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GC-MS AND *IN SILICO* ANALYSIS OF PHYTOCONSTITUENTS FROM THE ETHANOLIC EXTRACT OF *TERMINALIA CATAPPA* LEAVES AND *TERMINALIA CHEBULA* FRUITS

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ABSTRACT: Objective: To find the phytoconstituents present in the ethanolic extract of *Terminalia catappa* leaves and *Terminalia chebula* fruits by GC-MS method and then the active compound is subjected to the docking analysis. Material and Methods: The ethanolic extract is prepared by using soxhlet apparatus, then the extract is subjected to the GC-MS analysis. Then the *in silico* analysis done by using the complex structures were modeled using modeling software's Pymol. Result: *T. catappa* leaves, which contains seven phytoconstituents and *T. chebula* fruits contains twenty phytoconstituents and the squalene is present in both extracts and its docking results proves their binding activity. Conclusion: The ethanolic extract of both *T. catappa* leaves and *T. chebula* fruits contains large number of important phytoconstituents and their *in silico* reports proves the squalene cardioprotective activity. Hence, further *in vivo* studies are needed to find the cardioprotective activity of this plants.

KEYWORDS: *Terminalia catappa* leaves, *Terminalia chebula* fruits, Squalene, *In silico*.

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1. INTRODUCTION

Cardiovascular disease (CVD) is a common name in which the disease or disorders are related to the heart and blood vessels. It includes atherosclerosis, angina and the other CVD includes the cardiomyopathy, coronary artery disease (CAD), heart valve disorders and infections of the heart.

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The common symptoms of CVD are the chest pain, other symptoms are varied depending upon the specific type of CVD. The incidence of cardiovascular disorders in the recent years has increased steeply. But it is observed incidence of deaths due to coronary disorders has decreased at a reliable level by treatment with medicinal plant. A large number of medicinal plants are scientifically examined and found to be appropriate for the treatment of cardiovascular disorders. The previous study had discovered a few hypolipidemic herbs like *Aegle marmelos, Allium cepa, Nelumbo nucifera, Withania somnifera* etc. [1]. The isolated compounds like curcumin from *Curcuma longa*, S-allylcysteine from garlic, resveratrol from grape seeds, Mohanty *et al.* [2] has also been found to be cardioprotective effects. *Terminalia catappa* (*T. catappa*) belongs to the Combretaceae family. The leaves contain the anti-microbial activity [3, 4], anti-diabetic activity [5], anti-oxidant activity [6], hepatoprotective activity [7], and anti-cancer activity [8], *Terminalia chebula* (*T. chebula*) belongs to the Combretaceae family. The recent studies prove that the fruits which have the molluscicidal activity, anti-viral activity [9], anti-mutangenic activity and anti-cancer activity [10], and hepatoprotective activity [11]. In the present study to investigate the phytoconstituent present in the both the plants of *T. catappa* leaves and *T. chebula* fruits and then find the active compounds and their docking nature by *in silico* analytic method.

2. MATERIALS AND METHODS

GC-MS analysis was carried by comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32 mm, column length is 30m, column thickness 0.50 μm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml / minutes and an injection volume of 0.5 μI was employed (split ratio of 10:1) injector temperature 270ºC; ion-source temperature 200ºC. The oven temperature was programmed from 40ºC (isothermal for 2 minutes), with an increase of 8ºC / minutes, to 150ºC, then 8ºC /minutes to 250ºC, ending with a 20 minutes isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25 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 chromatograms was a Turbo Mass Ver 5.2 [12].

Identification of components

Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

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***In Silico* Analysis**

Complex structures were modeled using modeling software's Pymol (1.1 version, Delano Scientific LLC, San Carlos, CA, USA), Chimera (1.10.1 version UCSF Resources for bio-computing visualization and informatics, NIH, CA, USA) and Pose view installed on a desktop equipped with Pentium (R) Dual-E6600 at 3.05 GHz 3.06 GHz processor (2 GB RAM Core CPU) running the Ubuntu 12.01 (LINUX) and Windows XP SP3 operating system.

Docking Analysis

Analysis of binding affinity and the binding sites were performed in this research. LigPlot 1.4.5 was used to produce two dimensional docking representations of all ligands [13]. There are two types of interaction analyzed, hydrogen bond and hydrophobic interaction. Hydrogen bonds are indicated by green dotted lines whereas the spoke arcs represent protein residues making non-bonded (hydrophobic) contacts with the inhibitor. Interaction occurred between amino acid residue on the target side and functional group on the ligand side. Binding site similarity analysis the test ligands were performed in this research. Blue spheres indicate "good" pair potential per-atom score with the size of the sphere reflecting the strength of the interaction.

