Nat. Volatiles & Essent. Oils, 2021; 8(4): 16690-16703

NVEO
Natural Volatiles &
Essential Oils

Isolation, Characterization And In-Silico Studies Of Phytoconstituents Isolated From Chloroform Stem Bark Extract Of Holarrhena Pubescens

Bonagiri Sreedevi*[a] , Vijaya Kuchana[b] , S. Shobharani[c]

a-Department of Pharmaceutical chemistry, Teegala Ram Reddy College of pharmacy, Meerpet, Hyderabad, Telangana, India
b-Department of Pharmaceutical Chemistry, Teegala Krishna Reddy College of Pharmacy, Hyderabad.
c- Department of Pharmaceutical Chemistry, Center for pharmaceutical sciences, Institute of Sciences and Technology, JNTU, Hyderabad.

Abstract

Holarrhena pubescens (HP) commonly known as Tellicherry bark which has been used by tribals for many therapeutic ailments. Stem bark of HP was collected from local area and extracted using chloroform by soxhlation process and isolated the phytoconstituents from the chloroform extract using column chromatography method by gradient elution technique. Five compounds were isolated from the column out of which 3 compounds were found to be novel from the HP. Obtained phytoconstituents were checked for Insilco studies using reverse docking process with the help of Schrodinger software and the docking score indicates that the phytoconstituents were found to have very good antidiabetic action.

Keywords: Holarrhena pubescens, phytochemical isolation, Reverse docking, In-silico studies.

Introduction

Holarrhena pubescens commonly known as Tellicherry bark (English) or Kurchi (Hindi) is a flowering plant native to the Indian subcontinent, central and southern Africa, Indochina and parts of China (Department of AYUSH). It belongs to the Kingdom Plantae, Order Gentianales and Family Apocynaceae (India Biodiversity Portal). Parts of the plant like seeds and bark are used for therapeutic purposes in fever, dysentery, diarrhoea and amoebiasis. It has anti-malarial (Sarot C et. al., 2019), anti-diarrheal (Panda SK et.al., 2012), anti-bacterial (Dey A et.al.,2012) , anthelmintic (Patil R et.al., 2012), anti-amoebic (Shahabuddin KU et.al., 2006) , anti-oxidative(Anup B et.al., 2014), and blood purifying properties. It has no harmful side effects but includes some common effects like nausea and vomiting due to its bitter taste in some people. No adverse drug reactions were reported in pregnant and lactating women (Daniel M., 2006). Despite numerous therapeutic actions, no phytoconstituents were isolated from the stem bark hence an attempt has been made for isolation, characterisation and reverse docking of phytoconstituents.

Materials and methods

Authentication of Plant Material

The stem bark of Holarrhena pubescens (H.P) wall ex G.Don was collected from Nirmal district, Telangana, India during November 2016. The plant materials were authenticated by Dr.L.Rasingam, scientist In-charge at botanical survey of India, Deccan regional centre, Hyderabad, Telangana, India. Voucher specimen No: BSI/DRC/16-17/681, deposited at the Botanical survey of India.

Method of extraction

The plant part was washed and dried under shade. It was powdered using a pulveriser. Then the powder was placed in the Soxhlet apparatus using chloroform as menstrum it was subjected for 72hrs of extraction (Redfern J, et.al. 2014). At 40ºC the extract was concentrated in a Rotary evaporator to remove the excess of solvents obtained, solvent free extract was stored in a vacuum desiccator until further usage.

Isolation of phytoconstituents

Isolation of chemical constituents was carried from the obtained chloroform extract using column chromatography method (Sasidharan S et.al. 2011). Silica gel C is chosen as adsorbent for the stationary phase. Gradient elution techniques for elution using petroleum ether: chloroform: ethanol as mobile phase.

Column packing

The final concentrated solution of the chloroform extract of Holarrhena pubescens bark extract was mixed with column chromatography's silica gel (Eldahshan OA et.al. 2015). It was then mixed with the solvent, and the slurry was introduced at the top of the silica gel column, and a cotton plug was placed on it. Remaining empty space is filled with the mobile phase.

Gradient elution

The column was then eluted successively with petroleum ether: chloroform: ethanol, fractions were collected for 500ml portions mobile phase has been removed by using distillation method, final end condensed fraction is subjected to TLC using the eluent mobile phase concentration and Dragendroff's reagent and 5% sulphuric acid in ethanol is used as spraying reagent in order to mix the fractions of same compounds (Tsao R et. al., 2004). Isolated compounds listed at various mobile phases were given in the (Table: 1).

Characterisation: (Mallikharjuna P B et.al. 2007)

Five purified, isolated phytoconstituents were identified by FTIR, NMR, and MASS spectral characterisation process for further identification process the spectras were given from Fig: 1 to Fig15

In-silico studies: Reverse docking (Prime: v3.1; 2012, Maestro: v9.3; 2012):

The main technique used to screen anti-diabetic activity was structure-based drug design. The target proteins were obtained in their 3D forms from the RCSB PDB, and the binding affinities of putative ligand compounds to these protein targets were evaluated. Throughout the process, only the Schrodinger suite of applications was employed.

Software and software components

All software and software components were executed on the Schrodinger Maestro interface. Site Map was utilised to find ligand binding locations, and Receptor grid generation was used. LigPrep was utilized in the ligand preparation process. Glide was required to carry out the docking process, which is detailed in a separate section (Prime: v3.1; 2012, Maestro: v9.3; 2012).

Docking of ligands

The ligands Piceatannol and Resveratrol used in this research came from PubChem, a free database of 13 million commercially available molecules both academic and pharmaceutical researchers can use to conduct structural-based virtual screening studies (Irwin, J. J et.al. 2005). SDF files have been used to download individual ligands. Caavuranamide, Holadysenterine, and Mokluangin- D ligands were drawn in chemsketch and saved as MOL files.

Ligand preparation

To ensure that the desired ligands were in a low-energy state with the suitable stereochemistry because of its structure, Schrödinger's LigPrep was applied (Brooks, W. H; 2008). Brooks et al. emphasised the importance of stereochemistry in virtual screening, stating that including all stereoisomers of all prospective lead compounds was critical to avoid false negatives or losing a significant proportion of the potential leads (Du, J; 2011). LigPrep may also convert a 2D sketched structure into a 3D form, which can then be processed further in a sequence of processes to create a 3D ligand. The ligands' ionisation states, tautomers, ring conformations, molecular weights, and the quantity and types of functional groups were all checked as part of the preparation process. In table: 2 the details of the smile representation were given

Ligand docking

All ligands downloaded from Pubchem were first subjected to high throughput virtual screening (HTVS), then standard precision (SP), and finally extra precision (XP) docking (Glide: v5.8. 2012). These three docking modes were applied in this order to all docks in this work. The first docking mode to limit the amount of intermediate conformations throughout the docking funnel was HTVS docking. The SP mode was used to screen ligands that had adequate HTVS Glide scores based on the user's preferences. XP mode was created to screen false - positive and bioactive constituents that could bind to a specific conformation of the receptor using top-scoring ligand poses. Each docking job for the selected ligands was processed and divided into a number of sub jobs that could be handled by a restricted number of CPUs in the computer. Table 3 shows the docking results obtained.

