. It possesses an array of biological activities such as hepatoprotective, anti-inflammatory, anti-cancer, diuretics, nephroprotective, anti-oxidant, anti-viral, anti-bacterial, anti-hyperglycemic, anti-hypercholesterolemic etc. (Adeneye, 2006; Odukoya *et al.*,2007; Odetola and Akojenu, 2000; Anonymous, 2011).Recent report of anti-viral activity and its potential as a remedy for hepatitis B-virus infection has also attracted researchers to evaluate the potential of the plant (Martins *et al.*, 2011). phytoconstituents present in *P. amarus* Schum.& Thonn were investigated by using GC-MS analysis. GC-MS identify the chemicals like long chain hydrocarbons, alcohols, acids esters, alkaloids, steroids, amino acid and nitrogen compound.

### **Morphology:**

*Phyllanthus amarus* Schum & Thonn. Kongl. Danske Vidensk.Selsk. Skr. 4: 195.196. 1826; Airy Shaw in Kew Bull. 26:317. 1972. P.niruri auct.non L. 1753 p.p.; Hook. f. Fl. Brit. India 5: 298.1887; Cooke, Fl. Pres. Bombay 3: 84.1958 (Repr.).

Vernacular name: Bhui- awala( in Marathi )

### **Description:**

It is annual, multi branched herb, mostly erect with height up to 60-90 cm. Rounded, smooth, greenish stem with horizontal branching. Leaves have short petiole and are alternately arranged, elliptical to oblong in shape, pale yellow on dorsal side. Inflorescence is axillary, each axile having one male and one female flowers. Flowers are small and green to whitish in colour. Fruits are capsule with round shape and brown colour having 6 seeds inside the capsule.

Exsicata- Gautala Forest, Chhatrapati Sambhajinagar.

### **Materials and methods:**

#### **Collections of plant materials:**

Leaves of *Phyllanthus amarus* Schum.& Thonn was collected from different regions of Marathwada, it was also documented earlier ( Naik, 1998).

#### **Preparation of powder and extract**

The plant material of *Phyllanthus amarus* Schum.& Thonn was washed with sterile distilled water and was shade dried followed by pulverized to powder using a pestle and a mortar.

#### **Plant sample extraction:**

From the plant material 20g of powder was weighed and transferred to the soxhlet apparatus for extraction then pulverized powder was extracted exhaustively with 99% ethanol for 16 hr at 70<sup>0</sup> C. The crude extract obtained was dried between 40<sup>0</sup>-45<sup>0</sup>C using water bath. The final residue thus obtained was then sent to MIT Center for Analytical Research and Studies (MIT-CARS), Chhatrapati Sambhajnagar for GC-MS analysis.

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

GC-MS analysis was carried out on a GC-MS-QP 2020 Plus Scemadzu corp.0084 system and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column SH-Rxi-sil MS, electron ionization system with ionization energy of 70eV was used. Helium gas was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (Split ratio of 10:1) injector temperature-250<sup>0</sup>C; ion-source temperature 280<sup>0</sup>C. The oven temperature was programmed from 110<sup>0</sup>C (Isothermal for 2 min.) with an increase of 10<sup>0</sup>C / min to 200<sup>0</sup>C then 5<sup>0</sup>C / min. to 280<sup>0</sup>C / min, ending with a 9 min. isothermal at 280<sup>0</sup>C. Mass spectra were taken at **70 e V**; a **scan interval of 0.5s** and fragments from 40 to 550Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbo mass.