RESULTS AND DISCUSSION

Compound identification in *T. catappa* leaves by GC-MS method

Seven compounds were identified by GC-MS analysis of the *T. catappa* leaves is shown in the **Plate 1.** The compounds are bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)], n-hexadecanoic acid, pentalene, octahydro-, cis-, cyclooctene, 3-ethenyl-, 1,E-11,Z-13-octadecatriene, 13-(2-cyclopenten-1-yl) tridecanoic acid, squalene etc. Among these various compounds some are have highly medicinal activities. The compound of n-hexadecanoic acid have an anti-inflammatory activity [14], cyclooctane derivatives are reduces the mutagenicity [15], bicycloheptanes and its derivatives have neuro protective activity [16] and squalene have an anti-oxidant, anti-tumor and lipoxygenase inhibitors activity [17,18].

Compound identification in *T. chebula* fruits by GC-MS method

Plate 2 showed nearly 20 compounds were identified by GC-MS analysis in *T. chebula* fruits. The compounds are 1,2-benzene dicarboxylic acid, 1,3-dipheny 1,3,5,5-tetramethyl cyclotrisibxane, 2-benzofuranone, 2,6,10-trimethyl, 1,4-ethylene-14-pentadecane, hexadecanoic acid, 1,2-benzene dicarboxylic acid, 9,12-octa decadienoic acid, phytol, uridine, 9,12,15-octadecatrienoic acid and squalene. Among these various compounds some are have highly medicinal activities. The compound of 1, 2-benzene dicarboxylic acid diethyl ester which have the anti-microbial [19] and anti-fungal activity of the compound 3, 7, 11, 15-tetra methyl-2-hexadecane have an anti-microbial and anti-inflammatory and cancer preventive activity [20]. The compound of hexadecanoic acid, squalene, and phytol have an anti-oxidant and anti-cancer activity [21]. Phytol and octadecatrienoic

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acid which have an anti-inflammatory and cancer preventive activity [22]. Squalene have an anti-bacterial, anti-oxidant, anti-tumors, anti-cancer, immune stimulant, and chemo preventive activities [21]. In the present study, the *in silico* approach on phytochemicals squalene against cardiac target HMG CoA reductase is carried out using virtual screening, molecular docking and ADMET methods. Virtual screening of squalene compound showed the binding affinity towards target HMG CoA reductase. The compound was screened with binding affinity and compound was selected as hits pass resent study was to investigate the *in silico* cardio protective effects of squalene from the *T. catappa* leaves and *T. chebula* fruits. The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in **Table 1** and **Table 2**. The docking scores were Squalene was found by-8.99176Kcal/mol (**Figure 1, Figure 2** and **Figure 3**). The molecular docking of the hits showed the binding mode and interaction energy. The docking studies confirmed the inhibition of cardiac target protein HMG CoA reductase to show the cardioprotctive activity of squalene.