Results

Table: 1: phytoconstituents isolated at various ratios of mobile phases

Code Assigned	Ratio of mobile phase	Specifications
H.P-01	Pet ether 90%: chloroform 10%	Light yellow colour crystals formed (+ve for alkaloids)
H.P-02	Pet ether 35%: chloroform 65%	White amorphous (+ve for alkaloids)

H.P-03	Chloroform 100%	colourless crystals (+ve for Liebermann bur chard test)
H.P-04	Chloroform 75%: ethanol 35%	White amorphous ((+ve for Shinoda test))
H.P-05	Chloroform80%: ethanol 20%	White crystals (+ve for Shinoda test)

Characterization of compounds

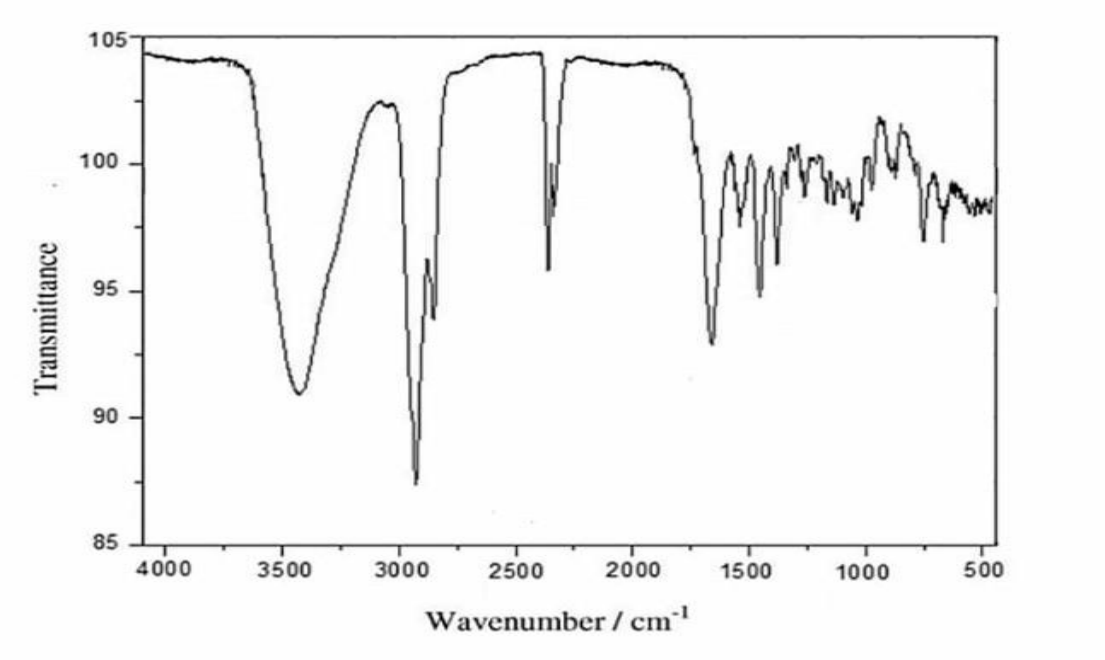

Fig: 1 FTIR spectra of H.P-01.

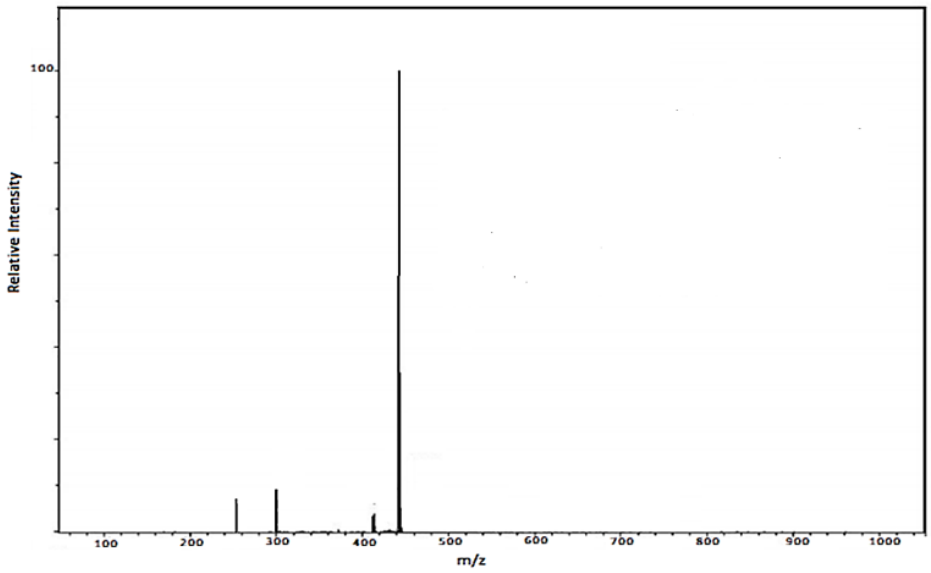

Fig: 02: Mass spectra of H.P-01.

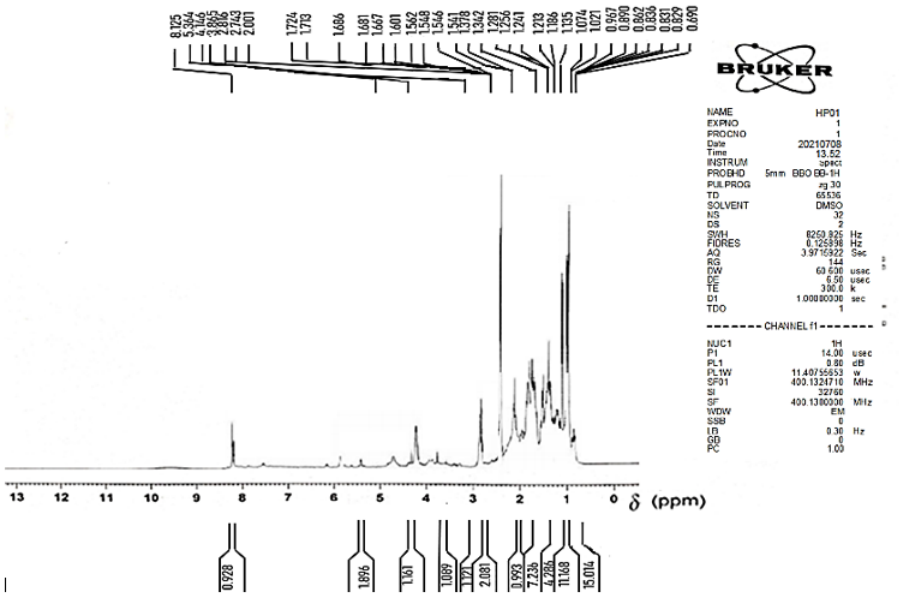

Fig: 03: NMR Spectra of H.P-01.

