Antimycobacterial Studies and Insilico Analysis of Phytochemicals Constituent of Croton bonplandianum Seeds

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ABSTRACT:

The aim of present investigation was to analyse the active phytochemical constituents of croton bonplandianum seeds by GC-MS analysis. By using qualitative method, it was checked for the presence of phytochemical substances. Totally 12 bioactive compounds were identified in aqueous methanol extract of croton bonplandianum seeds and solvent was removed to get crude extract. The occurrence of saponins, tannins, phenols and carboxylic acids were identified from the primary phytochemical tests. From the GC-MS studies, a new compound of Germacrene D was identified from the extracts of Croton bonplandianum seeds and secondary metabolites of twelve known compounds β-Caryophyllene, Borneol, Isobornyl acetate, α-Humulene, Chlorochin, Phorbol, Oleanolic acid, Ursolic acid, Carvone, Bisabolol and 9-Eicosene were also been identified. The extract also possessed potential antimycobacterium tuberculosis activity with 1.72 MIC µg/mL evaluated by MTB-MABAassay. The 6PBQ is an attractive protein used as a target for antituberculosis which contributes to its initiatives. In this experiment, the testing activity of natural compound in silico analysis of D-Germacene and 6PBQ. In silico docking between the D-Germacene from the croton bonplandianum seed extract into 6PBQ binding.

Keywords: *Antimycobacterium,Croton bonplandianum seeds, GC-MS studies, Insilco analysis, MTB-MABA assay, Tuberculosis, 6PBQ*

1. Introduction

For the first time in over a decade, the COVID -19 pandemic has changed the global process in embark upon tuberculosis. Deaths due to TB have increased, according to global TB report recently released by WHO 2021. India (41%), Indonesia (14%), the Philippines (12%), China (8%) and other 12 countries accounted for 93% According to the WHO, 4.1 million people are currently infected with tuberculosis but have yet to be diagnosed, up from 2.9 million in 2019. [1]. the treatment of the deadly disease has become a serious financial obstacle for the health care sector. As a consequence, there is immediate need for novel cost effective and eco-friendly alternative drugs with new mechanism to fight against the antimycobacterial species [2]. Recently there has been an increased interest in the plant based natural products to compete against the synthetic drugs with less side effects and innovative screening strategies in drug discovery. Among the plant species, one of the vital species of euphorbiacea family considered to be croton bonplandianum. It is an erect herb with plenty branched, clothed with branched hairs. Leaves crowded on the top, simple, ovate, and serrate. The flowering season

is from November to May. The leaves of the vegetation are simple, darkish green, leaves-alternately organized, 1.96 inches long, lance formed, milky, moderate furry stems & leaves, woody. Flowers are located to be tiny, peach color, 12 racemes, and tiny globe formed flower buds organized in upright spike with green fruits [3]. Plant acquires many biological activities such as anti-inflammatory [4,5], cytotoxic activity [6], amoebicidal activity [7,8,9], antioxidant [10,11], antimicrobial [12,14], wound healing [13], bactericidal [16-22] activity, larvicidal activity [23] and to treat liver disorders and skin diseases [24].

Owing to complications in obtaining the natural products from natural sources or complex synthetic process, the predicted in silico activity/binding will assist drug optimization prior to further studies. Although Insilco and invitro studies are not always interrelated, but aid in validation and optimization of new and prediction of novel anti-TB natural products.

2. METHODS AND MATERIALS

2.1 Plant Material

Croton bonplandianum seeds were collected from the Sri Paramakalyani College premises, Alwarkurichi, Tirunelveli.

2.2 Preparation of aqueous methanolic extract of Croton bonplandianum seeds

Croton bonplandianum seeds (100 g) were boiled in 500 L aqueous methanol till the green color was completely removed (6 h). The methanolic solution was primarily filtered using sterile muslin cloth and again filtered under stringent aseptic conditions, Whatman filter paper no. 1 was used.The filtrate was distilled under vacuum to remove the solvent to get the crude extract (27 g). The crude extract was collected in bottles and kept at 4 degrees Celsius until needed.

