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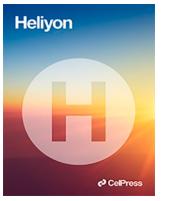

Research article

Phytochemical characterization of *Thevetia peruviana* (lucky nut) bark extracts by GC-MS analysis, along with evaluation of its biological activities, and molecular docking study

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ABSTRACT

Thevetia peruviana (T. peruviana; Family: Apocynaceae), commonly known as Lucky Nut, is a traditionally and medicinally important plant, and the barks of the plant are traditionally used as anti-inflammatory, anti-diabetic, and antibacterial remedies. Thus, this study aimed to evaluate bioactive phytochemicals and *in*-vitro biological activities from the bark of T. peruviana using methanolic (TPM) and dichloromethane (TPD) extracts. The GC-MS analysis showed the presence of 54 and 39 bioactive compounds in TPM and TPD, respectively. The TPM extract has a higher level of total polyphenolic contents (TPC: 70.89 ± 1.08 and 51.07 ± 0.78 mg GAE/g extracts, while TFC: 56.89 ± 1.16 and 44.12 ± 1.76 Qu.E/g extracts for TPM and TPD, respectively). Herein, the results of antioxidant activities were also found in correlation with the total polyphenolic contents i.e., depicting the higher antioxidant potential of TPM compared to TPD. The significant inhibitory activities of extracts were observed against tyrosinase (TPM; 59.43 ± 2.87 %, TPD; 53.43 ± 2.65 %), lipoxygenase (TPM; 77.1 ± 1.2 %, TPD; 59.3 ± 0.1 %), and α-glucosidase (TPM; 71.32 ± 2.44 %, TPD; 67.86 ± 3.011 %). Furthermore, in comparison to co-amoxiclave, the antibacterial property against five bacterial strains was significant assayed. The compounds obtained through GC-MS analysis were subjected to *in*-silico molecular docking studies, and the phyto-constituents with maximum binding scores were then subjected to ADME analysis. The results of *in*-silico studies revealed that the binding affinity of several phyto-constituents was even greater than that of the standard inhibitory ligands. ADME analysis showed bioavailability radars of phyto-constituents having maximum docking scores in molecular docking. The results of this study indicated that T. peruviana has bioactive phytochemicals and therapeutic potential and may provide a basis for treating metabolic disorders (inflammatory diseases like rheumatism and diabetes), bacterial infections, and skin-related problems.

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1. Introduction

The researchers are particularly interested in examining underexplored medicinal plants, which are being utilized as traditional treatments for a variety of diseases due to their abundance of bioactive phytochemicals with antioxidant, antibacterial, and other medicinal properties [1]. Phytochemical and biological studies on horticultural plants have identified potentially bioactive secondary metabolites (phenolics, sterols, and alkaloids) with medicinal and health benefits (anticancer, antioxidant, anti-inflammatory, antidepressant, and hypoglycemic effects) (Liu et al., 2023). Previous research has shown that bioactive chemicals from plants are valuable in the development of therapeutic moieties, and they continue to serve as an important library for discovering new drug leads (Toiu et al., 2018). The World Health Organization (WHO) promotes ethnobotanical and ethnopharmacological considerations as a profitable way to value plants as a source of natural products and molecules for both traditional and modern medicine (Baessa et al., 2019). Furthermore, analyzing natural extracts to identify novel active chemicals is a useful approach for assessing biological activity for therapeutic applications in comparison to traditional uses. This demonstrated a link between medicinal plants' vast biological potential against many diseases and future medication development (Vital and Rivera 2009).

Thevetia peruviana (*T. peruviana*), often known as bush milk, lucky nut, or yellow oleander, is a dicotyledonous evergreen ornamental shrub. It belongs to the Apocyanaceae family (Bora et al., 2014). This plant is native to Central and South America. It is widely farmed in tropical and subtropical climates around the world, including South Asian countries such as Pakistan. For almost 50 years, Nigerian missionaries have planted and researched *T. peruviana* as an appealing plant for homes, schools, and churches. *T. peruviana's* blooms and fruits are available year-round, ensuring a steady supply of seeds. Several phytochemicals have been identified in the plant's seeds, including the vetin, digitoxigenin, perusitin, cerberin, theveridoside, theveside, and peruvoside. Researchers have found quercetin, kaempferol, and quercetin 7-*o*-glactoside (polyphenols) in fresh flowers. The presence of phenol, protein, and flavonol glycosides has also been observed in the leaf extract (Mathuravalli et al., 2012).

The plant has various traditional uses due to its high bioactive ingredient content, including antipyretic, emetic, purgative, antibacterial, anti-inflammatory, and anthelmintic properties for intestinal worms and skin disorders (Zibbu, 2011). *T. peruviana* bark is being used to treat a variety of symptoms, including fever, purgation, sores, amenorrhea, and emesis (Silalahi 2022). *T. peruviana* bark decoctions have traditionally been utilized in India to treat inflammatory illnesses as well as diabetes (Basu and Tripura 2021). Although, the bark of many medicinal plants has been reported to have substantial therapeutic potential due to the high concentration of bioactive chemicals (Jamieson et al., 2022), however, to the best of our knowledge, no research has been undertaken in Pakistan on the phytochemicals and therapeutic benefits of *T. peruviana*'s bark extracts. Based on the facts, the current study was focused on the determination of phytoconstituents/chemical composition and biological importance of *T. peruviana's* TPM and TPD via both experimental and computational investigations. Initially, the plant extracts were prepared and preliminary screening for the presence of chemical constituents was done via basic tests followed by the characterization of constituents using gas chromatography-mass spectrometry (GC-MS) technique. After that, the biological importance of both extracts was determined against the targeted enzymes and different bacterial stains, using well established protocols. Both extracts exhibited significant anti-tyrosinase, anti-lipoxygenase and anti-α-glucosidase and anti-bacterial potential. The results were supported by in-silico molecular docking studies. In order to enhance comprehension of the enzyme binding mode and inhibition mechanisms, a molecular modeling study was conducted followed by molecular dynamics simulations and pharmacokinetics (ADME) analysis, with the aim of facilitating the development of better inhibitors against targeted enzymes.

2. Materials and methods

2.1. Preparation and extraction of T. peruviana bark

The plant was collected from the premises of Bahauddin Zakaria University (BZU), Multan, Pakistan. Dr. Zafarullah Zafar, "Institute of Pure and Applied Biology" of (BZU), Multan, Pakistan, has identified this plant as *T. peruviana*, and voucher number 81009-2022-12 was assigned to the plant.

After identification, the bark was separated from the plant and shade-dried for 45 days at 25 ^{o}C after thorough washing. After grinding and crushing, the dried bark was converted into powder form. The powdered bark weight was 1 kg, which was divided into two parts. The powder was soaked separately in methanol and dichloromethane (DCM) with 1:3 (material: solvent) for 7 days with occasional stirring. Soaked materials were filtered using a muslin cloth, and it was further filtered by running through filter paper (No. 1) using Buckner funnel. This filtration was repeated 3 times. The filtrate was evaporated by a rotary evaporator (Buchi, Flwali, Switzerland, at the speed of 120 cycles per minute) at 45 ^{o}C. 65 g of methanol extract was obtained after complete drying, while 55 g of DCM extract was obtained and both extracts were labeled as TPM and TPD, respectively.

2.2. Analysis of phytochemicals

2.2.1. Preliminary analysis of phytochemicals

The plant extracts were examined for their primary and secondary metabolites by phytochemical assays, described in the literature [2]. In order to confirm the existence of numerous primary and secondary metabolites i.e., alkaloids, glycosides, saponins, phenols, steroids, resins, tannins and flavonoids, the preliminary phytochemical analysis for each class has been performed. The thorough procedure has been incorporated in supplementary data file.

2.2.2. Spectroscopic analysis of total phenolic contents (TPC)

To find out the TPC in the TPM and TPD extracts of *T. peruviana* has been performed using the Folin Ciocalteu method described in the literature [3] but with some minor changes. For this purpose, the dried extract was dissolved in methanol and dichloromethane, yielding a stock solution with a concentration of 1 mg per mL. The gallic acid solution was also used with concentrations of 10, 20, 40, 80, 100, and 200 μg/mL. Gallic acid was used as the standard solution. To prepare the solutions, an Eppendorf tube was filled with 200 μL of Folin Ciocalteu reagent (FCR) and 200 μL of the extracts and standard. Vertexing was used to thoroughly mix the material. Following mixing, 0.8 mL Na_2CO_3 was added to the mixture, which were incubated for 2 hours at room temperature. A BioTek Synergy HT microplate reader (Winooski, VT, USA) was used to calculate the absorbance at 760 nm after 200 μL of the combined solution was transferred to a 96-well microtiter plate. The standard used to calculate the total phenolic contents was gallic acid and the unit "milligrams of gallic acid equivalent per gram of dry extract" abbreviated as mg GAE/g D.E±S.D was used to express TPC values.

2.2.3. Spectroscopic analysis of total flavonoid content (TFC)

Using the technique described in the literature with a minor modification, TFC were calculated [3]. Methanol and dichloromethane were used to make the extract stock solutions of TPM and TPD with concentration 1 mg/mL. The Eppendorf tubes were filled with 1 mL solution of the extract, 4 mL deionized water, 300 μL of $NaNO_3$ and 300 μL of 10% $AlCl_3$ solution. At room temperature, the mixture was incubated for 6 min. 2.4 mL of deionized water was added to the solution that had been incubated. Using Bio Teck Synergy HT microplate reader from Winooski, Vermont, USA, 200 μL of reaction mixture was placed in 96-well microtiter plate to measure the absorbance at the wavelength of 510 nm. For measuring flavonoid content, quercetin was employed as the reference standard. The concentrations of TFC were reported as mg of quercetin equivalents per gram of dry extract (mg Qu.E/g D.E±S.D).

2.2.4. Phytochemical characterization of TPM and TPD by GC-MS analysis

TPM and TPD extracts of *T. peruviana* were analysed by using a Gas chromatography-Mass spectroscopy (GC-MS) Agilent 7890B (santa Clara, CA, USA) and Mass hunter acquisition software. With the proportion of 30 mm × 0.25 mm ID × 0.25 μm film, the instrument's HP-5MS ultra inert capillary with non-polar column were used. Helium served as a carrier gas with a flow rate of 1.0 mL/min. The injector was activated at 250 °C, and the oven temperature was initially set at 50 °C for almost 5 min before being gradually increased to 250 °C at 10 °C per minute and finally to 3000 °C for 10 min at 70 °C per minute. The NIST 14 library data were used for tentative identification of the metabolites [4,5].

2.3. Biological activities of T. peruviana

To determine the biological potential of *T. preuviana*, the antioxidant, enzyme inhibitory and antibacterial activities were performed.

2.3.1. Free radical scavenging antioxidant activity

For the purpose of determining the scavenging capability of the extract 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays were carried out. With a few modifications, the protocols for the ABTS and DPPH assays were used [6].

In a 96-well microtiter plate, 10.0 μL of the extract solution and 90.0 μL of DPPH were combined for the DPPH radical scavenging assay. For 30 min in complete darkness, this combination was incubated at room temperature. By using a Micro Tek Synergy HT (Winooski, VT, USA) microplate reader, absorbance was measured at a wavelength of 517 nm. The results of DPPH assay were expressed as mg of Trolox equivalents per gram of dry extract (TE/g D.E±S.D) was used to express the results.

For the ABTS reducing power assay, a mixture of 2.45 mM potassium persulfate and 7.0 mM ABTS was prepared and was incubated at room temperature in dark. ABTS radical cations were produced. The ABTS stock solution was mixed with a methanol to dilute until its absorption of 734 nm was 0.007 ± 0.02. In a 96-well microtitre plate, 10.0 μl of extract solutions and 20.0 μl of previously diluted ABTS solution were mixed. The plate was allowed to incubate for 30 min at room temperature. BioTek synergy HT microplate reader (Winooski, vt, USA) was used for calculating the absorbance at 734 nm. The results of ABTS assay were reported as mg of Trolox equivalents per gram of dry extract (TE/g D.E±S.D).

2.3.2. Reducing capacities antioxidant behavior

FRAP and CUPRAC assays have been performed to assess the reductive capability of extracts by following the procedure explained by Ref. [7] with minor modification. For FRAP assay, 0.5 mL volume of extract solution that had been dissolved in methanol was vortexed with 2 mL of the FRAP reagent. Then 225 μL water was added and mixture was incubated at 37 °C for 30 min. The absorbance was measured at the wavelength of 593 nm. The results were computed as mg TEs/g D.E±S.D. For the CUPRAC assay, 200 μL of $CuCl_2$ (10 mm), 200 μL of neocuproine (7.5 mm), and 200 μL of ammonium acetate buffer (1 M, pH 7.0) were added to form a reaction mixture. The reaction mixture was incubated for 30 min at room temperature, and the absorbance was measured at 450 n. The results were computed as mg TEs/g D.E±S.D.

2.3.3. Enzyme inhibition potential of T. peruviana

2.3.3.1. Tyrosinase inhibitory activity. The tyrosine inhibitory activity of the TPM and TPD has been carried out by the procedure

adopted by Ahmed et al. [3] with minor modifications. The 10 μL of assay compound (5 mg/mL in DMSO), 150 μL phosphate buffer (50 mM of pH 6.8) and 20 μL of tyrosinase enzyme (0.34 mg in 2 mL of phosphate buffer containing 5771 units/mg) were mixed in each well and allowed to incubate at 30 °C for 15 min. After incubation period pre-read was taken and 1 mM of substrate was added in each well and allowed to re-incubate at 30 °C for 30 min at same conditions. After re-incubation at the wavelength of 480 nm after read was taken. For positive control and for negative control, kojic acid and methanol was used respectively. Equation (1) was used to determine the % inhibition of tyrosinase.

$$(\%)\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Aborbance of assay sample}}{\text{Aborbance of control}} \text{ x } 100 \quad (1)$$

2.3.3.2. α-glucosidase inhibitory activity. The α-glucosidase inhibition assay was carried out as per protocols described by Ref. [6] with minor modifications. A 10 μL mixture of enzyme soln. (1 U/mL), 50 μL of50 mM phosphate buffer having pH 6.8 and 20 μL aliquots of the TPM and TPD were incubated in 96 microtitre plate at 37 °C for 15 min. Then at 405 nm, pre-read absorbance was measured. 20 μL solution of p- α-d-glucopyranoside (0.5 mM) was added to the above mixture as a substrate. The reaction mixture was further incubated at 37 °C for 30 min. Then at 405 nm, post-read absorbance was measured after reading. The same procedure was repeated for positive control (Acarbose) and negative control (methanol). Equation (2) was used to calculate the % inhibition of α-glucosidase;

$$(\%)\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of assay sample}}{\text{Absorbance of control}} \text{ x } 100 \quad (2)$$

2.3.3.3. Lipoxygenase (LOX) inhibition assay. The *In vitro* anti-inflammatory activity of the studied plant extracts was determined using the lipoxygenase Inhibitor Screening Assay as described by Olech with minor changes. 10 μL of the sample was mixed with 90 μL of a lipoxygenase solution. After 5 min of incubation at room temperature, a solution of arachidonic acid was added and the plate was shaken for 10 min. 100 μL of chromogen was added, and the mixture was shaken for another 5 min. Absorbance was measured at 500 nm. Assay buffer instead of extract was used in the negative control. NDGA (nordihydroguaiaretic acid) was used as a positive control [8]. Equation (3) was used to determine the % inhibition of lipoxygenase;

$$(\%)\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of assay sample}}{\text{Absorbance of control}} \text{ x } 100 \quad (3)$$

2.3.4. Antibacterial activity of T. peruviana

Three gram-positive and two gram-negative strains were employed to assay the antibacterial properties of the TPM and TPD extracts. Disc diffusion method was used to find out the inhibition zones in mm. . In this assay, the extracts sample and standard sample (antibiotic) were diffused from a disc (loaded with extracts sample or antibiotic sample) over the medium of nutrient agar, making a concentration gradient. Discs (5 mm in diameter) were made up of filter paper and utilized to load the sample. A known concentration of the sample (10, 20, and 40 mg per mL) was dissolved in DMSO and infused on every disc. The discs were put on nutrient agar media that had already received an inoculation of assay bacterium strains. Co-amoxiclav was utilized as a standard antibacterial drug in the studies (1 mg/mL), and a disc devoid of sample extracts/standard was considered a negative control after being loaded with the solvent only (DMSO). These Petri dishes were placed for 12 h (4 ^{o}C) to ensure the diffusion of extracts/drugs from discs into the surrounding agar medium. The dishes were then placed in the incubator for 24 h at 37 °C to provide the best condition for the growth of bacteria. The "zone of inhibition" is a clear or distinct zone that exhibits no bacterial growth, and the diameter of this zone was measured in millimeters to determine the antibacterial potential of the samples (TPM and TPD) as compared to the standard.