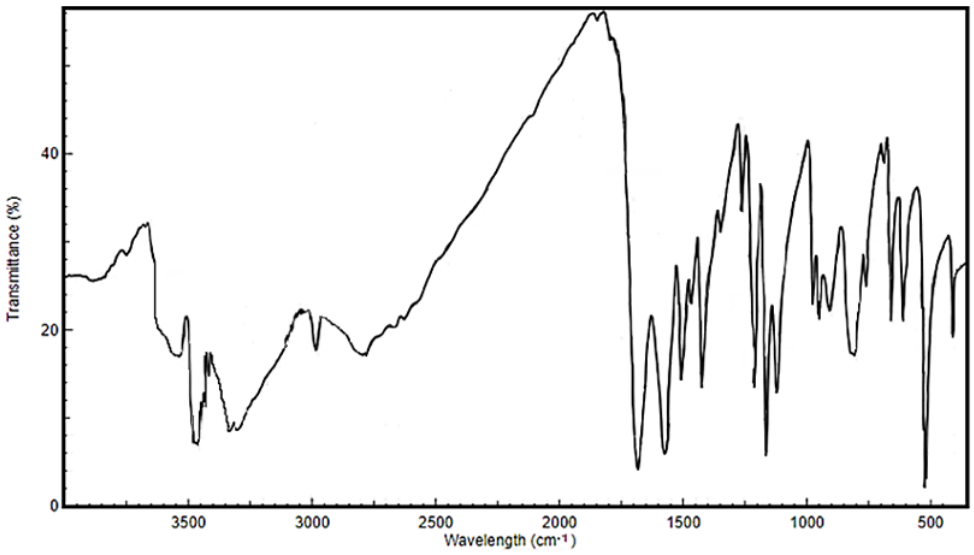

Fig: 04: FTIR Spectra of H.P-02.

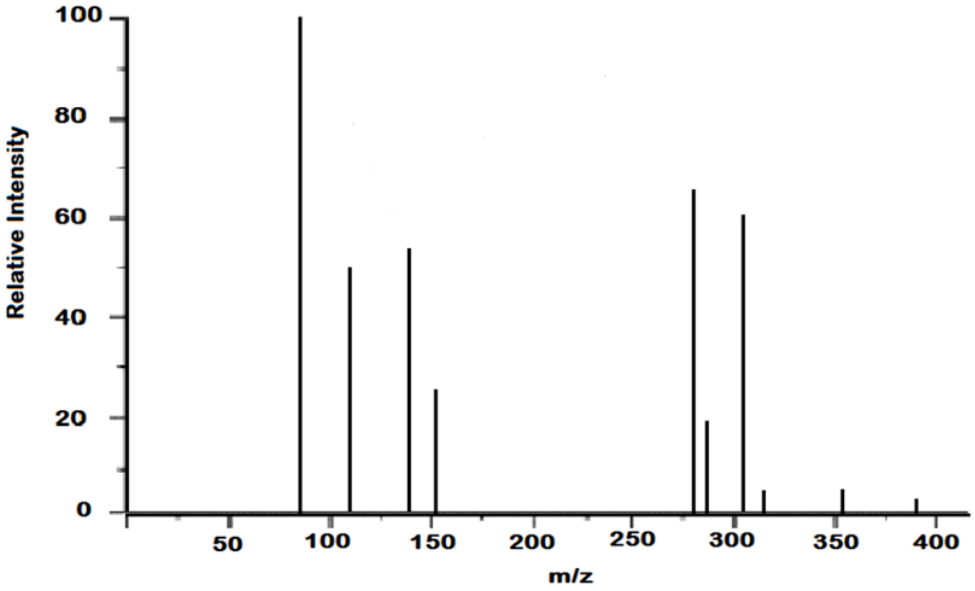

Fig: 05 Mass spectra of H.P-02.

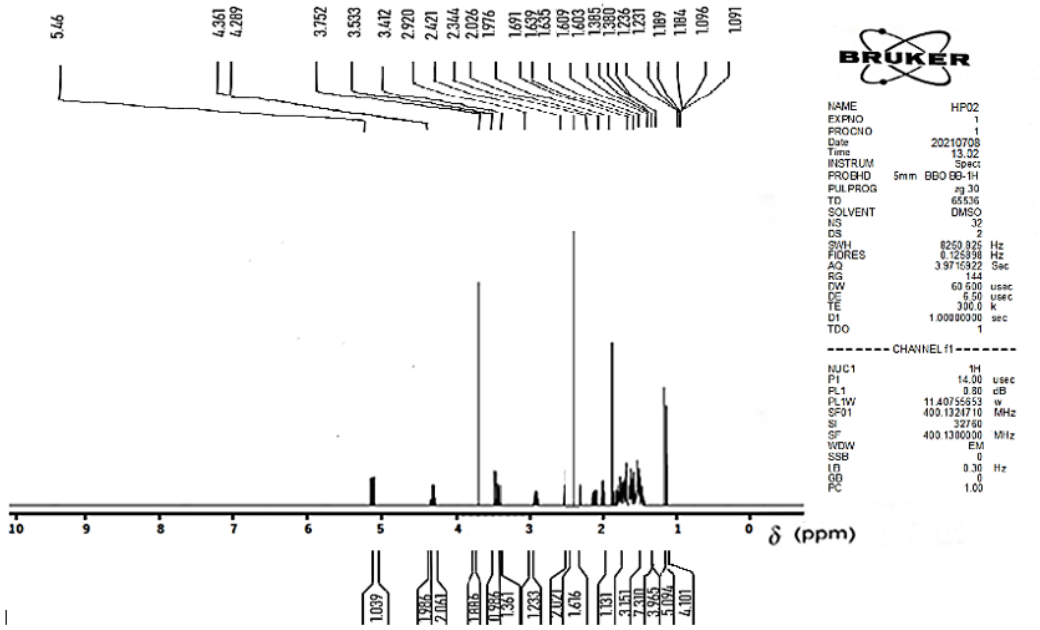

Fig: 06 NMR spectra of H.P-02.