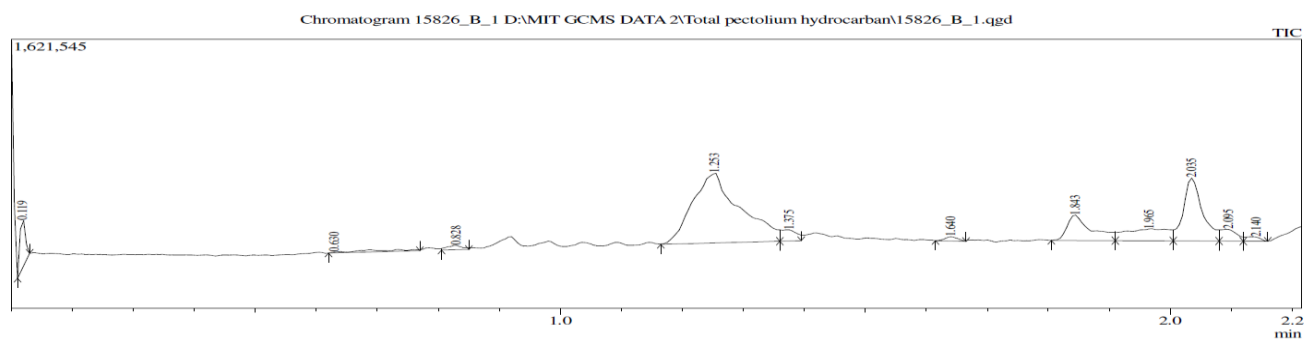
**Table 1. Phytochemicals present in *Phyllanthus amarus* Schum.& Thonn**

Sr. No	Compound Name	Retention Time	Pick Area (%)	Molecular Formula	Molecular weights
1.	Borane carbonyl	0.119	4.6172	CH <sub>3</sub> BO	42
2.	[1,2,3,4]Tetrazolo[1,5-b][1,2,4]triazine,	0.630	1.5098	C <sub>3</sub> H <sub>6</sub> N <sub>6</sub>	126
3.	Benzoic acid	0.828	1.0573	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	122
4.	Benzofuran,2,3-dihydro-	1.253	<b>50.2157</b>	C <sub>8</sub> H <sub>8</sub> O	120
5.	N-(Furan-3-ylmethylene-hydrazinocarbonylme	1.375	2.527	C <sub>14</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub>	271
6.	1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1	1.640	0.8012	C <sub>13</sub> H <sub>22</sub> O <sub>2</sub>	210
7.	4-Hydroxy-2-methylacetophenone	1.843	9.5352	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	150
8	3-Butyn-1-ol	1.965	7.9971	C <sub>4</sub> H <sub>6</sub> O	70
9	2,6-dimethoxy- Phenol,	2.035	18.0085	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	154

10	Nonanoic acid, hexyl ester	2.095	2.8108	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242
11	Carbonochloridic acid,decyl ester	2.140	0.9200	C <sub>11</sub> H <sub>21</sub> ClO <sub>2</sub>	220

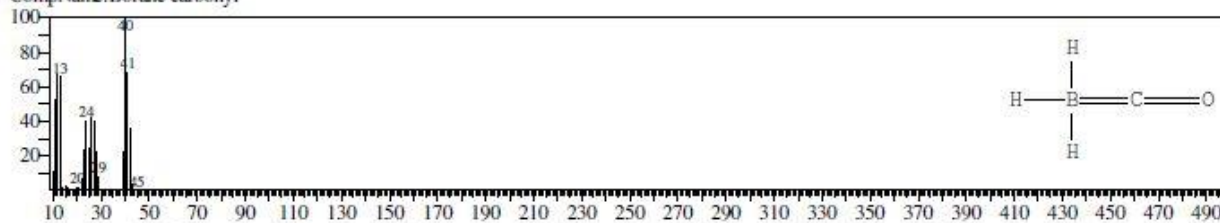
## Result and Discussion:

The present study was carried out on plant *Phyllanthus amarus* Schumach. & Thonn showed the medicinally important bio-active components. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and technique (NIST14). The spectrum of unknown components stored in the NIST14. The molecular weight, Molecular formula and Structure of component of test materials were identified.