2.3 Phytochemical reactions for Croton bonplandianum seeds of methanol extract

The crude extract was found to be a mixture of components by TLC and it was analyzed for primary phytochemical tests in order to find out the nature of compounds present in it. The characteristic colour reactions were carried out in order to find out the nature of compounds present in the crude extract.

2.4. GC-MS Analysis

The crude extracts of C. bonplandianum seed were subjected into GC-MS analysis at IICPT, Thanjavur. For this analysis (GCMS) an Agilent gas chromatograph (GC- 5890B) equipped with Mass Spectrometer (MS-5975) Mass Selective Detector was used. The column was fused silica capillary, HP-5 column (30m x 0.25 mm i.d x 0.25 µm film thickness).The carrier gas was helium with a flow rate of 1.5 mL in with the oven temperature programmed from 50 °C (held for 2 min) to 280 °C (held for 10 min) 14 at a rate of 20 degrees Celsius per minute. The injection and interface temperatures were set at 250 °C and 280 °C, respectively. 1-mL of the sample was injected in split less mode and was analyzed in MS full scan mode (m/z 40-650). The electron ionization was fixed at 70 eV. Acquisition of data was performed using the Chemsation software. Quantitative data were obtained by software integration of total ion chromatogram and mass spectrum. Respective compounds based on their retention times (RT) of Total ion current (TIC) chromatogram and the mass spectra of these compounds were matched with those found in the NIST spectral databases.

2.3 Anti-mycobacterium tuberculosis studies

The anti-mycobacterium activities of the crude extract of Croton bonplandianum seeds were determined by invitro MTB MABA assay [25]. Briefly, the inoculums was prepared from fresh LJ medium re-suspended in7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a Mc Farland tube No. 1, and diluted 1:20; 100 µL was used as inoculum. Each medication stock solution was thawed and diluted four times in 7H9-S to get the highest concentration tested. Using 100 L 7H9-S, serial two-fold dilutions of each medication were made immediately in a sterile 96-well microtiter plate.On each plate, there was also a growth control without antibiotics and a sterile control.To prevent evaporation during the incubation, sterile water was added to all perimeter wells.The plate was covered, sealed in plastic bags, and incubated in a normal environment at 37°C. Following the 7-day incubation period, 30 mL of alamar blue solution was added to each well, and the plate was re-incubated overnight.The MIC was defined as the lowest dose of medicine that prevented a change in colour from blue (oxidised state) to pink (reduced state), which indicated the growth of bacteria

2.5 In Silico Analysis:

Insilco studies were done to predict the potential ligand. Molecular docking procedures are followed to synthesize the most promising compound. The active compounds and its three-dimensional structure of ligand and receptor can be solved. The structure allows the observation of several intermolecular features supporting the process of molecular recognition. Basic structural descriptions of ligand-receptor complexes are vital for the investigation of binding conformations, depicting of key intermolecular interactions, characterization of unknown binding sites, mechanistic studies and the elucidation of ligand-induced conformational changes.

2.5.1 Preparation of the small molecules

The molecule D-Germacene was taken and 3D structures were sketched using biovia discovery visualizer.

2.5.2 Preparation of the protein

The crystal structure of 6PBQ with high resolution was recovered from Protein data bank. (pdb code: 6pbq). The structure was prepared in the procedure by protein preparation, including adding polar hydrogens, assigning partial charges and resulting structure was used as a receptor model [26].

2.5.3 Molecular docking:

For small molecule ligands to macromolecular targets, computational docking can be utilised to predict bound conformations and binding free energies. Docking analysis was done for the one target proteins with one of the new compound D-germacene present in the seed extract of croton bonplandianum using Auto Dock vina tools. An optimal conformation for the protein and the ligand, as well as the relative orientation between the protein and the ligand were discovered as a result of this research, lowering the total system's free energy. Data

from the docking results of all docking processes were tabulated and best confirmations were selected.