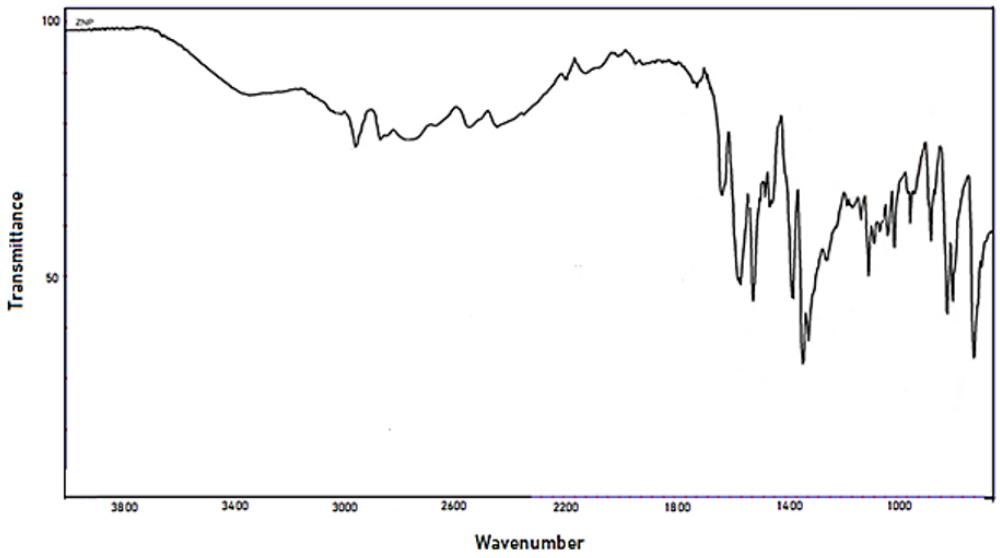

Fig: 07 FTIR Spectra of H.P-03.

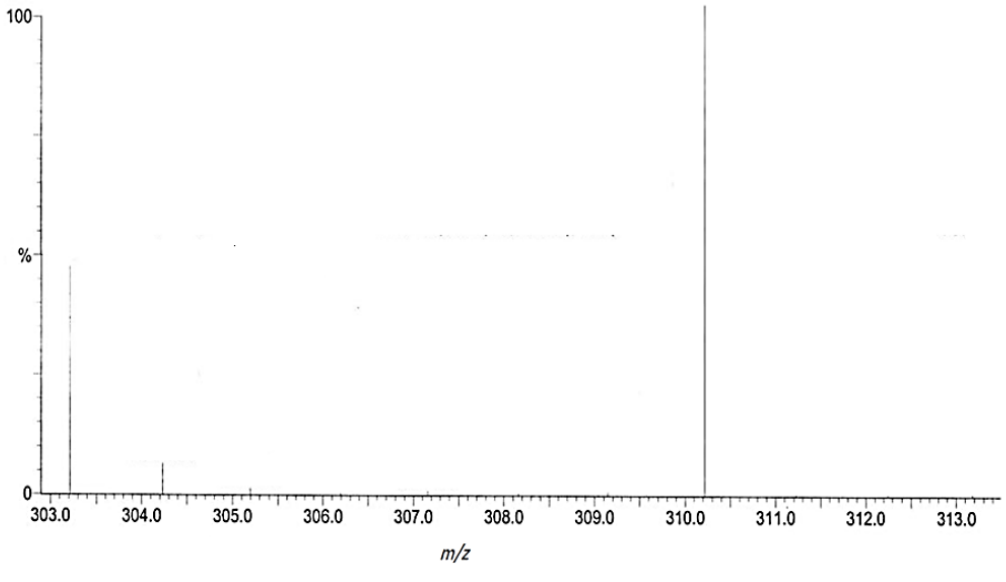

FIG: 08 MASS spectra of H.P-03.

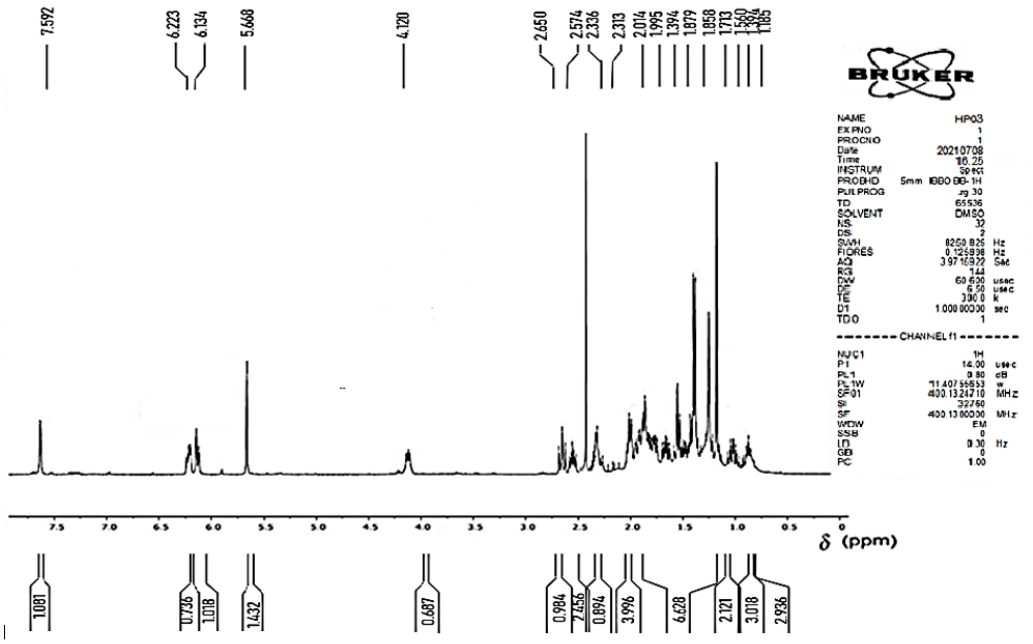

FIG: 09: NMR SPECTRA OF H.P-03.