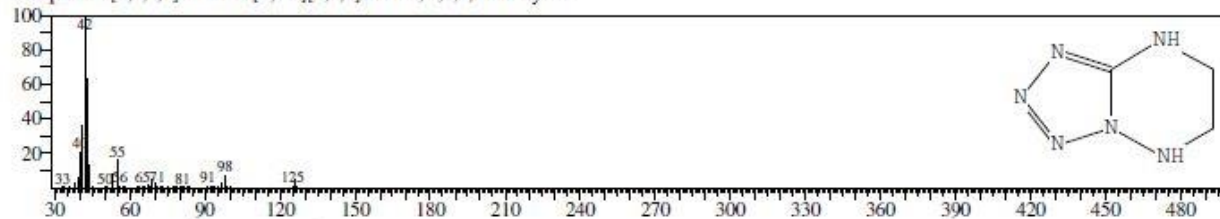


## Chromatogram of *Phyllanthus amarus* Schum.& Thonn

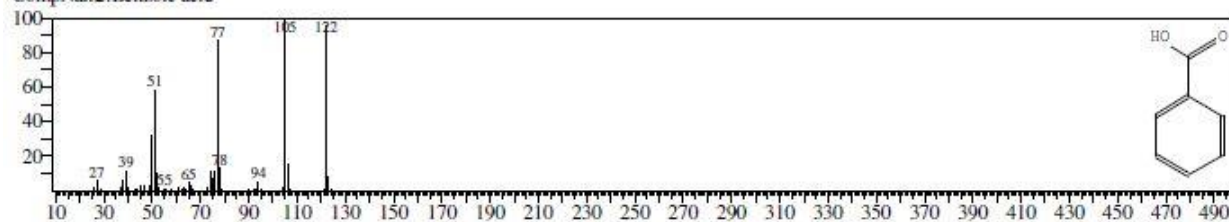
Hit#1 Entry:59 Library:NIST14.lib  
 SI:81 Formula:CH<sub>3</sub>BO CAS:13205-44-2 MolWeight:42 RetIndex:0  
 CompName: Borane carbonyl



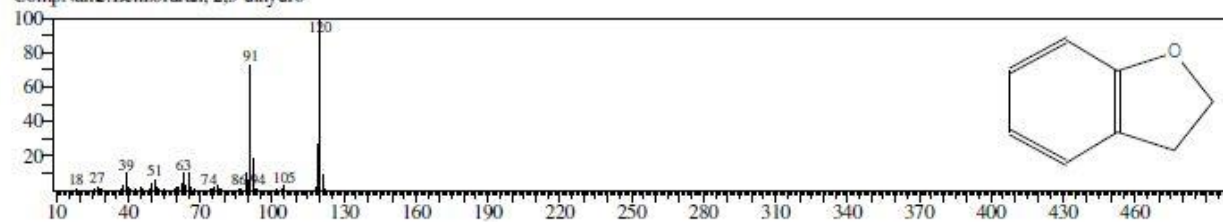
Hit#1 Entry:11180 Library:NIST14.lib  
 SI:68 Formula:C<sub>3</sub>H<sub>6</sub>N<sub>6</sub> CAS:0-00-0 MolWeight:126 RetIndex:0  
 CompName:[1,2,3,4]Tetrazolo[1,5-b][1,2,4]triazine, 5,6,7,8-tetrahydro-



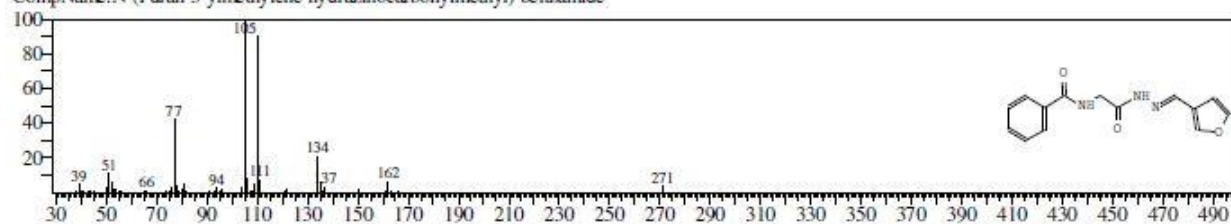
Hit#1 Entry:10012 Library:NIST14.lib  
 SE:84 Formula:C7H6O2 CAS:65-85-0 MolWeight:122 RetIndex:1150  
 CompName:Benzoic acid