2.4. Molecular docking

In the drug design and development, molecular docking is a helpful technique. The structure of the targeted proteins were downloaded from Protein Data Bank (PDB) website (https://www.rcsb.org/) in PDB format. The (PDB) ID of lipoxygenase, α-glucosidase and tyrosinase were 1yge (resolution: 1.40 Å), 5zcb (resolution: 2.50 Å) and 2y9x (resolution: 2.78 Å) respectively. The protein preparation was done using Discovery Studio 2021 Client where the removal of heteroatoms and water molecules was done followed by the addition of polar hydrogen, and the resulting structures were saved as a (PDB) file[37,132]. Secondary metabolites chosen from GC-MS analytical technique as shown in (Table 4) and standard compounds were downloaded from the PubChem database in SDF (structure-data file) format which were further saved to PDB format using Open Babel software (O'Boyle, 2011). PyRx program, in conjunction with Autodock vina, was utilized in order to accomplish the task of docking the ligands to the active site of the targeted enzymes [9]. The exhaustiveness parameter that controls the extent of the search was chosen as 8, and 9 modes were generated for each ligand. The best ligand pose selection for the receptor was done based on the docking score. The Discovery Studio Visualizer was utilized in order to study the interactions that occurred between the ligand-receptor complexes (Biovia. 2017).

2.5. Molecular dynamics simulation studies

The Molecular dynamic simulation is a computational tool used to identify the binding interactions and stability of the protein-ligand complex under accelerated conditions. In this study MD calculations were performed for the best docked pose of α-amyrone against α-glucosidase, lipoxygenase and tyrosinase complex using Nano scale molecular dynamic (NAMD) software on a CUDA-

Table 1
Phytochemical analysis of *T. peruviana* extract.

Sr. no.	Metabolite	Test	TPM	TPD
Primary metabolites				
	Carbohydrate	Molish Test	+	+
		Fehling test	+	+
	Amino acid	Ninhydrin test	–	–
	Protien	Biuret test	–	–
	Lipids	Sponification	+	+
Secondary metabolites				
	Alkaloids	Mayer's Test	+	+
		Wagner Test	+	+
	Phenols	Ferric Chloride test	+	+
	Tannins	Lead Acetate Test	+	+
	Resin	Acetic Anhydride Test	+	+
	Flavonoids	Alkaline Reagent Test	+	+
	Saponins	Froth Test	+	+
	Steroids	Salkowaski's Test	+	+
	Caradiac Glycoside	Keller-Killiani Test	+	+
	Glycoside	Erdmann's Test	+	+

TPM: *T. peruviana* methanolic extract, TPD: *T. peruviana* dicholoromethane extract, +: present, and -: absent.

accelerated GPU machine with a 16 core CPU and 64 GB RAM memory (Abida Ejaz, 2023). The TIP3P water model was used to conduct the simulations where CHARMM36 forcefield was used to simulate the system. The neutralization of the system was done at 0.15 M sodium chloride concentration. The system was equilibrated under isothermal and isobaric (NPT) conditions at a temperature of 300 K and a pressure of 1.01 bar. The system was equilibrated in an NVT ensemble for 500000 steps, followed by another 500000 steps in an NPT ensemble. After that, the simulation was run for 50 ns under periodic boundary conditions. Various analytical metrics, including root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF), were employed to assess the stability of the protein-ligand complex. The electrostatic interactions observed during MD simulations were taken into account using Particle Mesh Ewald summation method. All output files were visualized using VMD 1.9.3 (Humphrey. 1996).

2.6. ADME analysis

ADME analysis of the selected phytocompounds with maximum binding affinity were executed by using SWISS ADME online software (https://www.swissadme.ch/) tool [5].

2.7. Toxicity evaluation

The toxicity of the phytoconstituents were virtually predicted using onlinee tool ADMETlab 2.0. ADMETlab 2.0 is an enhanced version of the widely used ADMETlab for systematic evaluation of physicochemical properties, ADMET properties and medicinal chemistry friendliness. ADMETlab 2.0 has a greater capacity to assist medicinal chemists in accelerating the drug research and development process. The SMILES string of each compound was pasted separately and submitted. Skin Sensization, arcinogencity, Eye Irritation and Respiratory Toxicity scores were predicted using this tool (James, 2023).

2.8. Statistical evaluation

For each experiment or activity, biological duplicate extracts were investigated. Results were represented after subtracting the values for the negative control/blank solution (without extract), and these outcomes were disclosed as the standard deviation (SD) of the mean. One-way analysis of variance was performed on the data obtained from the quantitative analysis.

3. Results and discussion

3.1. Analysis of phytochemicals

3.1.1. Preliminary phytochemical analysis

TPM and TPD extracts of *T. peruviana* were investigated for preliminary phytochemical examination. Numerous primary and secondary metabolites were found; according to this study, proteins and amino acids were not found in any of the extracts. However, a trace amount of carbohydrate content was identified. Lipid levels were shown to be found in the plant. Alkaloids were discovered to be among the secondary metabolites in TPM and TPD; both extracts contain phenols and tannins. There was also evidence of flavonoids and saponins in TPM and TPD. The acetic anhydride assay for both extracts revealed the presence of steroids, cardiac glycosides, and glycosides, as well as resins (Table 1). The findings of current study were aligned with a similar study conducted by Meena et al. In their research, they reported the presence of various phytochemicals belonging to different chemical classes of bioactive compounds [10].

Table 2
Total Polyphenol contents and antioxidant potential of *T. peruviana*.

Sample	TPC (mg GA E/g D.E ±S.D)	TFC (mg Qu. E/g D.E ±S.D)	DPPH (mg TEs/g D.E ±S.D)	ABTS (mg TEs/g D.E ±S.D)	FRAP (mg TEs/g D.E ±S.D)	CUPRAC (mg TEs/g D.E ±S.D)
TPM	70.89 ± 1.08	56.89 ± 1.16	99 ± 0.51	86 ± 1.01	189 ± 1.50	195 ± 1.58
TPD	51.07 ± 0.78	44.12 ± 1.76	60 ± 0.69	52 ± 0.47	139 ± 1.18	168 ± 1.61

Total phenolic content (TPC) and Total flavonoid content (TFC) of TPM (methanolic extract) and TPD (dichloromethane extract) from *T. peruviana* bark. DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) FRAP (ferric ion reducing antioxidant power), CUPRAC (cupric ion reducing antioxidant capacity) are presenting the antioxidant activities of *T. peruviana*, while all the results are the means of triplicates along with their standard deviations.

3.1.2. Spectroscopic analysis of total phenol & flavonoid contents

TPC of TPM extract was calculated as recorded to be 70.89 ± 1.08 mg of gallic acid equivalent per gram of dry extract (mg GAE/g extract). In contrast, TPD extract has total phenolic content of 51.07 ± 0.78 mg of gallic acid equivalent per gram of dry extract (mg GA E/g extract), as shown in Table 2. Our study's findings were in agreement with the reports of Mendoza et al., from *T. peruviana*. They reported the significant values of TPC and TFC from the 50 % alcoholic and ethyl acetate extracts of callus cell suspension of this plant [11]. Total flavonoid content of TPM was (56.89 ± 1.16 mg Qu.E/g extract), while TPD had (44.12 ± 2.76 mg Qu.E/g extract), as shown in Table 2.

Among the most significant types of secondary metabolites in plants are phenolic compounds. These substances have connections to the systems of stress and environmental adaptation. However, the quality and quantity of TPC and other secondary metabolites biosynthesized in natural products vary massively. Moreover, *T. peruviana* has been subjected to prior investigation, which revealed that many phenols and flavonoids were found (Charnley and volume). There hasn't been a thorough scientific study on the total phenolic and flavonoid contents of *T. peruviana* bark yet. More polar solvents in the present study have a better ability to give polyphenols than the solvents with less or no polarity.

3.1.3. Phytochemical characterization by GC-MS analysis

TPM and TPD extracts of *T. peruviana* were possibly containing non-polar to mid-polar substances. For identification of non-polar molecules, it is recommended to employ GC-MS analysis. Numerous phytocompounds were tentatively identified using the NIST library. Fig. S1 and Fig. S2 show chromatograms of TPM and TPD, respectively, and phytocompounds were chosen with a similarity index of greater than 90 % to the reference compounds in the library. In Table 3 & Table 4, the secondary metabolites identified by GC-MS are listed together with their retention time, area percentage, compound name, chemical class, molecular formula and weight, pharmacological activity, and references from the literature. The major chemical class from TPM was terpenoids followed by steroids, fatty acids, and the other bioactive compounds (fatty acid esters, phenolic compounds, and alkenes, etc.). Moreover, tocopherols were also present in nearly, a prestigious class of phytochemicals with diverse biological activities. The findings of our study were also similar to a previous research in which the methanolic and aqueous extracts from *T. peruviana* seeds showed the presence of fatty acids such as n-hexadecanoic acid and other compounds [12].

The TPD extract also showed the presence of compounds of various chemical classes, mainly terpenoids followed by fatty acids, phytosteroids, and alkanes, while the minor classes included phenols aldehyde, tocopherol, and esters. The results of our study were also similar to a previous study conducted on dichloromethane extract from *T. peruviana* seeds, revealing the presence of terpenoids and fatty acids as main classes of phytochemicals [100]. Another evidence also substantiated our results, reporting the presence of various fatty acids, hydrocarbons, and sterols in the essential oils from leaves, flowers, and fruits of *T. peruviana* [101]

Phytochemical profiling by GC-MS also revealed many bioactive secondary metabolites, which have several biological activities, reported in the literature, including antioxidant, anti-inflammatory, anti-diabetic and antibacterial properties (Table 3, Table 4, S3 and S4). So, the phytochemical analysis of *T. peruviana* shows the presence of potentially bioactive phytochemicals, making it a good candidate for therapeutics and encouraging the isolation of novel molecules for drug discovery. And may serve as promising therapeutic agents for treating metabolic disorders such as diabetes, inflammatory diseases, skin related problems or infections and various bacterial infections.

3.2. Biological activities of T. peruviana

T. peruviana's biological activity was assessed using the antioxidant assay and by looking at the plant's potential for inhibiting enzymes and having antibacterial properties.

3.2.1. Antioxidant potential (free radical scavenging and reducing capacities)

The antioxidant potential was assessed by using the DPPH, FRAP, ABTS, and CUPRAC methods. Table 2 presents the findings of antioxidant studies, and Tables 3 and 4 shows their correlation with bioactive contents. When examined by the DPPH assay, the maximum value was obtained in the case of TPM as (99 ± 0.51 mg TE/g D.E±S.D) followed by TPD (60 ± 0.69 mg TE/g D.E±S.D). The values of both extracts were comparable and indicated that the plant has good antioxidant potential. The radical scavenging activities of our study were also in agreement with a shred of previous evidence reporting the significant DPPH scavenging results of *T. peruviana* in Egypt (Al-Rajhi, Yahya et al., 2022). Bioactive contents analysed in the study (TPC and TFC) may also contribute to TPM's higher

Table 3
Tentatively identified phytoconstituents by GC-MS analysis of TPM extract.

Sr. no.	RT	Area %	Tentative compound	Mol. Formula	Mol. Weight	Biological Activity	Nature of compounds
1	4.68	0.06	Vanillin	$C_8H_8O_3$	152.15	Anti-diabetic antioxidant, antimicrobial [13] anti-inflammatory [14,15]	Phenolic
2	5.25	0.05	Phenol, 2-methoxy-4-(1-propenyl)-, acetate, (Z)-	$C_{12}H_{14}O_3$	206.24	Antioxidant, antibacterial [16] Anti-bacterial (Paudel, Mukti Ram Chand, Mukesh Babu Pant, Basant Pant, Bijaya %J Biomolecules 2019)	Phenolic
3	8.10	0.05	4-Propenyl-2,6-dimethoxyphenol	$C_{11}H_{14}O_3$	194.23	Antimicrobial [17]	Phenolic
4	8.41	0.11	Methyl 12-methyltridecanoate	$C_{15}H_{30}O_2$	242.40	Antimicrobial [18]	Fatty acid esters
5	9.09	0.43	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Antioxidant, antimicrobial [19]	Fatty acids
6	10.02	0.10	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	Antifungal, insecticidal [20]	Fatty acids
7	10.60	0.77	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	Antimicrobial	Fatty acid esters
8	11.74	10.38	n-Hexadecanoic acid	$C_{16}H_{30}O_2$	254.41	Cytotoxic [21]	Fatty acids
9	12.32	0.05	*cis*-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	268.4	Antimicrobial (Di Pasqua, Betts et al., 2007)	Fatty acids
10	12.65	0.31	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.5	Antioxidant, cytotoxic [22]	Fatty acids
11	12.83	0.20	Methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$	294.5	Antimicrobial [23]	Fatty acid esters
12	12.99	0.45	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.5	Antimicrobial, antioxidant, anticancer [24]	Fatty acid esters
13	13.15	0.26	Phytol	$C_{20}H_{40}O$	296.5	Antidiabetic, antimycobacterial, antioxidant, antimicrobial, cytotoxic, antitumorous, antimutagenic, anti-teratogenic, antibiotic-chemotherapeutic, lipid lowering, antispasmodic, anticonvulsant, antinociceptive, anti-inflammatory, anxiolytic, antidepressant, immunoadjuvancy, hair growth facilitator, hair fall defense and antidandruff activities. [25] [26]	Terpenoids
14	13.46	0.21	Methyl stearate	$C_{19}H_{38}O_2$	298.5	Antimicrobial [27]	Fatty acid esters
15	14.57	0.85	9,17-Octadecadienal	$C_{18}H_{32}O$	264.4	Antimicrobial, antioxidant, anticancer [28]	Aldehyde
16	14.72	0.78	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	Antioxidant, antimicrobial [29]	Fatty acids
17	15.15	2.06	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	Antioxidant [30]	Fatty acids
18	15.28	0.10	Z,Z-10,12-Hexadecadien-1-ol acetat	$C_{18}H_{32}O_2$	280.4	Cytotoxic [31]	Ester
19	15.69	0.05	Oleic Acid	$C_{18}H_{34}O_2$	282.5	Antitumor, Anti-diabetic [32,33],	Fatty acids
20	16.02	0.09	10E,12Z-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	Antibacterial [34]	Fatty acids
21	17.24	0.26	Tricosane	$C_{23}H_{48}$	324.6	Antioxidant (Jing, Huang et al., 2019)	Alkanes
22	17.69	0.39	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.6	Antimicrobial [35]	Fatty acid esters
23	17.94	0.41	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324.5	Antimicrobial, antiviral, antifungal [36]	Fatty acid lactones
24	18.47	0.65	Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	Antimicrobial [37]	Fatty acids
25	18.79	0.13	Tetracosane	$C_{24}H_{50}$	338.7	Cytotoxic [38]	Alkanes
26	19.14	0.07	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340.6	Antioxidant [39]	Fatty acid esters
27	19.69	0.16	Heneicosanoic acid	$C_{21}H_{42}O_2$	326.6	Antioxidant [40]	Fatty acids
28	20.09	0.69	Heneicosane	$C_{21}H_{44}$	296.6	Antioxidant [41]	Alkanes
29	20.15	0.28	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.5	Antioxidant [42]	Fatty acid esters
30	20.36	0.04	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.6	Antimicrobial [43]	Fatty acid esters