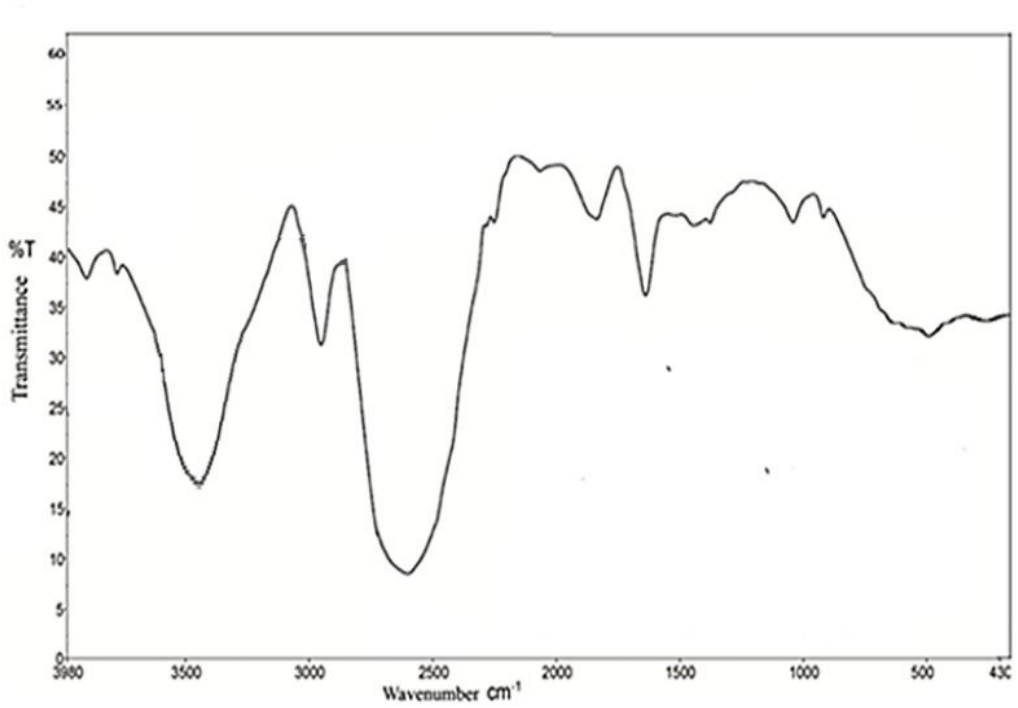

Fig: 10: FTIR spectra of H.P-04.

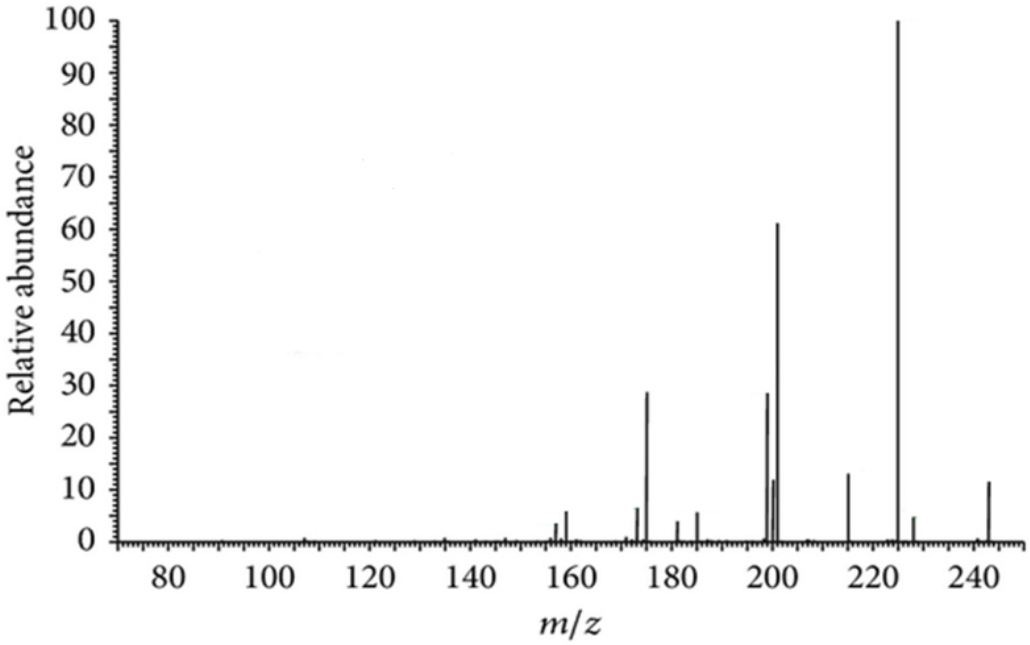

Fig: 11: Mass spectra of H.P-04.

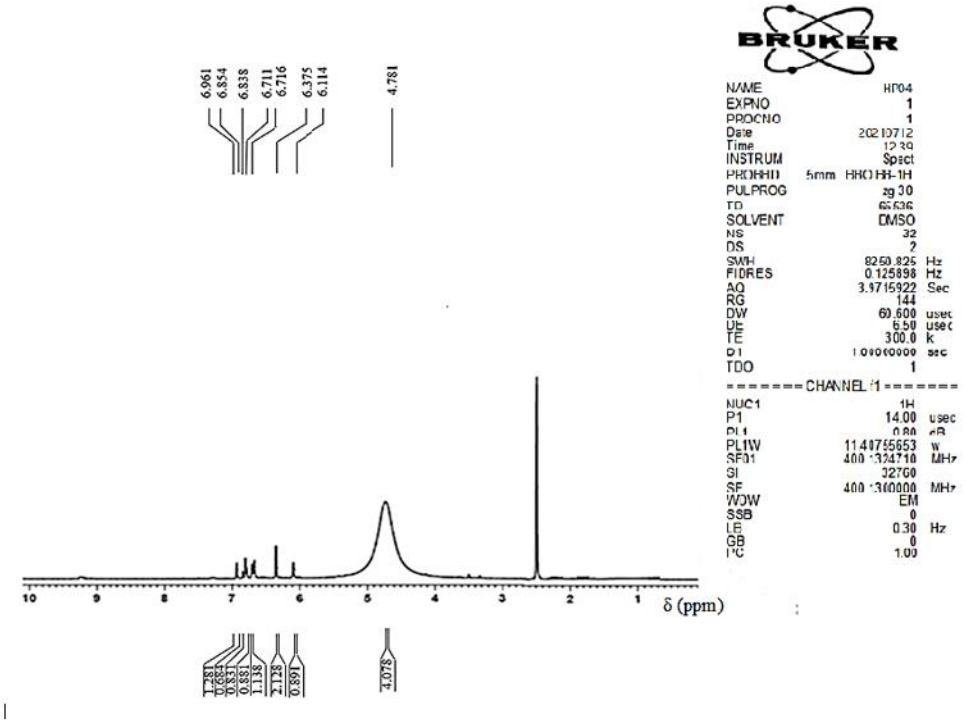

Fig: 12 NMR spectra of H.P-04.