TIC: 7813-184-0093.D/data.ms

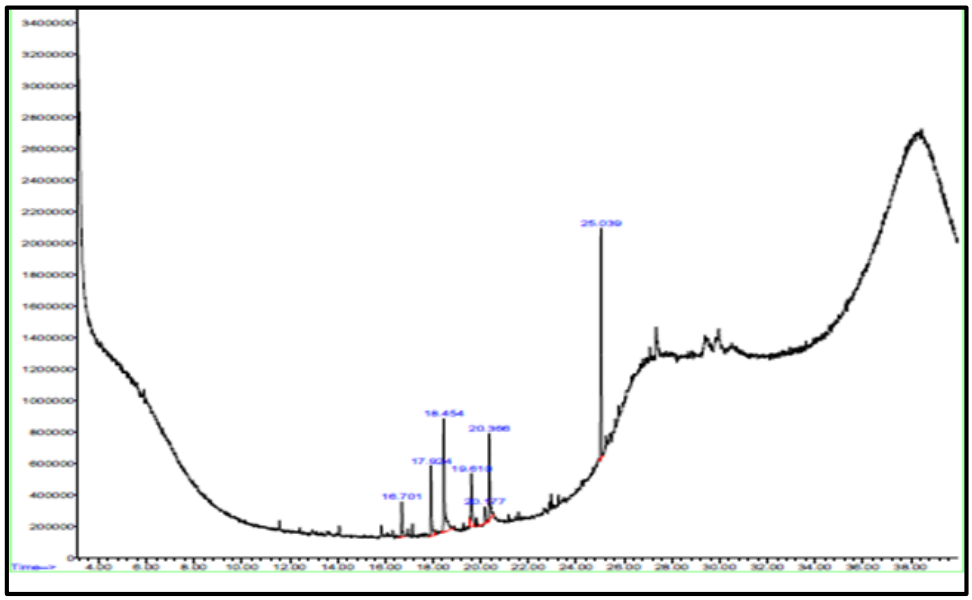

Plate 1: GC-MS for *T. catappa* leaves extract

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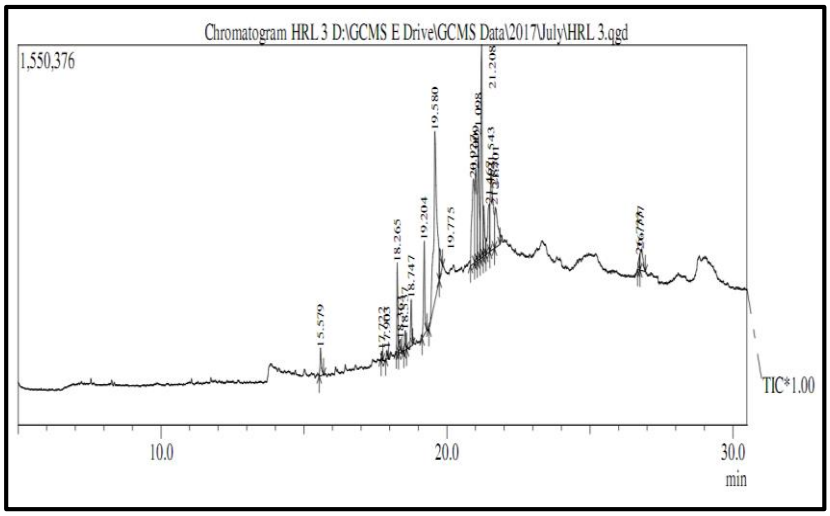
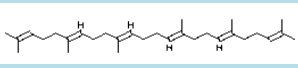


Plate 2: GC-MS for *T. chebula* fruits extract

Table 1: HMG CoA reductase enzyme binding sites

S. No.	Ligands	Amino acids in the binding pocket (Hydrophobic interactions)	Binding site amino acids in the structural unit
1.	Squalene	Asn529, IIe 531, 536, 762, Lys527, Gly532, Met534, Gln814, Tyr533, 517, Val538	Alpha helix

Table 2: Docking results of squalene against HMG CoA reductase enzyme

S. No.	Ligands	Structure	Molecular formula	Molecular weight (g/mol)	Hydrogen donor	Hydrogen acceptor	Docking energy level (*kcal/mol*)
1.	Squalene		$C_{30}H_{50}$	410.73	0	0	-8.99176

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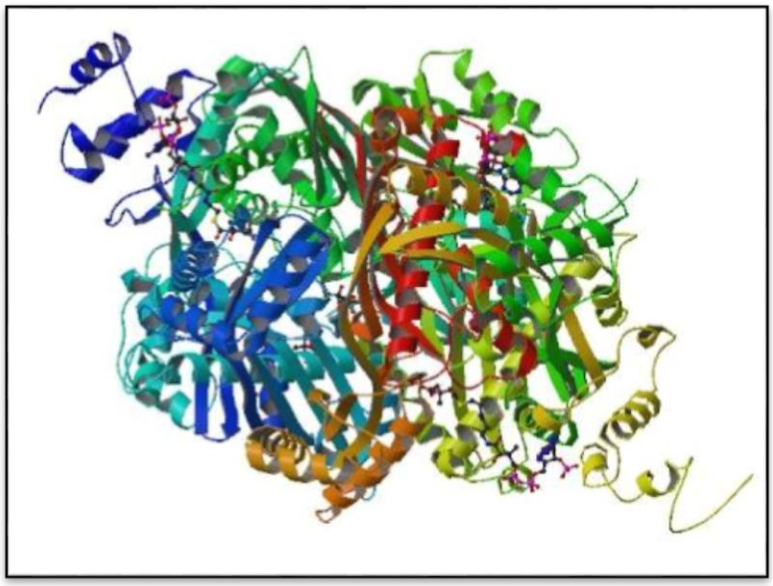