3.RESULT AND DISCUSSION

3.1 Primary Phytochemical Analysis

The screening of primary phytochemical analysis of methanol extract of Croton bonpladianum seed revealed the existence of Saponin, Tannins and Aromatic acids. The results of the primary phytochemical analysis are shown in Table 1

Table 1

Primary phytochemical screening of methanol extract of Croton bonplandianumseed

S.No	Phytochemical tested	Test results
1	Steroids	-
2	Triterpenoids	-
3	Reducing sugar	-
4	Alkaloids	−
5	Phenolic compound	−
6	Saponin	+
7	Xanthoproteins	−
8	Tannins	+
9	Flavonoids	−
10	Carboxylic acids	+

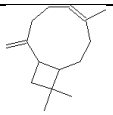
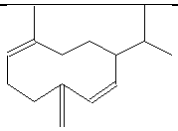
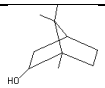
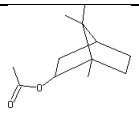
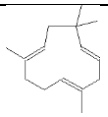
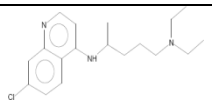
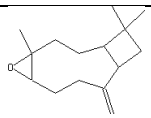
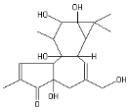
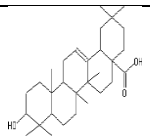
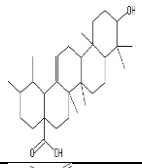
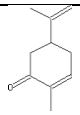
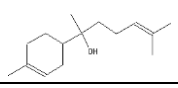
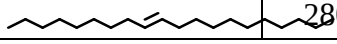
+ = *Positive* means present, − = *Negative* means absent

3.2 GC-MS Analysis

The results of GC-MS analysis showed that the Croton bonplandianum seed extract contained a total of 13 identified compounds based on their retention times (RT) and the mass spectra of these compounds with molecular ion peak (M) m/z and base peak m/z was matched with those found in the NIST spectral databases. A novel compound of Germacrene D was identified from the extracts of Croton bonplandianum seed and secondary metabolites of twelve known compounds β-Caryophyllene, Borneol, Isobornyl acetate, α-Humulene, Chlorochin, Phorbol, Oleanolic acid, Ursolic acid, Carvone, Bisabolol and 9-Eicosene were also been identified. The structure of the identified compounds is determined by the assistance with NIST spectral data bases. The retention time, name and structure of the identified compounds, molecular ion peak (M) m/z and base peak m/z are exhibited in Table- 2.

Table- 2. List of compounds identified in croton bonplandianum seed extract from GC –MS

Table- 2. List of compounds identified in croton bonplandianum seed extract from GC –MS

S.No	RT	Compound Name	Structure	Molecular ion peak (M)m/z	Base peak m/z
1	11.2	β-Caryophyllene		204	93
2	11.9	Germacrene D		204	161
3	12.1	Borneol		154	95
4	12.3	Isobornyl acetate		196	95
5	12.5	α-Humulene		204	93
6	12.9	Chlorochin		319	86
7	14.3	Caryophyllene oxide		220	43
8	15.2	Phorbol		364	83
9	15.9	Oleanolic acid		456	248
10	17.1	Ursolic acid		456	248
11	18.0	Carvone		152	95
12	19.8	Bisabolol		222	43
13	23.1	9-Eicosene		280	57

3.3 Antimycobacterium tuberculosis Activity

The antimycobacterium tuberculosis efficacy of aqueous alcoholic extract of Croton bonpladianumseeds studied against two different standard drugs namely Isoniazid and Ethambutol. The extract possessed prominent antimycobacterial activity
(1.72 MIC (µg/mL) and the results of Antimycobacterium tuberculosis studies are presented in Table 3.

Table: 3

Antimycobacterium tuberculosis studies on methanol extract of Croton bonplandianum seeds