(*continued on next page*)

Table 3 (*continued*)

Sr. no.	RT	Area %	Tentative compound	Mol. Formula	Mol. Weight	Biological Activity	Nature of compounds
31	20.82	0.36	Docosanoic acid	$C_{22}H_{43}O_2$	340.6	Antimicrobial [44]	Fatty acids
32	21.38	0.09	Tricosanoic acid, methyl ester	$C_{25}H_{50}O_3$	398.7		Fatty acid esters
33	21.64	0.08	1-Hexacosene	$C_{26}H_{52}$	364.7	Antibacterial, antioxidant [45]	Alkenes
34	21.81	0.05	Tricosanoic acid	$C_{23}H_{46}O_2$	354.6	Anticancer, antimicrobial, antioxidant [28]	Fatty acids
35	22.10	0.93	Heneicosane	$C_{21}H_{44}$	296.6	Antifungal, antimicrobial [46]	Alkanes
36	22.33	0.14	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.66	Antibacterial [47]	Fatty acid esters
37	23.20	0.44	α-Tocospiro A	$C_{29}H_{50}O_4$	462.7	Antioxidant [48]	Terpenoids
38	23.37	0.35	α-Tocospiro B	$C_{29}H_{50}O_4$	462.7	Antioxidant [48]	Terpenoids
39	23.86	3.55	Tricosane	$C_{23}H_{48}$	324.6	Antioxidant [49]	Alkanes
40	24.00	0.19	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	410.7	Antibacterial [50]	Fatty acid esters
41	24.53	0.68	Hexacosane	$C_{26}H_{54}$	366.7	Antioxidant, antimicrobial [51]	Alkanes
42	24.80	0.22	β-Tocopherol	$C_{28}H_{48}O_2$	416.7	Antioxidant [52]	Vitamin E
43	26.04	0.51	Ergost-5-en-3-ol, (3beta,24R)-	$C_{28}H_{48}O$	400.7	Anticancer, antioxidant [53]	Steroids
44	26.15	1.21	Campesterol	$C_{28}H_{48}O$	400.7	Antioxidant Anti-diabetic, Anticancer [54] [55,56,57].	Steroids
45	26.31	0.43	Stigmasterol	$C_{29}H_{48}O$	412.7	Antimicrobial, Anti-diabetic [58]; Abbasi, Khan et al., 2021)	Steroids
46	26.44	0.25	Ursa-9(11),12-dien-3-ol	$C_{30}H_{48}O$	424.7	Antioxidant [59] antimicrobial [60]. Anti-diabetic [61]	Terpenoids
47	26.94	7.52	γ-Sitosterol	$C_{29}H_{50}O$	414.7	Antibacterial, cytotoxic, Anti-diabetic [62,63]	Steroids
48	27.38	1.29	β-Amyrin	$C_{30}H_{50}O$	426.7	Antimicrobial [64]	Terpenoids
49	27.48	10.49	α-Amyrone	$C_{30}H_{48}O$	424.7	Antimicrobial [65]	Terpenoids
50	27.93	10.49	Lupeol	$C_{30}H_{50}O$	426.7	Anti-inflammatory, anti-arthritic, anti-diabetic [54,66]	Terpenoids
51	28.38	10.52	Olean-12-en-3-ol, acetate, (3.beta)	$C_{32}H_{52}O_2$	468.8	Antidiabetic [61]	Terpenoids
52	29.18	28.22	Urs-12-en-24-oic acid, 3-oxo-, methyl ester	$C_{31}H_{48}O_3$	468.7	Antimicrobial [67]	Terpenoids
53	29.83	0.90	Epilupeol (20[29]-Lupen-3αol) acetate	$C_{32}H_{52}O_2$	468.8	–	Terpenoids
54	29.94	0.05	Lup-20(29)-en-3-ol, acetate)	$C_{32}H_{52}O_2$	468.8	Antimicrobial [68]	Terpenoids

RT:retention time.

activities. Moreover, many compounds were identified by GC-MS from both extracts (TPM and TPD) with established antioxidant properties (Table 2).

ABTS had a scavenging potential for TPM of (86 ± 1.01 mg TE/g D.E±S.D), and the TPD value was measured as (52 ± 0.47 mg TE/g D.E±S.D). It further depicted the radical scavenging potential of the studied extracts. A piece of evidence also supports the antioxidant effects of the studied plant in which the significant inhibition values of ABTS were found (mg of Trolox equivalents). They also reported that the significant radical scavenging may be because of phenolic and flavonoid contents [102].

When evaluated for FRAP, the extract's reduction potential showed the maximum reduction potential of TPM to be (189 ± 1.50 mg TE/g D.E±S.D); for TPD, its value was (139 ± 1.18 mg TE/g D.E±S.D). A report by Arias et al. also reported the significant results of reducing capacity from the ethanol extract of *T. peruviana* and found them in correlation with TPC and TFC [102]. Our results of GC-MS analysis (Tables 3 and 4) also showed the presence of various bioactive phytocompounds with antioxidant effects, which may also have the reducing power of the extracts. However, a significantly high amount of TPC and TFC in the TPM compared to TPD may contribute to TPM's significantly higher antioxidant activities.

The maximum reducing potential of TPM was found by CUPRAC assay as (195 ± 1.58 mg TE/g D.E±S.D), and the reducing potential for TPD extract was (168 ± 1.61 mg TE/g D.E±S.D). Again, the higher reducing activities of TPM may be related to its higher values of polyphenolic contents. Moreover, a previous study on the hexane extract of leaves and fruit rind showed antioxidant potential when estimated through the antioxidant enzymes (*In vivo* rat model) [103]. It further attributes the role of non-polar constituents in both the studied extracts. According to our knowledge, we, for the first time, investigated the antioxidant potential of *T. peruviana* bark in this study.

Reactive oxygen species (ROS), the most significant of the detrimental reactive species (RS), are combated by a complex and multilayer network of antioxidant systems (AOS) in plants. The metabolic processes often result in the production of ROS. Inflammation and tissue damage are due to the accumulation of ROS, which causes negative effects on proteins, DNA, and fatty acids (Nagavarma, Yadav et al.). Therefore, antioxidants should be used to detoxify these ROS to strengthen the immune system. Antioxidants made from synthetic sources are less desirable than those made from natural sources due to their unfavorable side effects. Many plants, which are employed for a variety of biological functions, contain natural-origin antioxidants in large quantities (Béduneau, Saulnier et al.). Plants contain physiologically active substances called polyphenols. Antibacterial, antioxidant, antiviral, anti-diabetic, and anticancer properties are only a few of the outcomes that have been linked to polyphenol intake [23]. Previous research on

Table 4
Tentatively identified phytoconstituents by GC-MS analysis of (TPD) extract.

Sr. no.	RT	Area %	Tentative compound	Mol. formula	Mol. weight	Biological Activities	Nature of compounds
1	3.31	0.21	Thymol	C10H14O	150.22	Antimicrobial, Antineoplastic, Anti-inflammatory, Antirheumatic, Anti-neurodegenerative [7,69]	Phenolic
2	5.54	0.16	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202.33	Antimicrobial and anti-inflammatory (Ali, Mohammed et al., 2016)	Terpenoids
3	3.80	1.12	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	Anti-diabetic, Antioxidant, Anti-inflammatory Antimicrobial [1,69]	Phenolic
4	6.15	0.11	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180.24	Anti-diabetic, Anticancer Antitubercular Anti-inflammatory [70]	Benzofurans
5	6.53	0.67	Phenol, 4-ethenyl-2,6-dimethoxy-	$C_{10}H_{12}O_3$	180.20	Antioxidant, COX-2 inhibitor, inhibit tumor cell growth while inducing cell apoptosis [71]	Phenolic
6	8.18	0.72	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	$C_{13}H_{22}O_2$	210.30	Antioxidant [72]	Alkenes
7	8.39	0.59	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.40	Antimicrobial [73]	Fatty acid esters
8	8.58	0.13	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.20	Antibacterial [74]	Phenolic
9	8.93	1.41	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Anti-diabetic, Antibacterial Antifungal [75, 76,76,77]	Fatty acids
10 Antibacterial	9.64 Terpenoids	1.20	Neophytadiene	$C_{20}H_{38}$	278.5	Anti-cancer [78]	
11	10.57	1.71	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	Antibacterial Anti fungal (Chandrasekaran, Senthilkumar et al., 2011 [79],	Fatty acid esters
12	11.27	6.29	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Antimicrobial Anticancer Antioxidant Hupocholesteremic activity [80,76]	Fatty acids
13	13.06	0.35	Phytol	$C_{20}H_{40}O$	296.5	Antidiabetic cytotoxic, antioxidant, antimicrobial, cytotoxic, antitumorous, antimutagenic,anti-teratogenic,antibiotic-chemotherapeutic,lipid lowering, antispasmodic,anticonvulsant, antinociceptive,anti-inflammatory, anxiolytic, antidepressant, immunoadjuvancy, hair growth facilitator, hair fall defense and antidandruff activities. (Islam, Ali et al., 2018) [25]	Terpenoids
14	13.39	0.17	Methyl stearate	$C_{19}H_{38}O_2$	298.5	Antibacterial [27]	Fatty acid esters
15	18.25	0.32	Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	Anti-diabetic activity insulin stimulation, and α-glucosidase inhibitors. Antioxidant, antitumor [37]	Fatty acids
16	19.57	0.12	Heneicosanoic acid	$C_{21}H_{42}O_2$	326.6	Cytotoxic effect [81]	Fatty acids
17	20.05	0.52	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.5	Antibacterial [82]	Fatty acids
18	20.71	0.30	Docosanoic acid	$C_{22}H_{44}O_2$	340.6	α-Glucosidase inhibitory activity [83]	Fatty acids
19	21.35	0.14	Tricosanoic acid,	$C_{24}H_{48}O_2$	368.6	Antimicrobial, antioxidant [28]	Fatty acid esters
20	21.90	0.15	Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl-	$C_{19}H_{34}$	262.5		Alkene
21	22.13	0.08	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.5	Antibacterial, cytotoxic [30]	Fatty acid esters
22	22.63	0.20	Tetracosanoic acid	$C_{24}H4_8O_2$	368.6	Anti-diabetic Antimicrobial [17,84]	Fatty acids
23	22.96	0.23	Squalene	$C_{30}H_{50}$	410.7	Anti-diabetic Anticancer, Antiinflammatory, Antioxidant, Emollient and antioxidant [85, 86]	Terpenoids
24	23.14	0.15	α-Tocospiro B	$C_{29}H_{50}O_4$	462.7	Antioxidant [87]	Terpenoids
25	24.74	0.14	γ-Tocopherol	$C_{28}H_{48}O_2$	416.7	Anti-inflammation, antioxidant [88]	Tocopherols
26	25.32	0.83	Vitamin E	$C_{29}H_{50}O_2$	430.7	Anti-diabetic antioxidant [89,90]	Tocopherols

(*continued on next page*)

Table 4 (*continued*)

Sr. no.	RT	Area %	Tentative compound	Mol. formula	Mol. weight	Biological Activities	Nature of compounds
27	25.50	0.14	Ergosta-5,7,9(11),22-tetraen-3-ol, (3. beta.,22E)-	$C_{28}H_{42}O$	394.6	Anti-tumor [91]	Steroids
28	26.01	4.11	Campesterol	$C_{28}H_{48}O$	400.68	Anti-diabetic, Antiangiogenic cholesterol lowering and antifungal anti-cardiovascular, anti-cancer, and anti-microbial properties [92,93].	Phytosterols
29	26.21	0.40	Stigmasterol	$C_{29}H_{48}O$	412.6	Anti-diabetic, antimutagenic, antioxidant, anti-cardiovascular, anti-cancer, and anti-microbial [94,93]	Phytosterols
30	26.31	0.26	Ursa-9(11),12-dien-3-ol	$C_{30}H_{48}O$	424.7	Antimicrobial [60]	Terpenoids
31	26.95	3.34	γ-Sitosterol	$C_{29}H_{50}O$	414.7	Antibacterial, Antidiabetic, anticancer [53, 63]	Phytosterols
32	27.07	1.86	Stigmasta-5,24(28)-dien-3-ol, eta.,24Z)-	$C_{29}H_{48}O$	412.7	Antibacterial [95]	Phytosterols
33	27.24	3.15	β-Amyrin	$C_{30}H_{50}O$	426.7	Antidiabetic, Antimicrobial, Hypolipidemic effect [96,97]	Terpenoids
34	27.37	2.06	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424.7	Vasodepressor effects (Barla, Bİrman et al., 2006)	Terpenoids
35	28.12	11.83	24-Noroleana-3,12-diene	$C_{29}H_{46}$	394.7	Antimicrobial [98]	Terpenoids
36	28.30	0.29	Stigmasta-4,24(28)-dien-3-one, E)-	$C_{29}H_{46}O$	410.7	Antibacterial [99]	Terpenoids
37	28.74	18.54	Urs-12-en-24-oic acid,3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468.7	Antimicrobial [43]	Terpenoids
38	29.51	0.85	Epilupeol; 20(29)-Lupen-3αol, acetate (isomer 1)	$C_{32}H_{52}O_2$	468.8	Antimicrobial (Ferdous, Hossain et al., 2010)	Terpenoids
39	29.81	1.42	11-Oxo-β-amyrin	$C_{30}H_{48}O_2$	440.7	Anti-inflammatory [5]	Terpenoids

Table 5
Enzyme inhibition activity of *T. peruviana* against tyrosinase, α-glucosidase and lipoxygenase.