Figure 1: Crystallographic structure of HMG CoA reductase with PDB ID 1DQ9

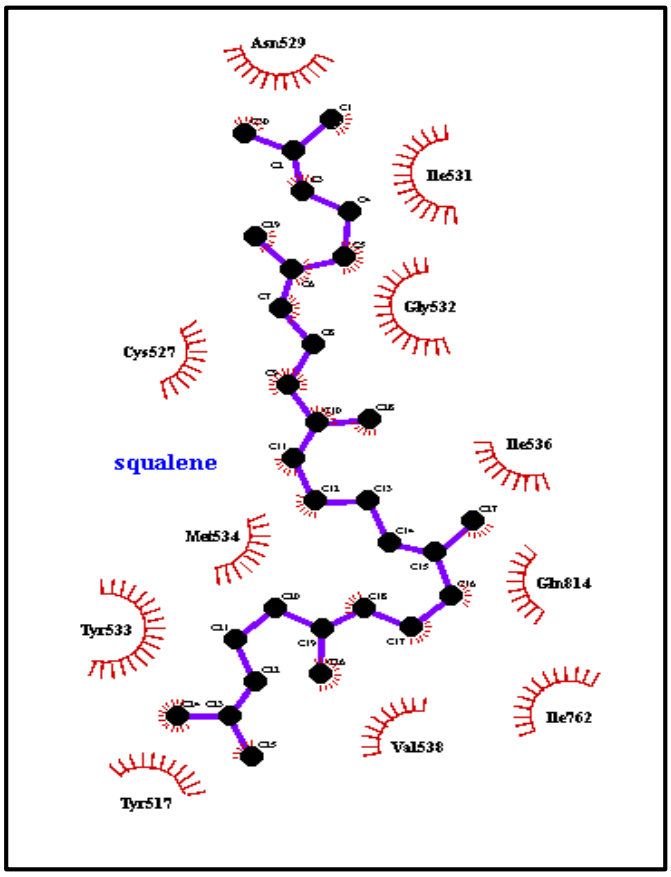

Figure 2: Interaction of Squalene with respective amino acids in HMG CoA reductasess

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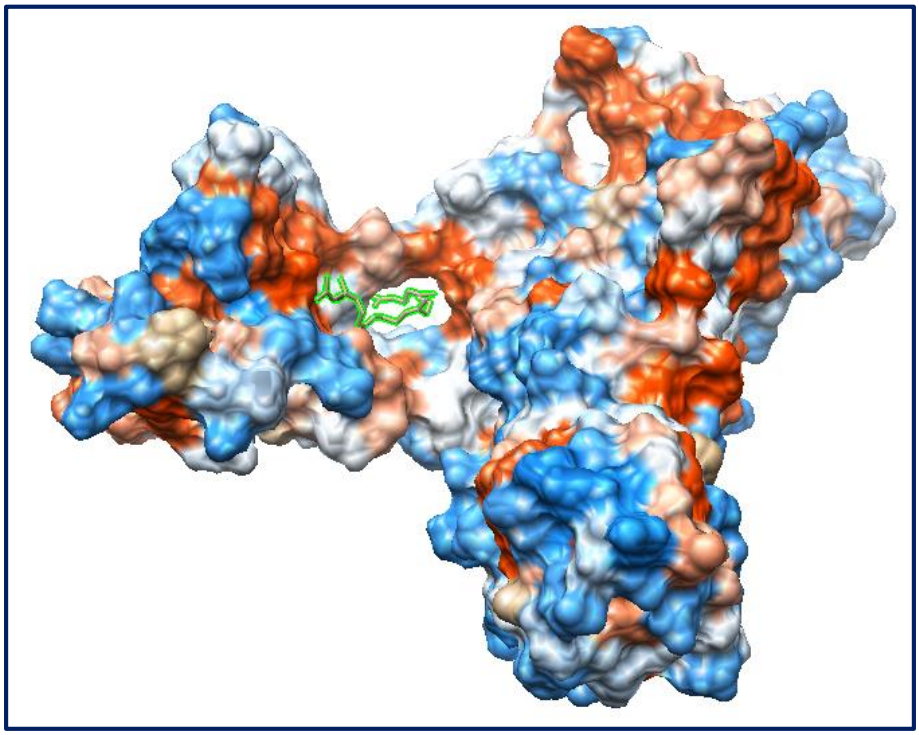

Figure 3: Electrostatic surface of HMG CoA reductase alongside of the amino acids motif with Squalene

4. CONCLUSION

The ethanolic extract of both *T. catappa* leaves and *T. chebula* fruits contains large number of important phytoconstituents and their *in silico* reports proves the squalene have a cardioprotective activity. Hence, further *in vivo* studies are needed to find the cardioprotective activity of this plants.

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CONFLICT OF INTEREST

The authors have no conflict of interest

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