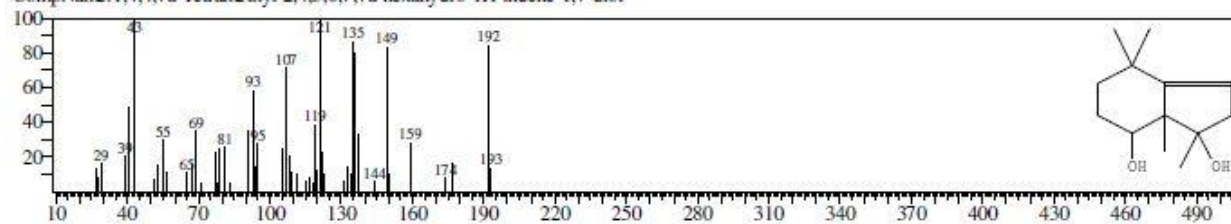
Hit#1 Entry:9561 Library:NIST14.lib  
 SE:89 Formula:C8H8O CAS:496-16-2 MolWeight:120 RetIndex:1036  
 CompName:Benzoofuran, 2,3-dihydro-



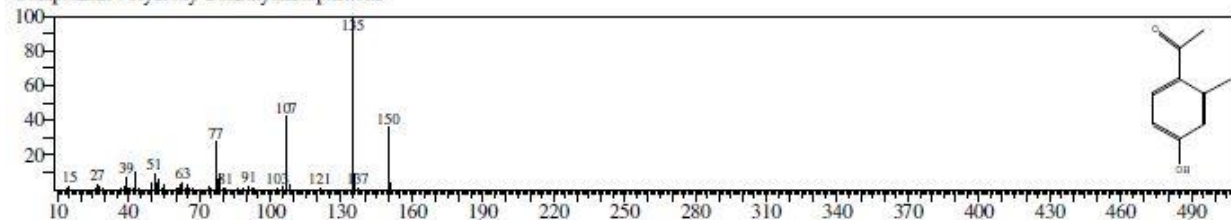
Hit#1 Entry:131416 Library:NIST14.lib  
 SE:71 Formula:C14H13N3O3 CAS:318259-19-7 MolWeight:271 RetIndex:2630  
 CompName:N-(Furan-3-ylmethylene-hydrazinocarbonylmethyl)-benzamide



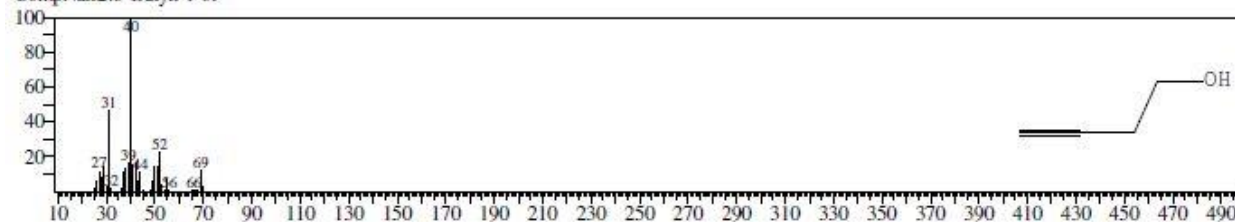
Hit#1 Entry:74698 Library:NIST14.lib  
 SE:69 Formula:C13H22O2 CAS:121747-53-3 MolWeight:210 RetIndex:1600  
 CompName:1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol



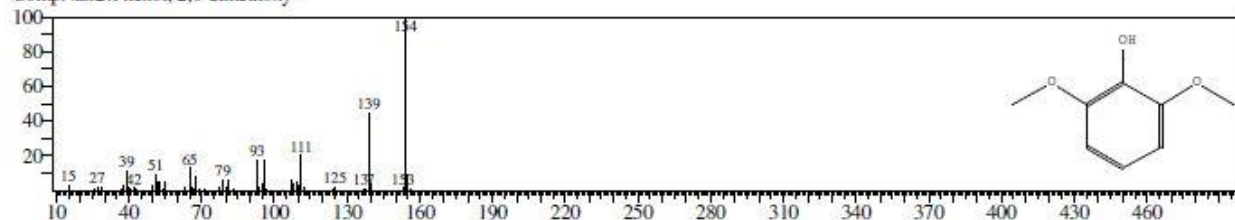
Hit#1 Entry:24788 Library:NIST14.lib  
 SE:86 Formula:C9H10O2 CAS:875-59-2 MolWeight:150 RetIndex:1363  
 CompName:4-Hydroxy-2-methylacetophenone