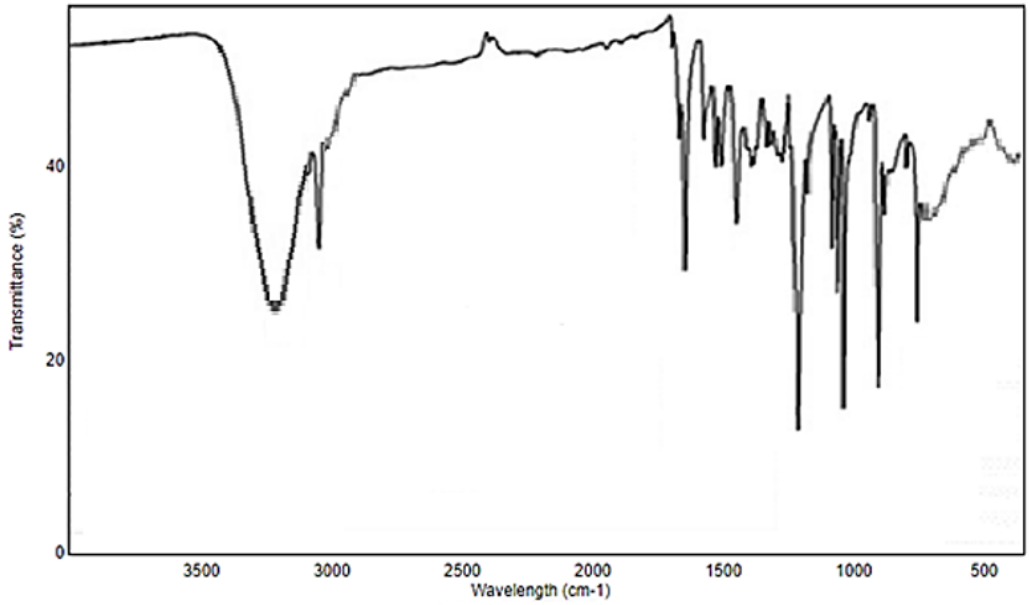

Fig: 13 FTIR spectra of H.P-05.

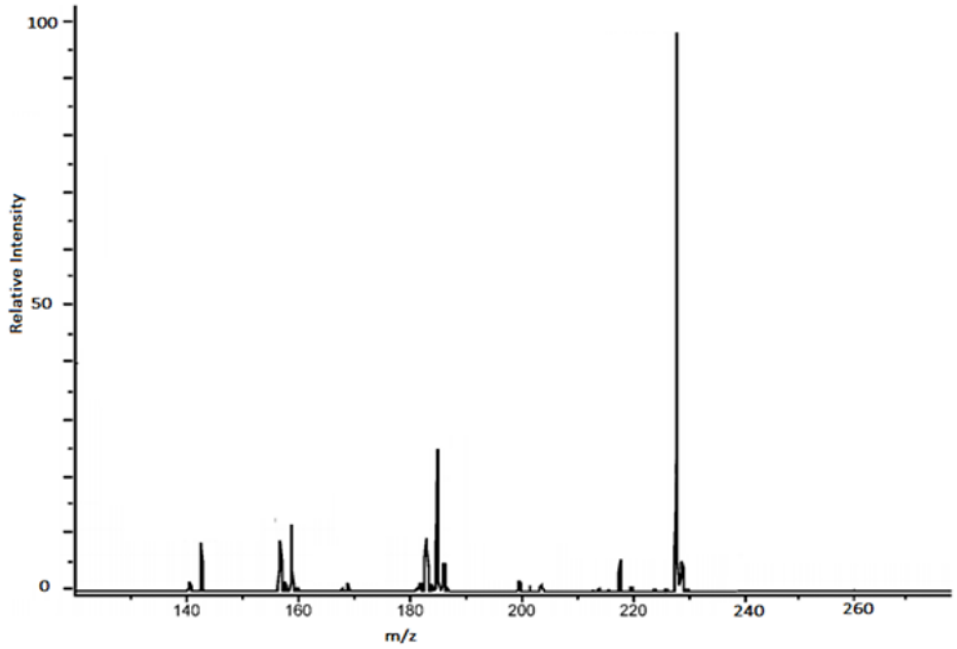

Fig 14: MASS spectra of H.P-05.

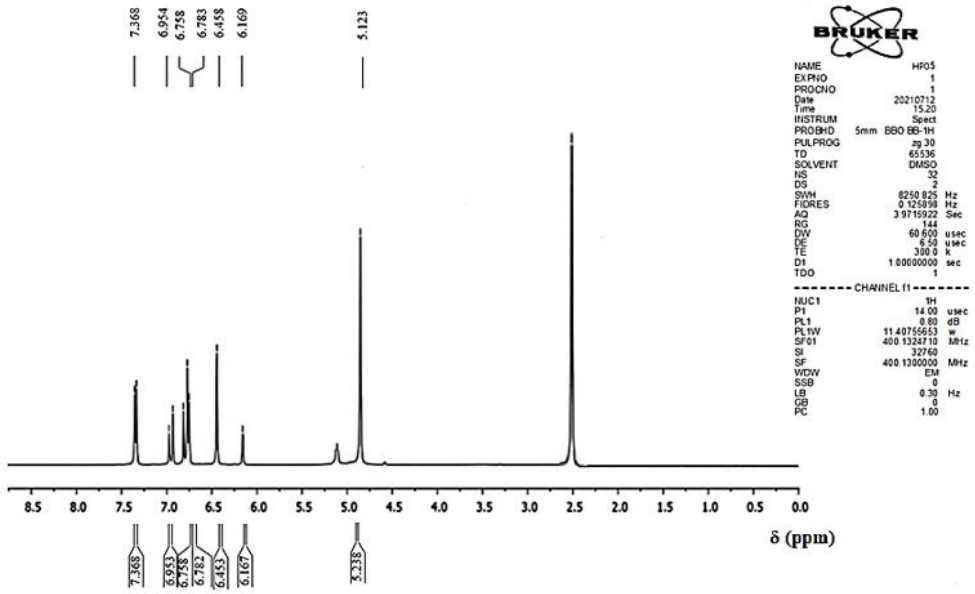

Fig15: NMR spectra of H.P-05.