Sample	Tyrosinase inhibition (%)	α-glucosidase inhibition (%)	Lipoxygenase inhibition (%)
TPM	59.43 ± 2.87	71.32 ± 2.44	77.1 ± 1.2
TPD	53.43 ± 2.65	67.86 ± 3.011	59.3 ± 0.1
Standard	89.16 ± 2.083[a]	86.07 ± 2.62[b]	89.06 ± 0.2[c]

Values are mean ± SD for tyrosinase and α-glucosidase inhibition by extracts of *T. peruviana* (TPM: methanolic extract and TPD: Dichloromethane extract), as well as standard inhibitors (a: kojic acid, b: acarbose, c: baicalein).

phenolic compounds showed a clear correlation between antioxidant activities [104] and a similar correlation was observed for the studied extracts. The higher concentration of phenolic and flavonoid-containing extract demonstrated a substantial antioxidant potential [105].

3.2.2. Enzyme inhibition activities of T. peruviana

The enzyme inhibition of *T. peruviana* was evaluated for its potential use in cosmeceuticals as a skin-lightning agent (tyrosinase inhibitory effect), as anti-inflammatory agent (lipoxygenase inhibition) and anti-diabetic properties (α-glucosidase inhibition).

3.2.2.1. Tyrosinase inhibitory potential T. peruviana. The spectrophotometry method was used to assess the tyrosinase inhibition from TPM and TPD extracts of *T. peruviana*. The percentage of inhibition of both extracts was shown to have tyrosinase inhibition potentials of (59.43 ± 2.87 %) for TPM and (53.43 ± 2.65 %) for TPD, respectively. Herein, the results were noteworthy when compared to the inhibition of kojic acid (89.16 ± 2.083 %) as the value calculated for this standard. These findings demonstrate the potential effectiveness of *T. peruviana* as a tyrosinase enzyme inhibitor (Table 5). Furthermore, these findings are compatible with the previous evidence describing the significant anti-tyrosinase activities of this plant. They further explained the possibility of tyrosinase inhibition due to the good antioxidant activities and bioactive contents [106].

The results of tyrosinase inhibition activity exhibited a correlation in the bioactive contents, antioxidant activities, and tyrosinase inhibition (Table 5). Lup-20(29)-en-3-ol, acetate) and α-Amyrone have a strong affinity for the tyrosinase enzyme, and possibly other compounds in these extracts may be responsible for significant tyrosinase inhibitory potential of TPM, as revealed by GC-MS profiling. Moreover, the existence of antibacterial, antioxidant, and anti-inflammatory compounds may also contribute to tackling skin diseases. Tyrosine is essential for melanin production. Due to the overexpression of tyrosinase, excessive melanin causes age spots. In the food and cosmetic industries, tyrosinase inhibitors and antioxidants are preferred preservatives and skin-protecting ingredient [107]. Many solutions for skin whitening have been launched in the market during the last decades, but these treatments have yet to reveal significant results. This frequently occurs because these bleaching agents have more toxicity and mutagenic effects, as demonstrated with

Table 6
Antibacterial activity of *T. peruviana*.

Bacterial strain	Zone of inhibition (mm) of Standard (co-amoxiclav) (Conc. = 1000 μg/mL)	Conc. (mg/mL)	Zone of Inhibition of TPM extract (mm)	Zone of Inhibition of TPD extract (mm)
Bacillus subtilis	22	10	8	6
		20	12	9
		40	19	17
Staphylococ-cus epidermidis	23	10	7	–
		20	11	7
		40	14	10
Staphylococ-cus aureus	21	10	7	6
		20	14	14
		40	16	15
Escherichia coli	24	10	5	5
		20	9	7
		40	12	10
Pseudomon-as aeruginosa	19	10	–	–
		20	–	–
		40	8	6

TPM: methanolic extract of *T. peruviana*; TPD: dichloromethane extract of *T. peruviana*.

hydroquinone [108]. Novel natural tyrosinase inhibitors with increased therapeutic efficacy, excellent skin penetration, and fewer side effects are currently being sought after, which increased the significance of the study design.

3.2.2.2. α-glucosidase inhibitory potential of T. peruviana. α-glucosidase inhibition assay was used to assess the anti-diabetic potential of TPM and TPD extracts of the studied plant, and the results are presented in Table 5. The maximum value of % inhibition was depicted by TPM, which was found to be (73.32 ± 2.44 %), and the value of inhibition for TPD was (67.86 ± 3.011 %). The calculated value of the standard drug acarbose was (86.07 ± 2.62 %) [109]. Rauf et al. also reported α-glucosidase inhibition by methanolic extract of *T. peruviana*. They claimed the significant anti-diabetic potential of methanolic extract of this plant among the four studied plants. So, the results of our study were found compatible with that study and revealed good anti-diabetic potential of methanolic extract compared to dichloromethane extract as well as standard (acarbose).

The bioactive compounds such as polyphenols may be contributing to the anti-diabetic properties of the extracts with a possible reduction in oxidative stress. Many phytoconstituents tentatively identified from GCMS analysis (Tables 3 and 4) are phytocompounds with the reported anti-diabetic activity, which may be contributing to the activity of TPM and TPD. Moreover, the higher quantity of polyphenols may also be contributing to the higher activity of TPM due to a possible synergism with terpenoids and other non-polar constituents. One of the most prevalent endocrine metabolic diseases, diabetes mellitus, has been linked to considerable morbidity and death owing to microvascular (retinopathy, neuropathy, and nephropathy) [9] and macrovascular (heart stroke, and peripheral vascular disease) consequences (Nazir, ur [103]. α-glucosidase, often occurring in the upper small intestine, raises the glycemic level by converting the carbohydrates into absorbable sugars and results in hyperglycemia. An intriguing function of plant extract is its ability to reduce postprandial glucose levels by inhibiting the in intestinal α-glucosidase complex. Moreover, the production of active oxygen free radicals has a role in the development of several degenerative illnesses, including diabetes, so the antioxidant effects of plants investigated in the current study may also help in the management of diabetes.

3.2.2.3. Lipoxygenase inhibitory potential of T. peruviana. To assess the anti-inflammatory potential of *T. peruviana,* lipoxygenase Inhibitor Screening Assay was performed as described by Ref. [8]. The findings of the assay revealed that TPM significantly inhibited lipoxygenase with % inhibition (77.1 ± 1.2) followed by TPD (59.3 ± 0.1) as shown in Table 5. Lipoxygenase is an enzyme that catalyzes the oxidation of polyunsaturated fatty acids to produce leukotrienes and other biologically active compounds. Inhibiting lipoxygenase activity can be useful in treating inflammatory disorders such as asthma and arthritis [110]. There is no reported literature available regarding lipoxygenase inhibition activity of *T. peruviana.* Natural products are rich sources of fatty acids, phenols, flavonoids and terpenoides and have reported biological activities such as antioxidant, anti-inflammatory activities (Rao, Ahmad et al., 2023). The biologically active phytoconstituents (phenols, fatty acid, flavonides and terpenoides) tentativly identified through GC-MS analysis may attribute to the lipoxygenase inhibition activity of the *T. peruviana.* The maximum lipoxygenase inhibition exhibited by TPM due to the tentatively detected phytoconstituents such as vanillin, phytol, lupeol.

3.2.3. Antibacterial activity of T. peruviana

In the evaluation for the antibacterial properties of TPM and TPD, the significant activity of both extracts was revealed, as shown in Table 6. The antibacterial activity of TPM and TPD was observed in a dose-dependent manner. The highest activity was observed with 40 mg/mL of samples, significantly close to the antibiotic used as standard (co-amoxiclav) in this study. Moreover, *B. subtilis* was found to be more susceptible than *S. aureus, S. epidermidis, E. coli,* and *P. aeruginosa* at the highest dose of both extracts. Maximum zones of inhibition were seen against *B. subtilis* (TPM; 19 mm, TPD; 17 mm) compared to co-amoxiclav (22 mm). Nearly equal inhibition was exhibited by both extracts towards *S. aureus*. Compatibility to our results was also observed from a previous study reporting the antibacterial activities from the latex of *T. peruviana*. Phytochemicals with documented antibacterial and antimicrobial properties

Table 7
Binding affinity scores (Kcal/mol) of tentatively identified phytoconstituents from TPM.

Sr. No.	Phytocompound	Tyrosinase	α-glucosidase	Lipoxygenase
1	Vanillin	−5.8	−5.4	−5.8
2	Phenol, 2-methoxy-4-(1-propenyl)-, acetate, (Z)-	−6.6	−5.4	−6.4
3	4-Propenyl-2,6-dimethoxyphenol	−5.9	−5.7	−6.3
4	Methyl 12-methyltridecanoate	−4.7	−4.8	−5.9
5	Tetradecanoic acid	−5.1	−4.7	−5.6
6	Pentadecanoic acid	−4.5	−4.9	−5.3
7	Hexadecanoic acid, methyl ester	−4.5	−4.1	−5.1
8	n-Hexadecanoic acid	−4.4	−4.1	−4.7
9	*cis*-10-Heptadecenoic acid	−5.1	−5.1	−5.8
10	Heptadecanoic acid	−4.9	−4.6	−6.3
11	Methyl octadeca-9,12-dienoate	−5.2	−5	−6.6
12	11-Octadecenoic acid methyl ester	−4.9	−4.6	−4.2
13	Phytol	−5.6	−4.4	−5.1
14	Methyl stearate	−4.8	−4.5	−6.4
15	9,17-Octadecadienal	−4.4	−4.9	−5.4
16	6-Octadecenoic acid	−5.6	−4.7	−5.6
17	Octadecanoic acid	−4.5	−4.4	−4.9
18	Z,Z-10,12-Hexadecadien-1-ol acetat	−4.9	−4.5	−5.3
19	Oleic Acid	−4.8	−4.8	−5
20	10E,12Z-Octadecadienoic acid	−6.4	−6.1	−9.2
21	Tricosane	−4	−4.4	−5.3
22	Eicosanoic acid, methyl ester	−4.7	−4.8	−6.3
23	4,8,12,16-Tetramethylheptadecan-4-olide	−5.7	−5	−5.8
24	Eicosanoic acid	−5.2	−4.6	−4.3
25	Tetracosane	−4.3	−4.2	−5.5
26	Heneicosanoic acid, methyl ester	−4.2	−4	−4.9
27	Heneicosanoic acid	−4.9	−5	−5.7
28	Heneicosane	−4.2	−4.4	−4.9
29	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	−4.6	−5	−5.8
30	Docosanoic acid, methyl ester	−4.1	−4.3	−6.2
31	Docosanoic acid	−4.6	−4.4	−5.1
32	Tricosanoic acid methyl ester			
33	1-Hexacosene	−4.3	−4.1	−5.8
34	Tricosanoic acid	−4.9	−4.4	−6.2
35	Heneicosane	−4.5	−3.9	−6.3
36	Tetracosanoic acid methyl ester	−5.4	−5.6	−7.2
37	αTocospiro-B	−6.7	−5.8	−8.8
38	ATocospiro A	−6.4	−6.2	−6.2
39	Tricosane	−4.3	−4.1	−5.8
40	Hexacosanoic acid, methyl ester	−4.4	−3.9	−5.8
41	Hexocosane	−7	−6.2	−6.9
42	beta-Tocopherol	−5.9	−6	−7.3
43	Ergost-5-en-3-ol,(3beta,24R)-	−6.4	−6.2	−8.3
44	Campesterol	−7.8	−7.2	−10
45	Stigmasterol	−8.1	−7.5	−9.9
46	**Ursa-9(11),12-dien-3-ol**	**−7.8**	**−8**	**−8.9**
47	gamma.-Sitosterol	−7.8	−7.2	−9.9
48	**beta-Amyrin**	**−8.7**	**−8.4**	**−9.4**
49	Lupeol	−8.6	−7.8	−10.1
50	**alpha.-Amyronee**	**−8.1**	**−13.3**	**−16.1**
51	Olean-12-en-3-ol, acetate, (3.beta)	−8	−8.3	−8.7
52	**Urs-12-en-24oic acid,3-oxo-,methyl ester**	**−7.8**	**−7.7**	**−8.7**
53	**Epilupeol; 20(29)-Lupen-3αol,acetate**	**−7.5**	**−7.6**	**−13.5**
54	**Lup-20(29)-en-3-ol, acetate)**	**−7.4**	**−12**	**−15.2**
55	Kojic acid (standard for tyrosinase)	−5.7		
56	Baicalein (standard for lipoxygenase)			−9.7
57	Acarbose (standard for α-glucosidase)		−7.7	

(Table 3) were identified from TPM by GC-MS (as shown in GC-MS chromatogram). While, the antimicrobial and antibacterial compounds from TPD extract may contribute to antibacterial potential of TPD (Table 4). It also correlates with the higher antibacterial activities of TPM extract; furthermore, the greater values of TPC and TFC may also be contributing to the greater antibacterial potency of TPM extract [111].

Extensive use of traditional antibiotics is leading to a gradual decline in their effectiveness against pathogenic bacteria, resulting in the potential of drug resistance, which may cause global health threats. Therefore, it is necessary to create new antibacterial agent to prevent the spread of mutating strains of bacteria [112]. Plants are the natural resources synthesizing the secondary metabolites, which may offer the defense against microbial infections. Plant-derived chemicals and their analogs are demonstrated to be efficient antibacterial agents or may work in concert with prevailing antibiotics to increase their potency, thereby reviving their use in medicine

Table 8
Binding affinity scores (Kcal/mol) of tentatively identified phytoconstituents from TPD.

Sr. No.	Phytocompound	Tyrosinase	α-glucosidase	Lipoxygenase
1.	Thymol	−5.8	−6.3	−6.4
2.	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	−6.9	–	−7.4
3.	2-Methoxy-4-vinylphenol	−6.2	−5.5	−5.8
4.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	−7	−6	−6.7
5.	Phenol, 4-ethenyl-2,6-dimethoxy-	−6.2	−5.4	−5.7
6	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	−6.7	−5.4	−5.9
7.	Methyl tetradecanoate	−4.7	−4.9	−5.2
8.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2- methoxyphenol	−6.5	−5.9	−6.2
9.	Tetradecanoic acid	−5.6	−4.3	−5.9
10.	Neophytadiene	−5.1	−4.4	−6.1
11.	Hexadecanoic acid, methyl ester	−4.3	−4.5	−5.8
12.	n-Hexadecanoic acid	−4.5	−4.4	−4.8
13.	Phytol	−5.6	−4.6	−6.4
14.	Methyl stearate	−4.6	−4	−4.9
15.	Eicosanoic acid	−4.2	−4	−6
16.	Heneicosanoic acid	−5	−4.2	−5.2
17.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	−4.3	−4.8	−5.6
18.	Docosanoic acid	−4.5	−4.7	−5.9
19.	Tricosanoic acid,	−4.1	−4.5	−5.4
20.	Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl-	−6.2	−5.6	−8.3
21.	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	−4.5	−4.6	−5.7
22.	Tetracosanoic acid	−4.6	−4.5	−5.1
23.	Squalene	−6	−5	−8.2
24.	alpha.-Tocospiro B	−6.7	−5.9	−7
25.	gamma.-Tocopherol	−6.2	−6.1	−8.2
26.	Vitamin E	−6	−6.9	−8.4
27.	**Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-**	**−8.2**	**−7.3**	**−10.7**
28.	Campesterol	−7.7	−7.3	−10
29.	Stigmasterol	−8	−7.4	−9.8
30.	Ursa-9(11),12-dien-3-ol	−8.1	−8.2	−9.6
31.	gamma.-Sitosterol	−7.7	−7.4	−10
32.	Stigmasta-5,24(28)-dien-3-ol, eta.,24Z)-	−8.1	−7	−10.3
33.	**beta.-Amyrin**	**−8.7**	**−8.4**	**−9.3**
34.	**Lup-20(29)-en-3-one**	**−7.7**	**−7.4**	**−8.1**
35.	24-Noroleana-3,12-diene	−8.6	−7.8	−10.1
36.	Stigmasta-4,24(28)-dien-3-one, E)-	−7.6	−6.8	−7.1
37.	**Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-**	**−8.5**	**−8.4**	**−10.7**
38.	**Epilupeol; 20(29)-Lupen-3αol, acetate (isomer 1)**	**−13**	**−7.6**	**−10.1**
39.	11-Oxo-.beta.-amyrin	−8.3	−8.4	−9.3
40.	Kojic acid (Standard for tyrosinase)	−5.7		
41	Baicalein (standard for lipoxygenase)			−9.7
42	Acarbose (Standard for α-glucosidase)		−7.7	

(Alibi, Crespo et al., 2021).