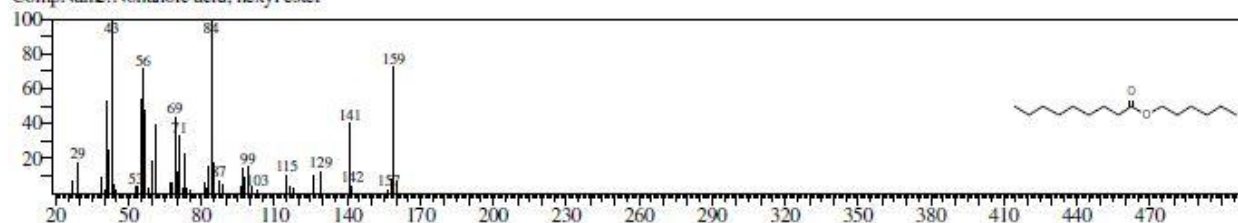
Hit#1 Entry:542 Library:NIST14.lib  
 SI:60 Formula:C<sub>4</sub>H<sub>6</sub>O CAS:927-74-2 MolWeight:70 RetIndex:659  
 CompName:3-Butyn-1-ol



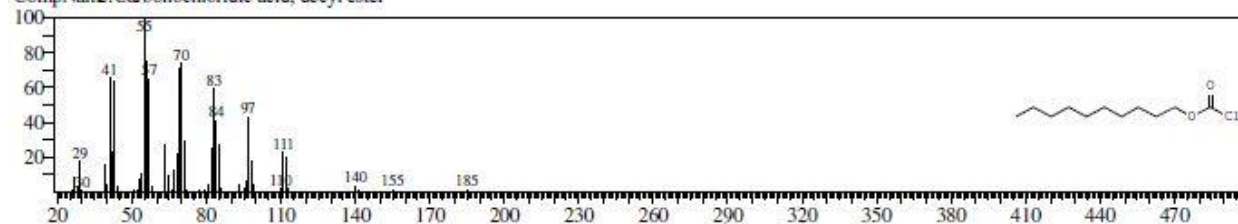
Hit#1 Entry:27835 Library:NIST14.lib  
 SI:92 Formula:C<sub>8</sub>H<sub>10</sub>O<sub>3</sub> CAS:91-10-1 MolWeight:154 RetIndex:1279  
 CompName:Phenol, 2,6-dimethoxy-



Hit#1 Entry:104473 Library:NIST14.lib  
 SI:68 Formula:C<sub>15</sub>H<sub>30</sub>O<sub>2</sub> CAS:6561-39-3 MolWeight:242 RetIndex:0  
 CompName:Nonanoic acid, hexyl ester



Hit#1 Entry:83195 Library:NIST14.lib  
 SI:73 Formula:C<sub>11</sub>H<sub>21</sub>ClO<sub>2</sub> CAS:55488-51-2 MolWeight:220 RetIndex:1458  
 CompName:Carbonochloridic acid, decyl ester



## GC-MS ANALYSIS

The active principles with their retention time (RT), molecular formula, molecular weight and percentage composition in the ethanol extract of leaves of *P. amarus* Schum.& Thonn is presented in Table 1.

In the present study includes characterisation of the chemical profile of *P. amarus* Schum.& Thonn by using GC-MS analysis. The chromatogram showed the relative concentration of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyses the compound extracted at different times to identify the nature and structure of the compounds.