S.No	Name	**MIC (µg/mL)**
1	Crude extract	1.72
2	Isoniazid	0.1
3	Ethambutol	1.56

3.4 Insilico analysis

The Anti- Tuberculosis revealed that the methanol extract of croton bonplandianum seed exhibited a good activity against MTB H37Rv. As the compound D-Germacene was newly discovered and there is no literature information regarding its biological function. The receptor selected for docking studies was 6PBQ. To predict whether the docking parameter are adequate flexible ligand-rigid enzyme simulation was performed with 6PBQ. The compound D-Germacene interaction with 6PBQ (Fig1).For the receptor structure, crystallographic and trajectory water molecules, ions and ligand compounds were removed. There is no literature information regarding their biological function. Prediction of Functional Spectrum for Material (PASS) was used to gain a sense of their potential activity online program. This instrument is invented to offer possible biological activity based on the compound's molecular structure. 2.97708A^0 and 3.34537 A^0. The molecule of D-Germacene is encapsulated in the hydrophobic cavity of formed the Ala84, Val63, Val 87 with a bond length of 5.28543A^0, 5.4443A^0, 5.00569A^0 respectively. It is vital highlight that the examined novel compound D-Germacene establishes conventional hydrogen bonds. These facts described the excellent anti- TB activity of D-Germacene compound extracted as a crucial compound from the seeds of croton bonplandianum

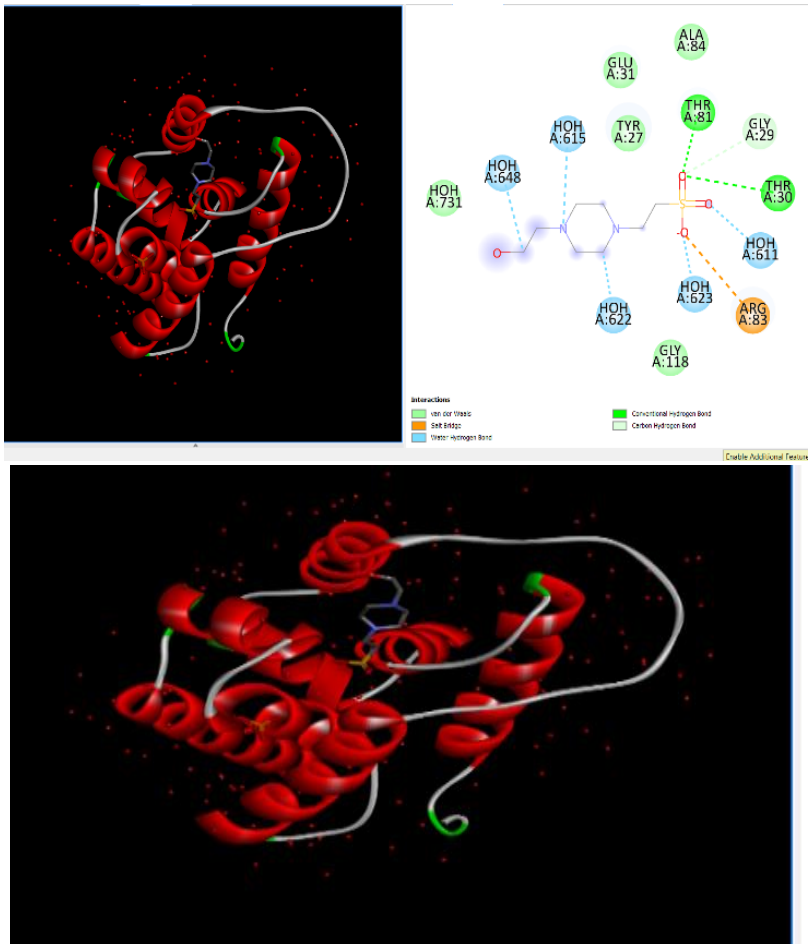


Fig. 1. D-Germacene interaction with 6PBQ

5.CONCLUSION

The results of GC-MS analysis showed that the croton bonplandianum seed extract contained a total of 13 identified compounds. A novel compound of Germacrene D was identified from the extracts of croton bonplandianum seed and secondary metabolites of twelve known compounds β-Caryophyllene, Borneol, Isobornyl acetate, α-Humulene, Chlorochin, Caryophyllene oxide, Phorbol, Oleanolic acid, Ursolic acid, Carvone, Bisabolol and 9-Eicosene were also been identified using GC-MS. The extract possessed potential antimycobacterium tuberculosis activity with 1.72 MIC µg/mL evaluated by MTB-MABAassay. Insilico analysis also favouredthe inhibition activity of the extract.

5.1 Declaration of Competing Interest

The authors declare no competing financial interest.

5.2 Acknowledgments

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