3.3. Molecular docking

In-silico studies on the phyto-constituents from TPM and TPD, obtained from the GC-MS analysis, was performed to understand the binding interaction of the identified compounds within the active pocket of the targeted enzymes i.e., Lipoxygenase, Tyrosinase and α-glucosidase. The results are shown in Tables 7 and 8. Prior to molecular docking studies, the active pocket amino acid residues of each protein were determined using discover studio 2021 [113]. The amino acid residues of active pocket of α-glucosidase were ASP21, ASN23, ASP25, ILE27,ASP29,ASP534, GLU537 and THR543, Similarly, the active pocket amino acid residues of tyrosinase were HIS61, CIS83, HIS85, HIS94, HIS295, ASN260, VAL283, ALA286, HIS296, ASP312, ASP336, GLN351 and ASP353 and amino acid residues of active pocket of lipoxygenase were HIS499, HIS504, ASN694 and ILE839. The docking procedure was validated to authenticated the results [114].

Total 39 compounds were identified during the GC-MS analysis of TPD extract and all the compounds were docked within the active pocket of the targeted enzymes. The results are shown in Table 8. From the results it was observed that Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)- and Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)- were the constituent which exhibited maximum potential against the targeted enzymes. But when the results of molecular docking investigations were compared with TPM extract it was observed that the α-amyrone is the compound which exhibited strong potential against all the ezymess and therefore can be suggested for futher isolation.

3.3.1. Molecular docking of tentatively identified phytoconstituents against tyrosinase enzyme

From the results mentioned in Tables 7 and 8 it was observed that phytochemical epilupeol; 20(29)-Lupen-3αol,acetate(isomer1)

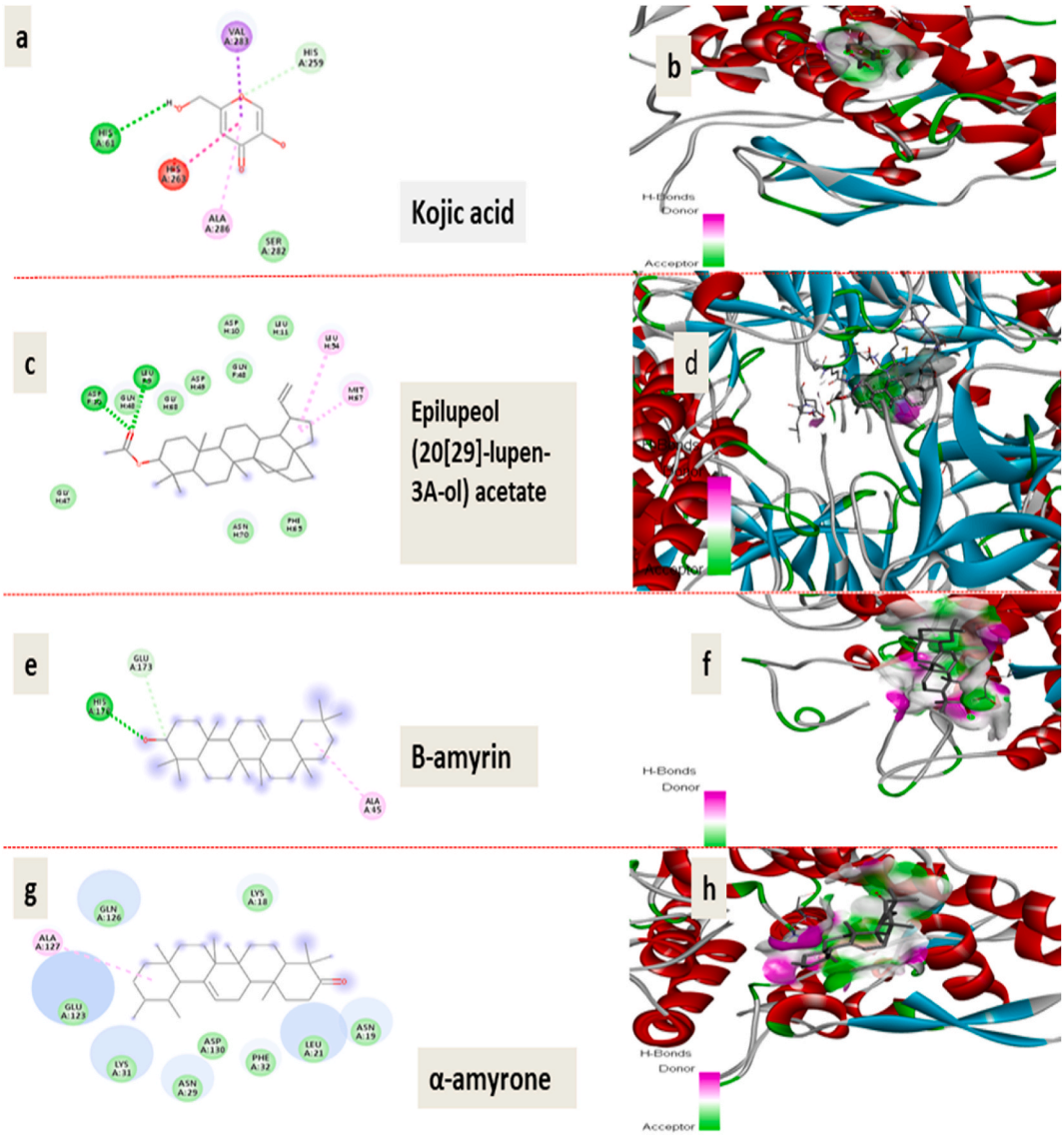

Fig. 1. The depiction of 2D and 3D structures of (a, b) kojic acid, (c, d) Epilupeol; 20(29)-Lupen-3αol, acetate (isomer 1), (e, f) β-amyrin and (g, h) α-amyrone within the active pocket of tyrosinase (PDB: 2y9x).

can bind with tyrosinase with highest docking score −13 kcal/mol which was found 2.3 folds better than kojic acid (standard) i.e., −5.7 kcal/mol.

The binding interactions of kojic acid (standard) were visualized Fig. 1 (a, b) and it was observed from the 2D figure that this compound formed five types of interactions within the active pocket of amino acid residues. Hydroxyl group attached at C-5 formed conventional hydrogen bond with amino acid residue HIS61. Hetero oxygen of pyran ring formed non-favorable interaction with HIS263. The amino acid residues VAL283 and ALA 286 formed pi-sigma interaction and pi-alkyl interaction, respectively with pyran ring of kojic acid. Moreover, it was observed that HIS 259 formed carbon hydrogen bond and SER282 amino acid residue was involved to form van der Waals interaction with the kojic acid.

As compare to standard, epilupeol; 20(29)-Lupen-3αol,acetate (isomer1) formed conventional hydrogen bond, alkyl bond and van der Waals interactions within the active pocket of enzyme as shown in Fig. 1 (c, d). Briefly the acetate group of compound formed two conventional hydrogen type interactions with amino acid residue LEU9 and ASP10 and two alkyl type interactions were formed between amino acid residue LEU54 and MET67 with cyclo pentane ring. Moreover, seven amino acid residues GLN48, GLY68, ASP49 GLN48, LEU11, ASN70 and PHE65 formed van dar Waals interactions with former compound.

The β-amyrin which was found in both TPM and TPD, exhibited second highest docking score of −8.7 kcal/mo.l. The binding interactions of β-amyrin Fig. 1 (e, f) suggested that this compound formed three types of interactions within active pocket amino acid residues. The binding interactions suggested that this compound has potential because of the formation of one strong hydrogen bond.

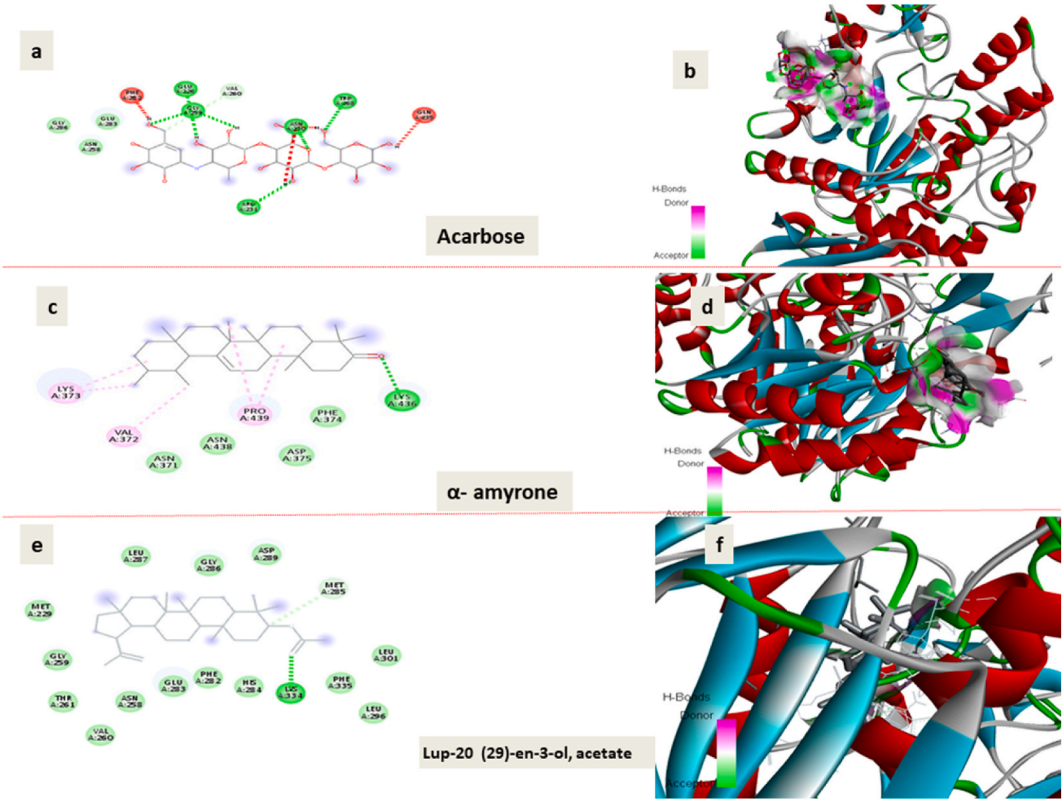

Fig. 2. The depiction of 2D and 3D structures of (a, b) acarbose, (c, d)) α-amyrone and (e, f) Lup-20(29)-en-3-ol, acetate) within the active pocket of α-glucosidase (PDB: 5zcb).

Briefly, icosahydropicen-3-ol formed one carbon hydrogen bond with GLU173. This ring also formed van der Waal interaction with HIS178 amino acid residue. Another cyclohexane ring formed one alkyl interaction with ALA45.

α-amyrone also yielded docking score −8.1 kcal/mol, which showed that this compound has potential to inhibit the enzyme. The binding interactions of α-amyrone were visualized Fig. 1 (g, h) and it was observed from the 2D Figure that this compound formed Vander Waal and alkyl type of interactions within the active pocket of enzyme. Briefly the cyclohexane ring containing one methyl group formed alkyl interaction with amino acid residue ALA127 and the amino acid residues including GLN126, LYS18, ASN19, LEU21, PHE32, ASP130, ASN29, LYS31 and GLU123 formed van der Waal interactions with different cyclohexane rings and carbonyl group of α-amyrone. The binding interactions of most potent compounds are given in Fig. 1 and the interactions of other compounds are given in supplementary file from Figure S5-S9 and S26-S35 respectively.

From the results it was observed that the phyto-constituents had great potential against the tyrosinase and most of the identified compounds showed more potential than that of the standard. The results were confirmed by re-docking method. Hence, this research supports the possibility of the extracts of the plant having a significant ability to block the enzyme tyrosinase.

3.3.2. Molecular docking of tentatively identified phytoconstituents against α-glucosidase enzyme

From the results mentioned in Tables 7 and 8 it was observed that the α-amyrone which was found in TPM exhibited significantly high docking score −13.3 kcal/mol as compare to other phytochemicals and it was also found that it is 1.72 folds better than acarbose i. e., −7.7kcal/mol.

The binding interaction of acrabose (standard) were visualized and shown in Fig. 2 (a, b). It was observed from the 2D figure that this compound formed van der Waal, carbon hydrogen and conventional hydrogen interaction within the active pocket of enzyme. Briefly the methanol group attached with pyran ring formed conventional hydrogen interaction with amino acid residues TRP268. In pyran ring hetero oxygen formed conventional hydrogen interaction with amino acid residue ASN230, hydroxyl group attached with same ring formed again conventional hydrogen bond with amino acid residue ARG 231. Hydroxyl groups attached with cyclohexane and cyclohexene ring also made conventional hydrogen bond with amino acid residue GLY259 which is attached to amino acid residue GLU226. GLY259 amino acid residue is linked with VAL260 amino acid residue via carbon hydrogen bond. GLU282, GLY286 and ASN 250 amino acid residues formed van der Waal interaction with cyclohexene ring of acrabose.

As compare to standard, the binding interaction of α-amyrone were visualized and shown in Fig. 2 (c, d). It was seen from 2D figure that α-amyrone formed van der Waals, alkyl and conventional hydrogen type interaction within active pockets of enzyme α-glucosidase. Carbonyl group attached with cyclohexane ring formed conventional hydrogen interaction with amino acid residue LYS436.