Several compounds were identified in the ethanolic extracts of leaves of *P. amarus*. The prevailing compounds were Borane carbonyl ; [1,2,3,4] Tetrazolo [1,5-b][1,2,4] triazine; Benzoic acid ;Benzofuran,2,3-dihydro-, N-(Furan-3-ylmethylene-hydrazinocarbonylme; 1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1;4-Hydroxy-2methyl acetophenone; 3-Butyn-1-ol ;2,6-dimethoxy Phenol; Nonanoic acid, hexyl ester; Carbonochloridic acid, decyl ester,etc. The spectrum profile of GC-MS confirmed the presence of 11 major components with the retention time 0.119, 0.630, 0.828, 1.253, 1.375, 1.640, 1.843, 1.965, 2.035, **2.095** and 2.140 mins respectively. Highest concentration of compound was found in Nonanoic acid, hexyl ester, with retention time of 2.095 mins. While percent peak area was 4.6172, 1.5098, 1.0573, 50.2157, 2.527, 0.8012, 9.5352, 7.9971, 18.0085, 2.8108, 0.9200 respectively. Highest percent peak area was in Benzofuran,2,3-dihydro- which is 50.21%.

## DISCUSSION

During the investigation the following compounds were identified like 2,6-dimethoxy-phenol which is a phenolic compound and it may be employed as an antioxidant, antimicrobial, antifungal and anti-inflammatory agents. Nonanoic acid is a fatty acid and it may be an active antimicrobial and anti-diarrhoeal agent. 3-Butyn-1-ol is suggested to be an aliphatic alcoholic compound and it employed as an antimicrobial (Duke, 2012).

By interpreting these compounds, it is found that *P.amarus* Schum.& Thonn possesses various therapeutical applications. In present investigation the standardization parameter of phytochemical study was used, which could help in authentication of *Phyllanthus amarus* Schum.& Thonn. For preparation of the monograph, result of present study will used as

reference material. Purification, isolation and identification of phyto-constituent results may be useful in the preparation of a unique drug.

## References:

1. Bose Mazumdar Ghosh A, Banerjee A, Chattopadhyay S. An insight into the potent medicinal plant *Phyllanthus amarus* Schum. and Thonn. Nucleus (Calcutta). 2022;65(3):437-472.
2. Calixto JB,Santos AR, Cechinel Filho V, Yunes RA. A review of the plants of the *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. Med Res Rev. 1998, 18:225–58.
3. Kathriarachchi H, Samuel R, Hoffmann P, Mlinarec J, Wurdack KJ, Ralimanana H, Stuessy TF, Chase MW. Phylogenetics of tribe Phyllantheae (Phyllanthaceae; Euphorbiaceae sensu lato) based on nrITS and plastid matK DNA sequence data. Am J Bot. 2006 Apr;93(4):637-55.
4. Anonymous: Tropical Plant Database (2011). <http://www.raintree.com/plant.htm> Access Date: 27/8/2011.
5. Adeneye AA (2006). Hypoglycemic and hypochloesterolemic activities of the leaf and seed extract of *Phyllanthus amarus* in mice. Fitoterapia. 77(7-8): 511 – 514
6. Odetola AA, Akojenu SM (2000). Antidiarrhoeal and gastrointestinal potentials of the aqueous extract of *Phyllanthus amarus*(Euphorbiaceae)-Afri. J. Med. Sci. 29 (2):119-122.
7. Odukoya OA, Inya-Agha SI, Ilori OO (2007). Immune boosting herbs: lipid per-oxidation in liver homogenate as index of activity J. Pharm. Toxicol. 2 (2):190-195.
8. Martins, L.R.R., Pereira-Filho, E.R., Cass, Q.B., 2011. Chromatographic profiles of *Phyllanthus* aqueous extracts samples: a proposition of classification using chemometric models. Anal. Bioanal. Chem. 400, 469–481.

9. NIST Standard Reference Database 1A NIST/EPA/NIH Mass Spectral Library (NIST 14) and NIST Mass Spectral Search Program (Version 2.2)
10. Naik V.N. (1998) "Flora of Marathwada "Vol. 1 Amrut Prakashan, Aurangabad.
11. N.P.Singh,P.Lakshminarasimhan,S.Karthikeyan & P.V.Prasanna "FLORA OF MAHARASHTRA STATE" DICOTYLEDONES,VOLUME 2 (Combretaceae to Ceratophyllaceae).BOTANICAL SURVEY OF INDIA
12. Duke JA (2012). Phytochemical and ethnobotanical databases. [http:// search.conduit.com/ results..](http://search.conduit.com/results..)