Table: 2 H.P isolated compounds along with their smile representation

S.No	Name of the Compound	Smile
1	Caavuranamide	C[C@H]1[C@H]2[C@H](C[C@H]3[C@@H]4CCC5C[C@@H](NC=O)CC[C@]5(C)[C@H]4CC[C@]23C)OC21CCC(C)CN2
2	Holadysenterine	CC(=O)N(O)C(C)C1CCC2C3CC=C4CC(N)CCC4(C)C3CCC12CO
3	Mokluangin-D	O=C1C=C2C=CCCC2(C)C2CCC34C=NC(C)C4CCC3C21
4	Piceatannol	C1=CC(=C(C=C1C=CC2=CC(=CC(=C2)O)O)O)O
5	Resveratrol	C1=CC(=CC=C1C=CC2=CC(=CC(=C2)O)O)O

Table. No: 3 - Docking scores (Glide Scores) of the Isolated compounds with Aldose Reductase, alpha amylase, alpha glucosidase and insulin receptor.

H.P					
S.no	Name of the Compound	Docking Score			
		Aldose Reductase - 6TUF	Alpha Amylase - 6Z8L	Alpha Glucosidase - 7KBR	Insulin Receptor - 7BW8
1	Caavuranamide	0.00	0.00	-4.19	-5.27
2	Holadysenterine	-2.34	-4.12	-4.37	-4.16
3	Mokluangin-D	-3.07	-5.99	-6.73	-4.95
4	Piceatannol	-8.69	-5.97	-7.00	-6.85

5	Resveratrol	-8.95	-5.43	-6.79	-5.55

Discussion

After extraction of the H.P stem bark extract using chloroform and ethanol solvents. As per the significant action identified in the pharmacological activities chloroform extract was found to be more significant therapeutically active, hence it was isolated using gradient elution technique, light yellow crystals which gave positive for alkaloids were isolated using the mobile phase at a concentration of 90% pet ether and 10% chloroform , based on spectral elucidation of the Fig:1, of FTIR spectra 3400 cm^{-1} indicated the functional group of NH, 2970 cm^{-1} indicates the functional group of CH_2 , 2850cm^{-1} indicates the functional group of -CH, 1270cm-1 indicates the functional group of CN, 1065cm^{-1} indicates functional group of -CO, As per the Fig :2 Molecular weight of the compound was found to be 443, and as per the NMR spectral characterisation given in Fig: 3 8.125 (s, 1H, CHO); 5.3 (s, 2H, NH);0.86-4.164 (29H, CH_2 & CH); 0.836 (d, 3H, CH_3); 0.831 (d, 3H, CH_3); 0.829 (s, 3H, CH_3); 0.69 (s, 3H, CH_3), as per the spectral characterisation information in fig:1, fig2 and fig 3 the compound was found to have molecular formula of $C_{28}H_{46}N_2O_2$ and was identified as "Caavuranamide".

As per the FTIR spectral information given in Fig: 4 at 3510 cm^{-1} -OH group was detected, at 3410 cm^{-1} -NH group was detected, at 2950 cm^{-1} -CH group was found, at 1680 cm^{-1} C=O stretching was detected, at 1070 cm^{-1} -CO stretching was found, as mass spectral characterisation was carried in the Fig: 5, 391.2892 m/z value was identified. As per the Fig 6 NMR characterisation at 1.09δppm a doubles was identified with -3H, and -CH_3, a singlet for 3H, CH_3 was found at 1.231 δppm region, a single of -3H, and -CH_3 was detected at 1.976 δppm region, a singlet was identified with groups of 2H and -CH_2 at a region of 2.920, a triplet of 2H atoms with -CH_2 at region of 2.421 δppm, at 2.344 δppm region a triplet, 2H, and -CH_2 were detected. The 2.026 δppm region at quartet with 2H, and -CH_2 from the peak region of 1.096-1.697 δppm 13H peaks with -CH and -CH_2 were found. At a 3.412 δppm 1H, -CH of the Methyl amide group was detected. At 3.533 δppm 1H, and -CH were characterized. A singlet was detected at 3.752 δppm region -2H, -CH_2. A singlet of 4.29 δppm region 1H, -OH group, 4.361 one singlet -2H, and NH_3 and 5.466 triplet was detected at -H, HC=CH. White amorphous powder, MP: 218-220 °C. Molecular formula: $C_{23}H_{38}N_2O_3$, Molecular weight: 390.6. Based on the spectral interpretation H.P-02 compound was found to be "Holadysenterine"

H.p-03 was found to be white solid crystals having a molecular formula: $C_{21}H_{27}NO$, with M.P. 198-200^0 C as per the Fig: 7 IR (KBr, cm^{-1}): 2890 (CH); 1700 (C=O), 1680 (C=N); 1585 (C=C) stretchings were detected. In the mass spectra Fig: 8 molecular wt of 311.1 $[M+H]^+$ was found. As per the Fig: 9 NMR characterization indicated the peaks at 7.59 (s, 1H, CH=CH); 6.22 (q, 1H, CH=CH); 6.13 (q, 1H, CH=CH); 5.66 (s,1H, CH=CH); 4.12 (m, 1H, CH); 2.65 (q, 1H, CH); 2.57 (d, 1H, CH); 2.33(q, 1H, CH); 2.313 (q, 2H, CH_2); 2.014 (t, 1H, CH_2); 1.995 (t, 1H, CH_2); 1.879 (t, 1H, CH_2); 1.858 (t, 2H, CH_2); 1.1713 (t, 1H, CH); 1.560 (q, 2H, CH_2); 1.394 (s, 3H, CH_3); 1.185 (d, 3H, CH_3). Based on the spectral data H.P-03 compound was found to be "Mokluangin D"

H.P-04 compound exhibited Pale white amorphous crystals having a Molecular formula: $C_{14}H_{12}O_4$, as per FTIR spectral interpretation fig: 10 the following stretchings 3522.73 (OH), 3005.21(Ar-CH), 1650(C=C), 1037(CO).at a m/z of 245.24 $[M+1]^+$ mass peak was identified as per the fig:11. The following peaks 4.781(s, 4H, OH); 6.114 (s, 1H, Ar-H); 6.375 (d, 2H, CH=CH); 6.716 (s, 1H, Ar-H); 6.711 (d, 1H, Ar-H); 6.838 (s, 1H, Ar-H); 6.854 (d, 1H, Ar-H); 6.961 (s, 1H, Ar-H) were indicated in the Fig:12

at NMR characterization process. Based on the above spectral data H.P-04 was found to be "Piceatannol ''.