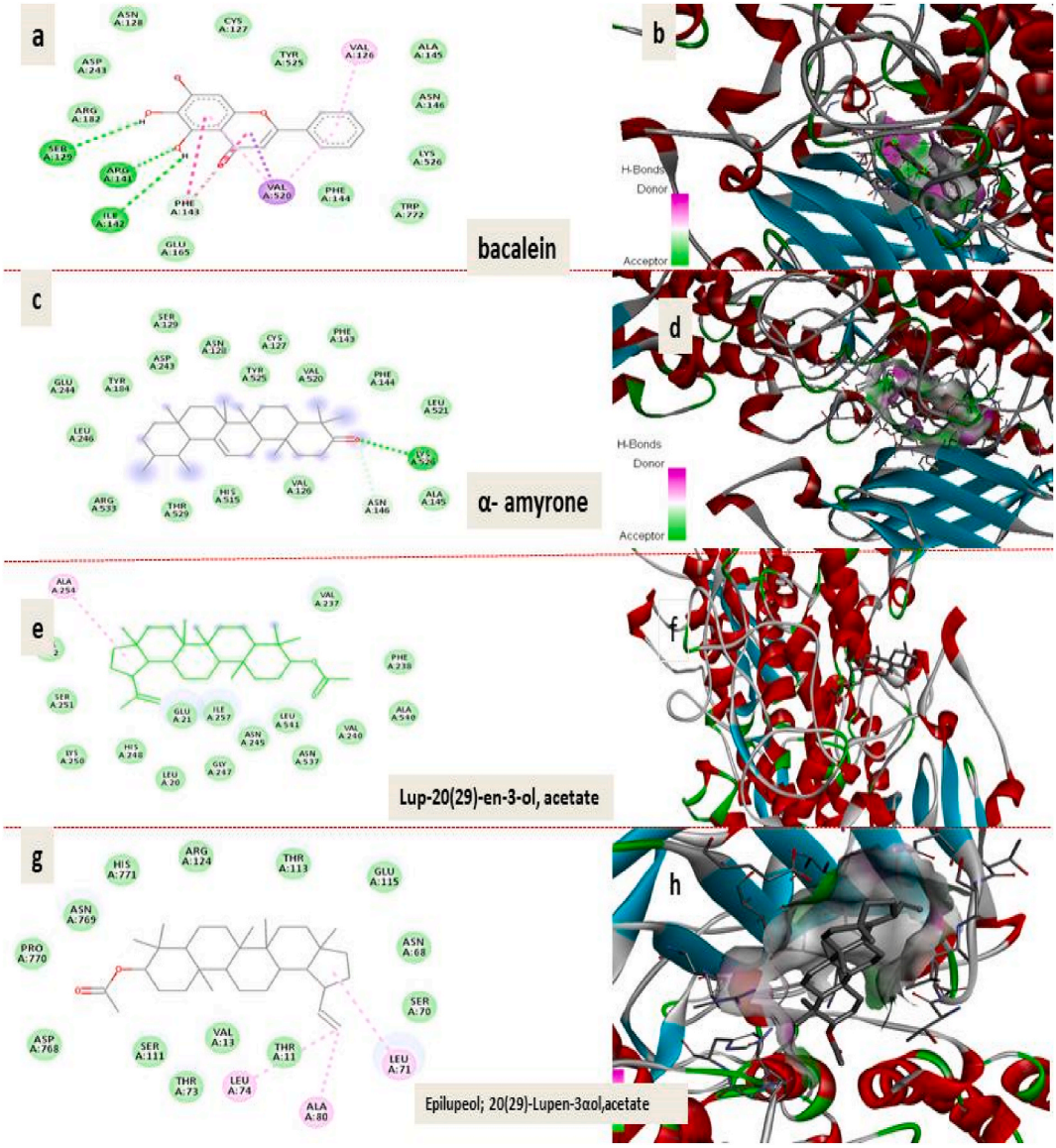

Fig. 3. The depiction of 2D and 3D structures of (a, b) bacalein (c,d) α-amyrone, (e, f) Lup-20(29)-en-3-ol, acetate) and (g, h) Epilupeol; 20(29)-Lupen-3αol,acetate within the active pocket of lipoxygenase (PDB: 1yge).

One of methyl group of cyclohexene ring and cyclohexane ring of α-amyrone formed alkyl interaction with amino acid residue PRO439. LYS373 amino acid residue formed interaction with another cyclohexane ring via two alkyl bonds. Methyl group attached with same ring formed alkyl interaction with VAL372. Amino acid residues ASN371, ASN438, ASP375 and PHE374 formed van der Waals interaction with different cyclohexane rings of α-amyrone.

An interesting effect was observed by Lup-20(29)-en-3-ol, acetate found in TPM, which exhibited docking score of −12.0 kcal/mol Fig. 2 (e, f). The former formed three types of binding interactions conventional hydrogen bond, carbon hydrogen bond and van der Waals within the active pocket of amino acids. Briefly, carbonyl group of cyclo-hexyl acetate ring exhibited one conventional hydrogen bond with amino acid residue LYS334 and also the same ring forms carbon hydrogen type interaction with MET285 residue. Compound formed fourteen interacting van der Waals bonds with LEU301, PHE335, LEU296, HIS284, PHE282, GLU283, ASN258, VAL260, THR261, GLY259, MET229, LEU287, GLY286 and ASP289 amino acid reidues. The binding interactions of most potent compounds are given in Fig. 2 and the interactions of other compounds are given in supplementary file from Figure S18-S25 and S45-S49 respectively.

3.3.3. Molecular docking of tentatively identified phytoconstituents against lipoxygenase enzyme

From the results mentioned in Tables 7 and 8 it was observed that the maximum/significant binding affinity against lipoxygenase

was found for α-amyrone which was found in TPM. This α-amyrone exhibited docking score of −16.7 kcal/mol which was found 1.65 folds better than the baicalein i.e., −9.7 kcal/mol.

When the binding interactions of standard baicalein Fig. 3 (a, b) with lipoxygenase were visualized, it was observed that this compound formed six types of interactions within active pocket amino acid residues. This complex showed conventional hydrogen bond, carbon hydrogen bond, pi-sigma, pi-pi stacked, pi-alkyl and van der Waals interactions. Briefly, one of the hydroxyl group of benzene ring formed conventional hydrogen bond type with SER129 amino acid residue, second hydroxyl group shared hydrogen bond with ARG141 and ILE142 amino acid residue. 4-H chromen-4-one ring and benzene ring formed pi-pi stacked interaction with VAL520 amino acid residue. Phenyl ring attached with 4-H-chromen-4-one ring formed pi-alkyl interaction with VAL126 and VAL520 amino acid residues. VAL520 amino acid residue also formed pi-alkyl interaction with tri hydroxyl benzene ring. Amino acid residues PHE144, TRP772, LYS526, ASN146, ALA145, TYR525, CYS127, ASN128, ASP243, ARG182 and GLU165 formed van der Waals interaction with compound.

As compared to standard, the binding interactions of α-amyrone, shown in Fig. 3 (c, d) suggested that this compound formed three types of interactions within active pocket of amino acid residues. The binding interactions suggested that this compound showed more potential because of the formation of one conventional hydrogen bond and one carbon hydrogen bond. Briefly, carbonyl group of cyclohexane formed conventional hydrogen interaction with amino acid residue LYS526, and amino acid residue ASN146 also interacts with same carbonyl carbon via carbon hydrogen bond. Seventeen amino acid residues LEU521, PHE144, PHE143, VAL520, CYS127, TYR525, ASN128, SER129, ASP243, TYR184, GLU244, LEU246, ARG533, THR529, HIS515 VAL126 and ALA145.

Phytochemicals from TPM extract i.e., Lup-20(29)-en-3-ol, acetate and Epilupeol; 20(29)-Lupen-3α ol,acetate exhibited docking score of −15.2 kcal/mol and −13.2 kcal/mol, respectively. Briefly, the binding interactions of Lup-20(29)-en-3-ol, acetate) with lipoxygenase were visualized as shown in Fig. 3 (e, f). The 2D structure showed that cyclo pentane ring of compound formed conventional alkyl interaction with ALA254. Moreover, amino acid residues VAL22, SER251, LYZ250, HIS248, LEU20, GLU21, ILE257, GLY247, ASN245, LEU541, ASN537, VAL240, ALA540, PHE238 and VAL237 formed van der Waal interactions with cyclohexane rings of compound.

Epilupeol; 20(29)-Lupen-3α ol,acetate exhibited two types of interactions i.e., alkyl and van der Waals interactions within the active pocket of amino acid as shown in Fig. 3 (g, h). Briefly, alkene group attached with cyclo pentane ring of former formed two interacting alkyl bonds with LEU74 and ALA80 amino acid residues, while one interacting alkyl bond was formed between cyclo pentane ring of compound and LEU71 residue. Furthermore, thirteen amino acid residues THR11, VAL13, THR73, SER111, ASP768, PRO770, ASN769, HIS771, ARG124, THR113, GLU115, ASN68 and SER70 were interacting with compound via van der Waals bonds.

The binding interactions of most potent compounds are given in Fig. 3 and the interactions of other compounds with tyrosinase, lipoxygenase and α-glucosidase are given in supplementary file from Figs. S3–S47.

Our study showed that TPM and TPD extracts of *T. peruviana* have α-glucosidase inhibition (71.32 % and 67.86 %) respectively. Among all the tentative compounds α-amyrone shows significant binding affinity scores (Kcal/mol) against all the three enzymes lipoxygenase (−16.1), α-glucosidase (-13.3) and tyrosinase (−8.1). α-amyrone is a pentacyclic triterpenoid of medicinal importance including anti hyperglycemic, anti-inflammatory (significant anti-inflammatory effect on paw edema and ear edema) [115,116]. When the α-glucosidase inhibitory activity of former compound was compared to the standard acarbose, the findings showed inhibition rate of (96.59 % ± 0.52) at a concentration of 1.6 μg/mL, whereas the standard (acarbose) showed an inhibition rate of 51.5 % at the concentration of 60 μg/mL (Ferreira, 2017), it has also reported in literature that α-amyrone has significant antidiabetic potential in terms of α-glucosidase inhibition [97]).

Our finding also revealed that both extracts (TPM and TPD) showed significant inhibition of lipoxygenase. Among the tentatively identified ***α***-amyrone has been reported to have significant potential in both acute and chronic inflammatory process (Quintão, 2014). 5-lipoxygenase inhibitors exert anti-inflammatory effects by suppress the activity of enzyme 5-lipoxygenase by inhibition the synthesis of proinflammatory mediators such as leukotriene (Luo, 2022; Vo, 2019). Pentacyclic triterpenes from various medicinal plants also showed potent inhibitory activity of mushroom tyrosinase [117]. The reported study suggested that pentacyclic triterpenes are powerful tyrosinase inhibitors and they have great potential for management of hyperpigmentation related to increase production of melanocytes (Ullah, 2007). Our study also supported that TPM and TPD extract of *T. peruviana* have tyrosinase inhibition (59.43 % and 53.43 %) respectively, which may be due to presence of triterpenoids like α-amyrone, α-amyrin, and β-amyrone.

In conclusion, the results of *in-silico* molecular docking showed how the ligands detected by GC-MS analysis interacted with the enzymes tyrosinase, lipoxygenase and α-glucosidase. These results support our discovery of the plant extract in terms of tyrosinase, lipoxygenase and α-glucoside inhibitions.

3.4. Molecular dynamics simulations

In order to validate the docking results the most potent compound with highest binding affinity i. e., α-amyrone against α-glucsidase, lipoxygenase and tyrosinase, each protein-ligand complex was simulated in aqueous environment stabilized, by incorporation of NaCl as counter ions. In molecular dynamic simulation studies RMSD and RMSF values are used to measure the stability of the system. Any protein-ligand complex with RMSD value lower than 2.0 Å is considered stable and strong. In this study the behavior of each protein and its ligand complex was studied to understand the stability and validity of docking results. The average RMSD value of protein ligand complex is considerably lower than protein with the mean value of 1.6 Å, 2.3 Å and 1.8 Å against α-glucosidase, lipoxygenase and tyrosinase, respectively. The graph of each complex show minor fluctuation for the initial 10–20 ns and then got stable afterwards. The RMSD value was calculated with respect to the initial conformations of complex. The simulation results effectively confirms the protein's and its complex's excellent stability in an aqueous media. Further analysis of MD simulation was carried out by

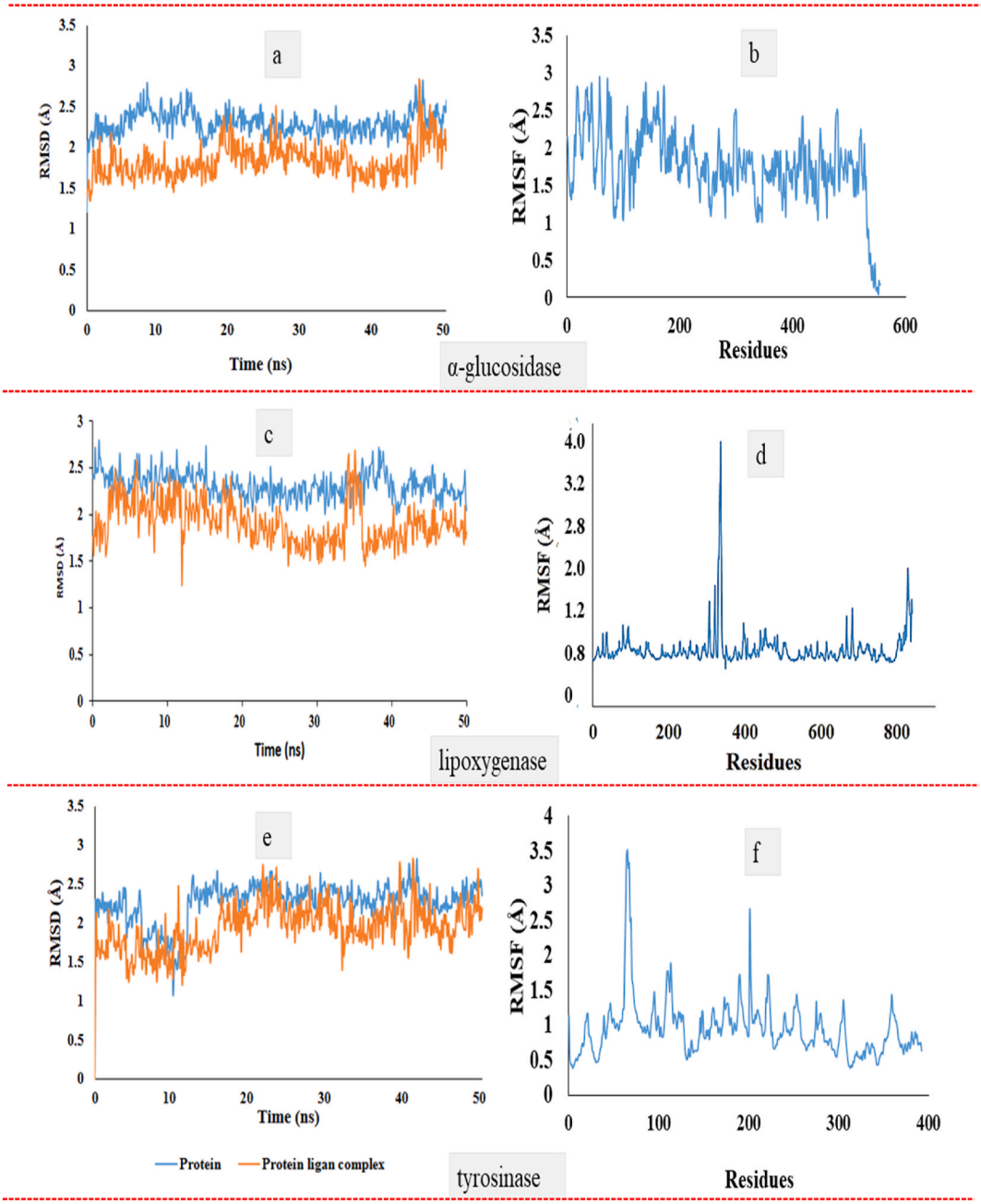

Fig. 4. (a) RMSD plots illustrating the dynamics of α-glucosidase and protein α-amyrone complex. (b) RMSF graph showing flexibility within the α-glucosidase protein. (c) RMSD plots illustrating the dynamics of lipoxygenase and protein α-amyrone complex. (b) RMSF graph showing flexibility within the lipoxygenase protein. (e) RMSD plots illustrating the dynamics of tyrosinase and protein α-amyrone complex. (f) RMSF graph showing flexibility within the tyrosinase protein.

generating RMSF plots, and the results are presented in Fig. 4(a–f). Any fluctuation in the amino acid residues of C and N terminal lobe in target protein can be identified by RMSF graphs. The average RMSF value for C_α chain with majority of amino acid residues of α-glucosidase, lipoxygenase and tyrosinase protein were found to be about 3.2 Å, 3.8 Å and 2.5 Å which is presented in the RMSF graph. The very few residues of the protein experienced small variations, which could be related to the hanging position. The RMSF data further established the stability of α-glucosidase, lipoxygenase and tyrosinase proteins and its complex in aqueous conditions. The RMSD and RMSF plots of all the targeted proteins and its complex is presented in Fig. 4(a–f) below.

In addition to this, the solvent accessible nm^2 surface area (SASA) of all the targeted proteins was also studied. This analysis has long been regarded as a key variable in research on protein folding and stability studies. It is described as the surface characterized by a

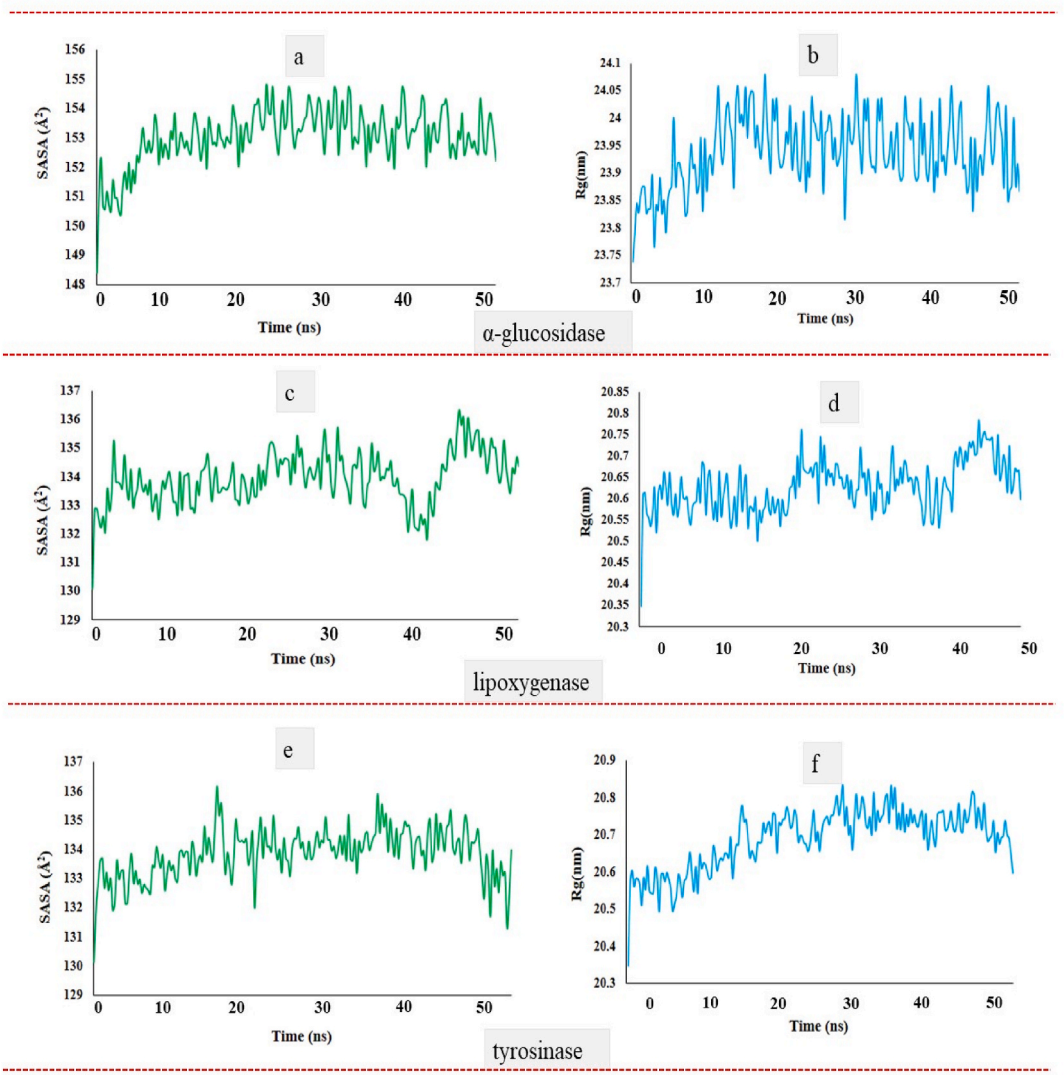

Fig. 5. (a) Time-course SASA plot depicting solvent exposure regions of the α-glucosidase protein. (b) Radius of Gyration (Rg) trajectory of the α-glucosidase protein (c) Time-course SASA plot depicting solvent exposure regions of the lipoxygenase protein. (d) Radius of Gyration (Rg) trajectory of the lipoxygenase protein (e) Time-course SASA plot depicting solvent exposure regions of the tyrosinase protein (f) Radius of Gyration (Rg) trajectory of the tyrosinase protein.

hypothetical solvent sphere's center and the molecule's van der Waals contact surface all around a protein. The results of MD simulations confirms the stability of targeted protein with average SASA value of 152 Å, 134 Å and 132 Å, respectively. The analysis of SASA reveals the uniform solvent dynamics with slight fluctuation at the end of simulation trajectory indicating the solvent penetration into specific cavities or binding pockets of the targeted protein (Bilal, 2022).

Similarly, the mass-weighted RMS distance of atomic cluster from their common center of mass is known as Radius of gyration (Rg). In MD simulation studies Rg is an important factor in finding the compactness of protein. Rg value of each protein complex was found to be slightly fluctuating throughout the simulation length, showing the flexibility of residues with an average value of 23.6 Å, 21.7 Å and 19.5 Å, respectively. The SASA analysis and radius of gyration results are shown in given below in Fig. 5(a–f).

3.5. ADME analysis of selected phytoconstituents with best docking score

The 11 phytoconstituents from TPM and 13 phytoconstituents from TPD were selected in terms of maximum binding affinity (best docking score), then were analysed further for ADME analysis by using SWISS ADME online software. This online tool may provide information about the pharmacokinetics, physicochemical properties and drug likeness attributes of the selected phytoconstituents with best docking scores. Lipinski's Rule of five states that when any phytoconstituent or drug fails to fulfil the two or more rule of Lipinski's Rule of Five, it may consider a non-oral drug or phytoconstituents.

Table 9
ADME analysis of selected phytoconstituents with high docking score of TPM of *T. peruviana.*

Sr. No.	Phytocompounds	Physicochemical Properties				Lipophilicity	Lipinski's Rule
		HBD	HBA	MWT	MR		
1	Campesterol	1	1	400.68	128.42	6.54	Yes, 1 violation
2	Stigmasterol	1	1	412.69	132.75	6.62	Yes, 2 violations
3	Ursa-9(11),12-dien-3-ol	1	1	424.70	134.67	6.82	Yes, 2 violations
4	gamma.-Sitosterol	1	1	414.71	133.23	6.73	Yes, 2 violations
5	beta-Amyrin	1	1	426.72	134.88	6.92	Yes, 2 violations
6	Lupeol	1	1	426.72	135.14	4.68	Yes, 1 violation
7	alpha.-Amyronee	0	1	424.70	134.18	6.82	Yes, 2 violations
8	Olean-12-en-3-ol, acetate, (3.beta)	0	2	468.75	144.62	7.08	Yes, 2 violations
9	Urs-12-en-24oic acid,3-oxo-,methyl ester	0	3	468.71	140.27	4.63	Yes, 1 violations
10	Epilupeol; 20(29)-Lupen-3αol,acetate (isomer 1)	0	2	468.75	144.88	7.08	Yes, 2 violations
11	Lup-20(29)-en-3-ol, acetate,ta.)	0	2	468.75	144.88	7.08	Yes, 2 violations

HBD; hydrogen bond doners, HBA; hydrogen bond acceptors, MWT; molecular weight, M.R; molar refractivity.

Table 10
ADME analysis of selected phytoconstituents with high docking score of TPD of *T. peruviana.*

Sr No.	Phytocompounds	Physicochemical Properties				Lipophilicity	Lipinski's Rule
		HBD	HBA	MWT	MR		
1	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-	1	1	394.63	127	6.24	Yes, 1 violation
2	Campesterol	1	1	400.68	128.42	6.54	Yes, 1 violation
3	Stigmasterol	1	1	412.69	132.75	6.62	Yes, 2 violations
4	Ursa-9(11),12-dien-3-ol	1	1	424.70	134.67	6.82	Yes, 2 violations
5	gamma.-Sitosterol	1	1	414.71	133.23	6.73	Yes, 2 violations
6	Stigmasta-5,24(28)-dien-3-ol, eta.,24Z)-	1	1	412.69	132.75	6.62	Yes, 2 violations
7	beta.-Amyrin	1	1	426.72	134.88	6.92	Yes, 2 violations
8	Lup-20(29)-en-3-one	0	1	424.70	134.18	6.82	Yes, 2 violations
9	24-Noroleana-3,12-diene	0	0	394.68	128.70	7.70	Yes, 1 violation
10	Stigmasta-4,24(28)-dien-3-one, E)-	0	1	410.67	131.79	4.67	Yes, 1 violation
11	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	0	3	468.71	140.27	5.95	Yes, 2 violations
12	Epilupeol; 20(29)-Lupen-3αol, acetate (isomer 1)	0	2	468.75	144.88	7.08	Yes, 2 violations
13	11-Oxo-.beta.-amyrin	1	2	440.70	135.08	5.89	Yes, 2 violations

HBD; hydrogen bond doners, HBA; hydrogen bond acceptors, MWT; molecular weight, M.R; molar refractivity.

In the current study, ADME analysis of selected phytoconstituents of TPM and TPD revealed that all selected phytoconstituents from TPM (violate two rules) except campesterol, lupeol and Urs-12-en-24oic acid,3-oxo-,methyl ester (violate one rule) and from TPD, all selected phytoconstituents (violate two rules) except Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-, Campesterol, 24-Noroleana-3,12-diene, and Stigmasta-4,24(28)-dien-3-one, E)- respectively, as described in Table 9 and Table 10. All the selected and analysed phytoconstituents appropriate and suitable for oral administration and possess orally active drug likeness features.

Campesterol, lupeol and Urs-12-en-24oic acid,3-oxo-,methyl ester from TPM and Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-, Campesterol, 24-Noroleana-3,12-diene, and Stigmasta-4,24(28)-dien-3-one, E)- from TPD are best because these phytoconstituents only violate one rule. The oral drug delivery system provide marvellous safety, patient compliance, avoidance of pain and have various advantages over different routes of drug administration system. Tables 9 and 10, describes the different physicochemical properties such as lipophilicity, pharmacokinetic behaviour, no. of bond rotations, molecular weight and no. of hydrogen bond donor and acceptor of selected and analysed phytoconstituents. Fig. 6 and Fig. 7 describe the bioavailability radar of selected phytoconstituents from TPM and TPD respectively. In the literature, there is no reported literature available regarding ADME analysis of *T. peruviana*.

The toxicity of the phytoconstituents were virtually predicted using online tool ADMETlab 2.0. The reference for the evaluation for toxicity was taken from ADMETlab 2.0 that mentions empirical decision of 0–0.3 as excellent toxicity, 0.3 to 0.7 as moderate toxicity, and 0.7 to 1 as poor toxicity (James, 2023). All compounds choosen from TPD showed skin sensization and carcinogencity ranging from 0.1 to 0.3, in case of eye irritation all compounds showed eye irritation except beta-Amyrin and 11-Oxo-beta-amyrin which showed moderate toxicity, Stigmasterol exhibited excellent respiratory toxicity and remaining compounds showed moderate and poor respiratory toxicity as shown in Table 11.

All compounds choosen from TPM showed excellent skin sensization and carcinogenicity ranging from 0.1 to 0.3, β-Amyrin and α-amyrone showed moderate eye irritation and other compounds showed excellent eye irritation. Stigmasterol exhibited excellent respiratory toxicity, Campesterol, gamma-Sitosterol, Epilupeol; 20(29)-Lupen-3alpha-ol, acetate (isomer 1), and Lup-20(29)-en-3-ol, acetate,ta.) showed moderate respiratory toxicity and remaing compounds showed poor respiratory toxicity as shown in Table 12.