H.P-05 exhibited White powder with slight yellow crystals having a molecular formula: $C_{14}H_{12}O_3$, with M.P:261-263^{0}C. As per the FTIR spectra Fig: 13: 3252 (OH), 3017 (=C-H Ar-H); 1611 (Ar C=C str), 1155 (C-O str) were detected. 227.0 $[M-H]^+$ molecular weight peak was detected as per the fig: 14. NMR spectra of the H.P-05 has revealed the peaks at 5.123 (s, 3H, OH); 6.169 (d, 2H, Ar-H); 6.458 (s, 1H, Ar-H); 6.783 (s, 2H, Ar-H); 6.758 (d, 2H, Ar-H); 6.954 (d, 1H, CH=CH); 7.368 (d, 1H, CH=CH) were represented in the Fig:15. Based on the above spectral data Hp-05 compound was found to be "Resveratrol".

Obtained compounds were checked through reverse docking for their binding activity towards to its anti-diabetic action confirmation with the help of the enzymes ``Aldose Reductase, Alpha amylase, Alpha glucosidase and Insulin receptor" using the Schrödinger's LigPrep was used to ensure the desired ligand was in a low-energy state with correct stereochemistry for its structure (Brooks, W. H; 2008). The importance of stereochemistry in virtual screening was emphasised by Brooks et.al. where inclusion of all stereoisomers of all potential lead compounds was crucial to minimise any false negatives or to prevent losing a significant proportion of the potential leads (Du, J; 2011). LigPrep can also convert a 2D drawn structure to its 3D form which would be further processed via a series of steps to prepare the 3D ligand. The preparatory steps involved ensuring the ligands existed in appropriate ionisation states, tautomers, ring conformations, molecular weights and also the number and types of functional groups.

All ligands downloaded from Pubchem were subjected directly to high throughput virtual screening (HTVS) docking initially, followed by standard precision (SP) and then extra precision (XP) docking. These three docking modes were applied to all docking carried out in this project in this order. HTVS docking was the first docking mode used to reduce the number of intermediate conformations throughout the docking funnel. The SP mode was used to screen the ligands with reasonable HTVS Glide scores as chosen by the user. XP mode was designed for use on top-scoring ligand poses to screen false positives and active compounds that could bind to a specific conformation of the receptor. Each docking job of the selected ligands was processed and separated into a number of sub jobs to be run by a limited number of central processing units (CPUs) in the computer.

As per the glide score displayed by the isolated compounds of H.P indicates the Docking of the compounds with enzymes of diabetes and best reverse docking score was exhibited by Mokluangin D at -5.99 to alpha amylase receptor, Piceatannol at -7.00 at alpha glucosidase and -6.85 to Insulin receptor. Resveratrol has bound to alpha reductase at -8.95.

Conclusion

From the phytochemical studies carried on H.P stem bark compounds of Caavuranamide, Piceatannol, and Resveratrol are found to be novel compounds from the H.P bark extract. Obtained phytochemicals with the Insilco studies proved to be exhibiting the anti-diabetic effect by interaction with Aldose Reductase (PBD ID - 6TUF), Alpha Amylase (PBD ID - 6Z8L), Alpha Glucosidase (PBD ID - 7KBR) and Insulin Receptor (PBD ID - 7BW8).

Conflicts of interest:

There is no conflicts of interest regarding the submitted article.

References

- Anup Bhusal, Nirmala Jamarkattel, Aasmin Shrestha, et al. Evaluation of antioxidative and antidiabetic activity of bark of Holarrhena pubescens wall. J Clin Diag Res.2014 sep;8(9):HC05-HC08
- Brooks, W. H.; Daniel, K. G.; Sung, S. S.; Guida, W. C.: Computational validation of the importance of absolute stereochemistry in virtual screening. Journal of chemical information and modeling 2008, 48, 639-45.
- Daniel M. Medicinal plants: chemistry and properties. Science, Enfield, NH 2006.
- Department of AYUSH. Ministry Of Health and Family Welfare. Government of India.
- Dey A, De JN. Ethnobotanical survey of Purulia district, West Bengal, India, for medicinal plants used against gastrointestinal disorders. J Ethnopharmacol.2012;143:68-80.
- Du, J.; Sun, H.; Xi, L.; Li, J.; Yang, Y.; Liu, H.; Yao, X.: Molecular modeling study of checkpoint kinase 1 inhibitors by multiple docking strategies and prime/MM-GBSA calculation. Journal of computational chemistry 2011, 32, 2800-9
- Eldahshan OA, Abdel-Daim MM. Phytochemical study, cytotoxic, analgesic, antipyretic and anti-inflammatory activities of Strychnos nux-vomica. Cytotechnology. 2015;67(5):831-844. doi:10.1007/s10616-014-9723-2
- Glide: v5.8, Schrödinger. LLC, New York, NY, 2012
- India Biodiversity Portal. Holarrhena pubescens Wall. Ex G Don.
- Irwin, J. J.; Shoichet, B. K.: ZINC--a free database of commercially available compounds for virtual screening. Journal of chemical information and modeling 2005, 45, 177-82
- LigPrep: v2.5, Schrödinger. LLC, New York, NY, 2012
- Maestro: v9.3, Schrödinger. LLC, New York, NY, 2012.
- Mallikharjuna P B, Rajanna L N, Seetharam Y N, Sharanabasappa G K. Phytochemical studies of Strychnos potatorum. Lf-A medicinal plant E-J Chem. 2007;4:510–518.
- Panda SK, Patra N, Sahoo G, Bastia AK, Dutta SK. Antidiarrhoeal activities of medicinal plants of similipal biosphere reserve, Odisha, India. Int J Med Arom Plants.2012;2(1):123-134.
- Patil R, Devkar S, Pawar P, Pattewar A. Invitro anthelminthic activity of Holarrhena antidysenterica bark. Int Pharma Res Dev.2012;4(3):147-150.
- Prime: v3.1, Schrödinger. LLC, New York, NY, 2012.
- Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J Microbiol Biol Educ. 2014;15(1):45-46. Published 2014 May 1. doi:10.1128/jmbe.v15i1.656.
- Sarot Cheenparacha, Pannnakorn Boapun, Thunwadee Limtharakul, Stephen G Pyne. Antimalarial and cytotoxic activities of pregnene-type steroidal alkaloids from Holarrhena pubescens roots. Natural Product Research. 2019; 33(6):782-788.
- Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1-10.
- Shahabuddin KU, Sarwar MS, Mohiuddin E. Clinical evaluation of some herbal medicine for amoebiasis. Pak J Pharmacol.2006; 23(2):9-12.
- Tsao R, Deng Z. Separation procedures for naturally occurring antioxidant phytochemicals. J Chromatogr B. 2004;812:85–99.