4. Conclusions

The current study investigated TPM and TPD extracts of *T. peruviana* for phytochemical investigation and biological potential. The

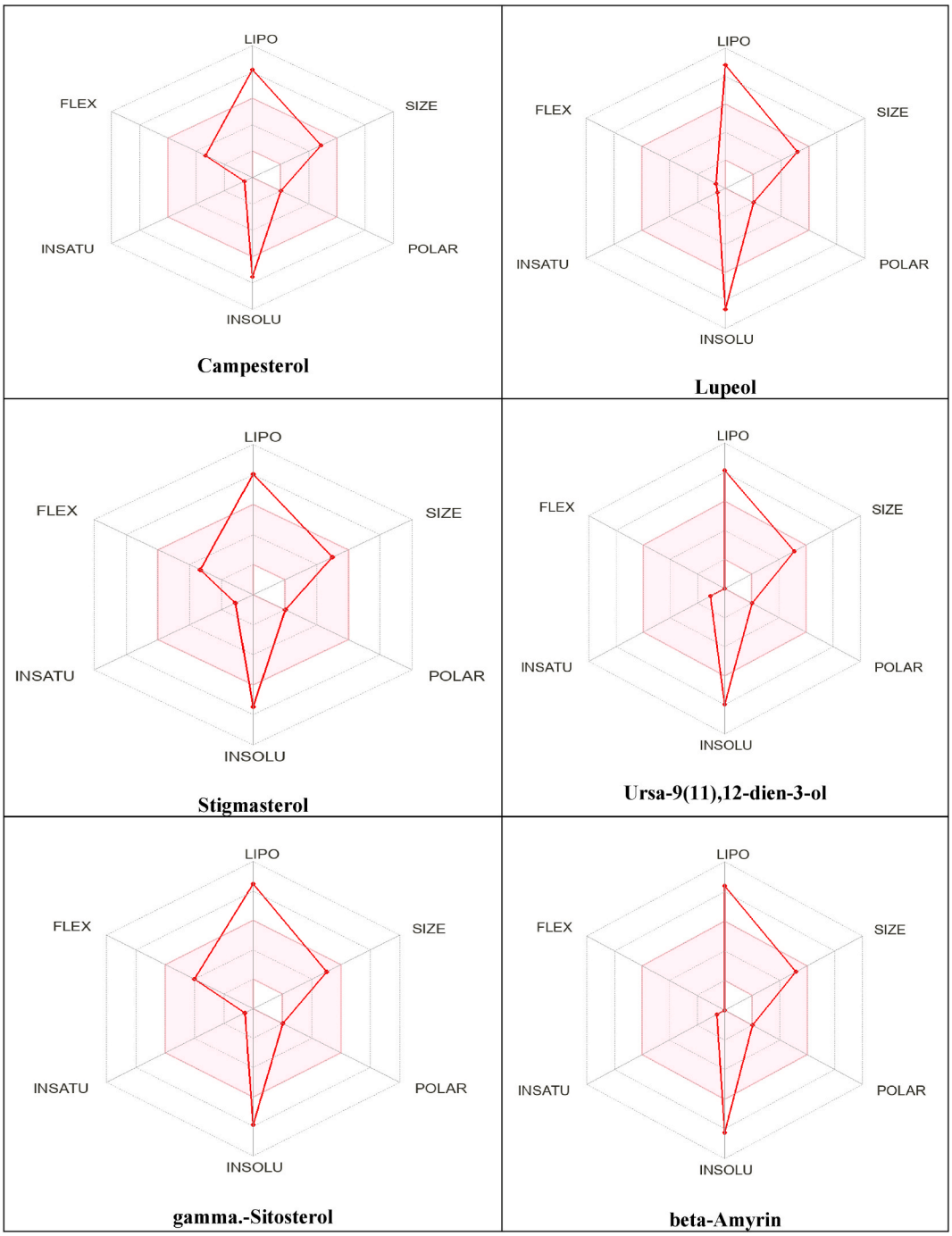

Fig. 6. Bioavailability radar of selected phytoconstituents from TPM.

comparative analysis revealed that TPM and TPD extracts contained the total phytochemicals, confirming the antioxidant, enzyme inhibition (tyrosinase, lipoxygenase and α-glucosidase) and anti-bacterial potential. The reported extract's GC-MS analysis revealed a tentative identification of the phytocompounds along with expected secondary metabolites. *T. peruviana* enzyme inhibitory potentials were supported further by *In-silico* molecular docking investigations. Furthermore, the MD stimulations studies in terms of RMSD, RMSF, RoG and SASA validated the most effective molecule with the highest binding affinity, α-amyrone, which was docked against α-glucosidase, lipoxygenase, and tyrosinase. The current study revealed the medicinal potential of *T. peruviana* bark, making its way to industrial exploitation and can be used as a future molecule for synthesis of new molecules that can be used in treatment of those

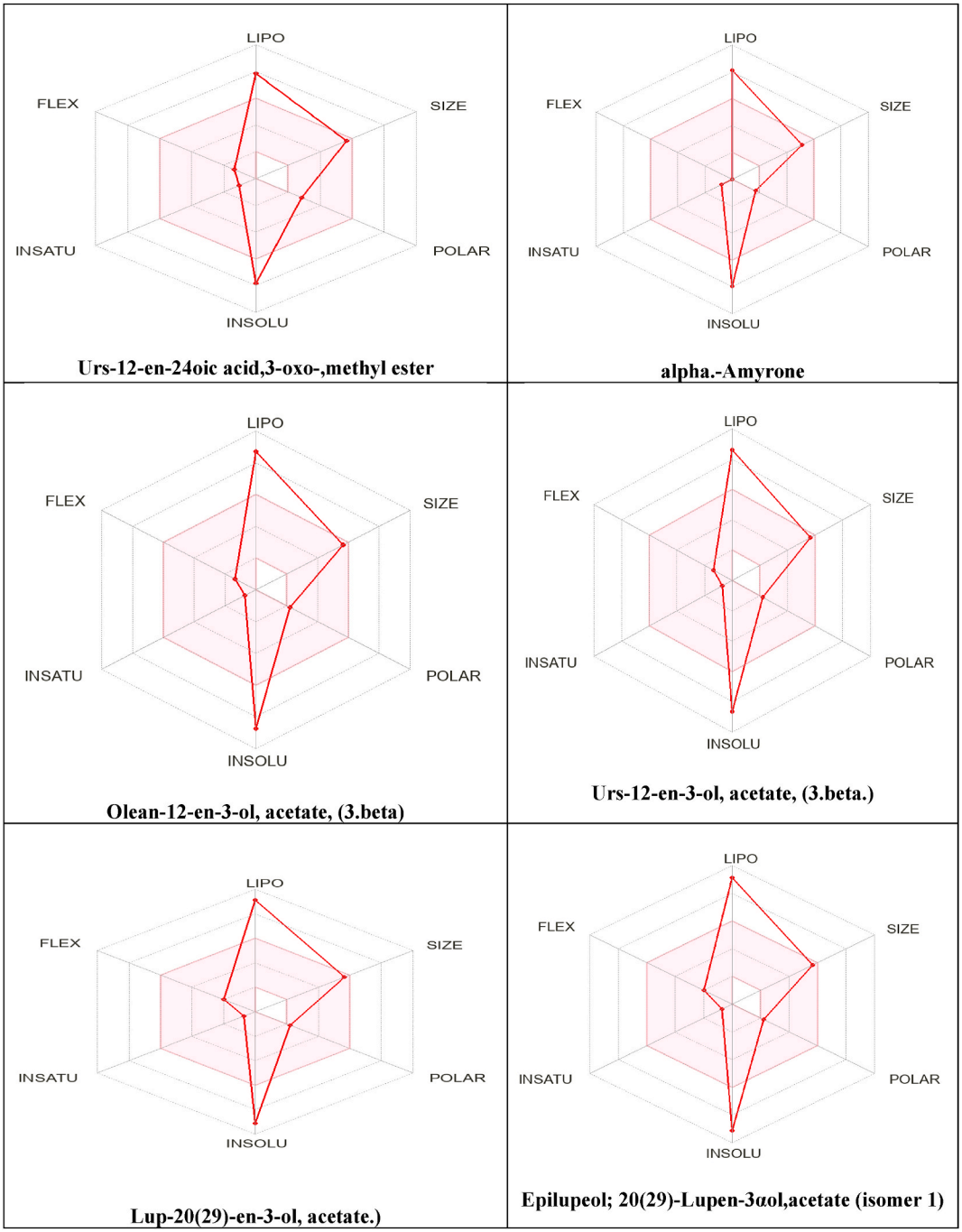

Fig. 6. (*continued*).

dieases where these targeted enzymes are involved. More studies targeted for isolation of novel bioactive phytoconstituents, and structural elucidation of pure phytochemicals especially α-amyrone from *T. peruviana* extract/fractions could be a potential candidate to serve as a lead for novel anti-diabetic, anti-inflammatory and antibacterial drugs. In addition to this, *In-vivo*, toxicological studies may be done for exploitation of the extracts in skin ailments, inflammatory disorders and diabetes.

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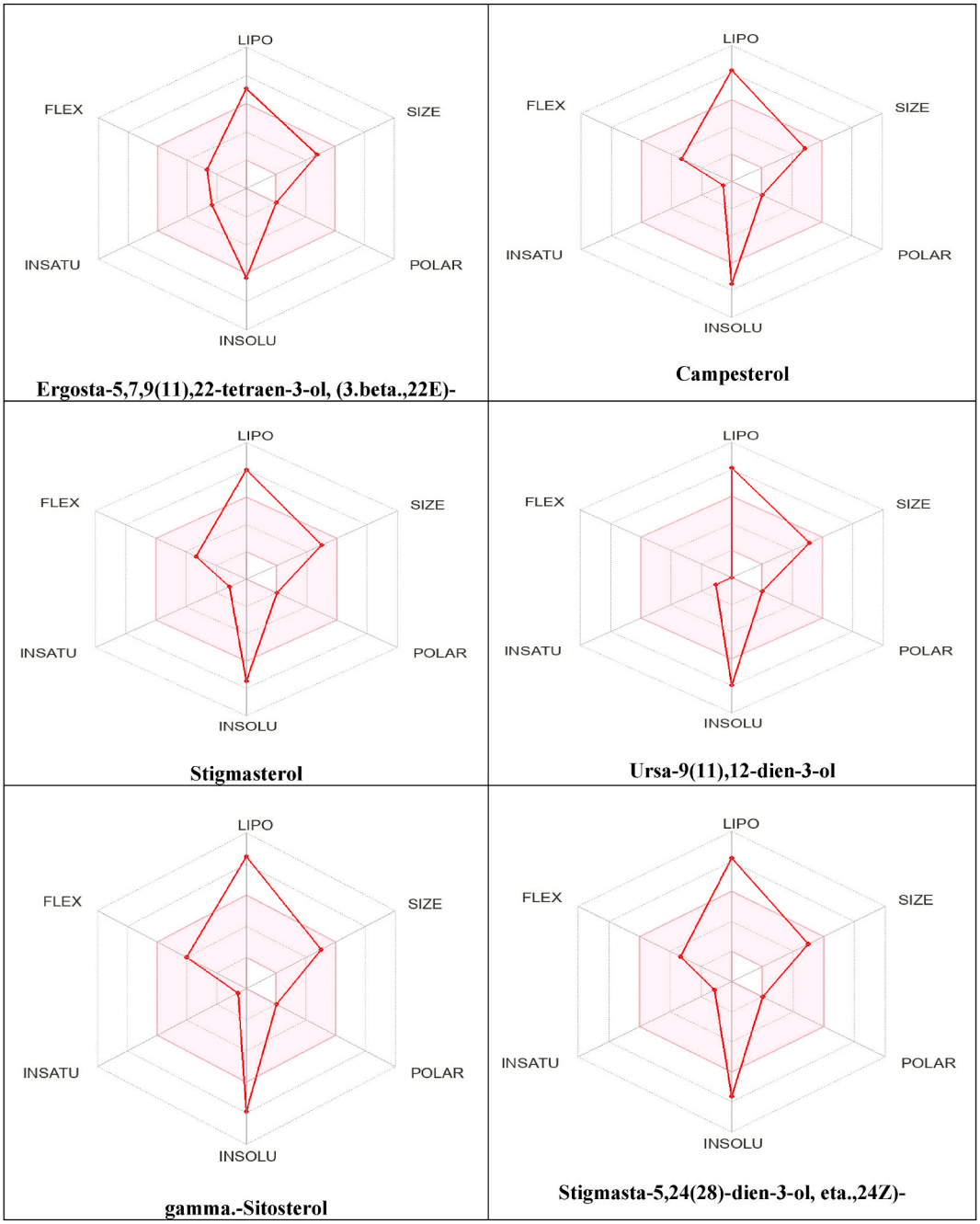

Fig. 7. Bioavailability radar of selected phytoconstituents from TPD.

Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

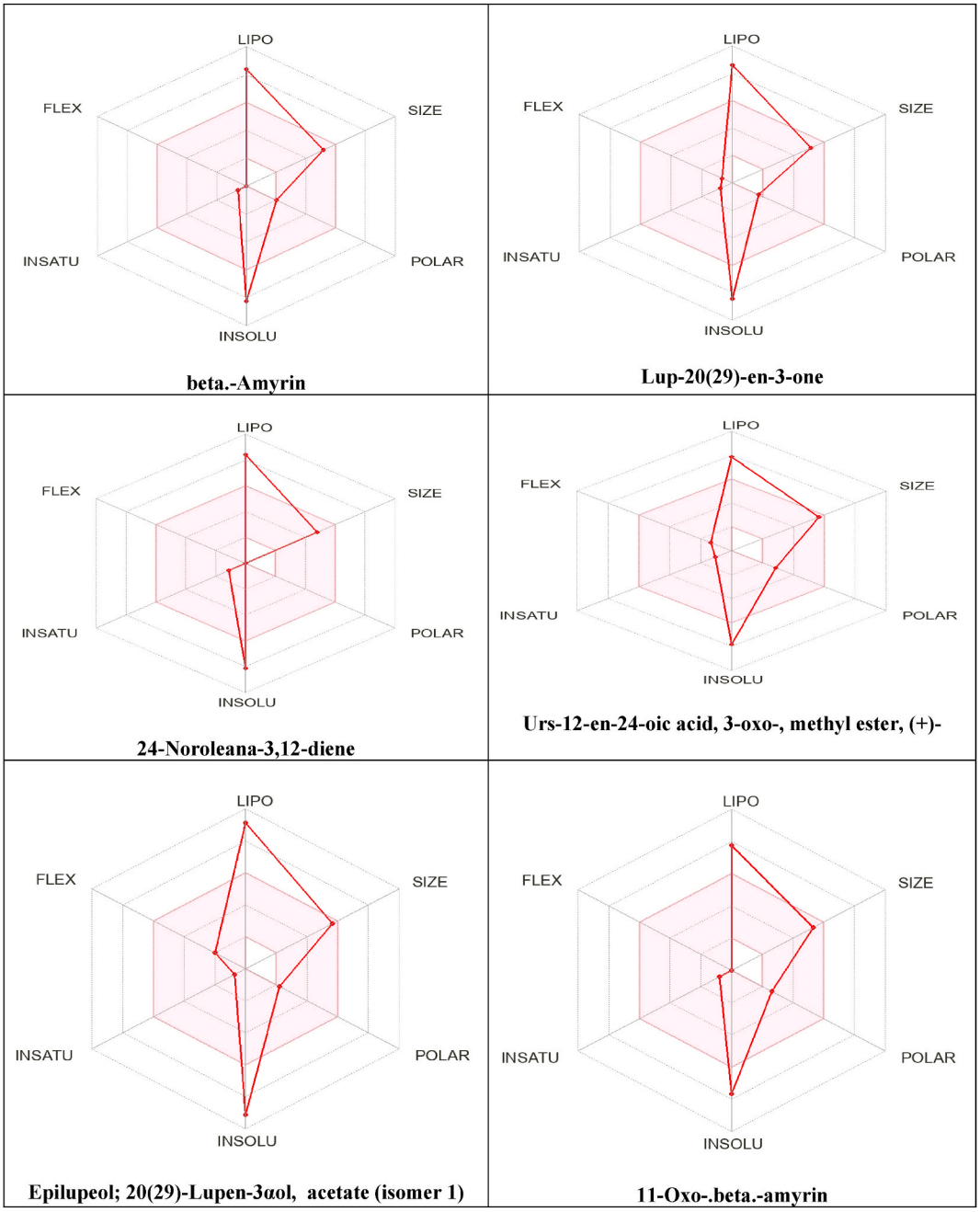

Fig. 7. (*continued*).

Data availability statement

All relevant research data have been included in the manuscript and supplementary file. No separate repository is applicable.

CRediT authorship contribution statement

Rao Anum Rehman Khan: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Samina Afzal:** Supervision, Project administration. **Hanan Y. Aati:** Funding acquisition. **Sultan Aati:** Funding acquisition, Data curation. **Huma Rao:** Writing – review & editing, Software, Investigation. **Saeed Ahmad:** Validation, Formal analysis. **Musaddique Hussain:** Visualization. **Kashif ur Rehman Khan:** Visualization, Validation, Funding acquisition.

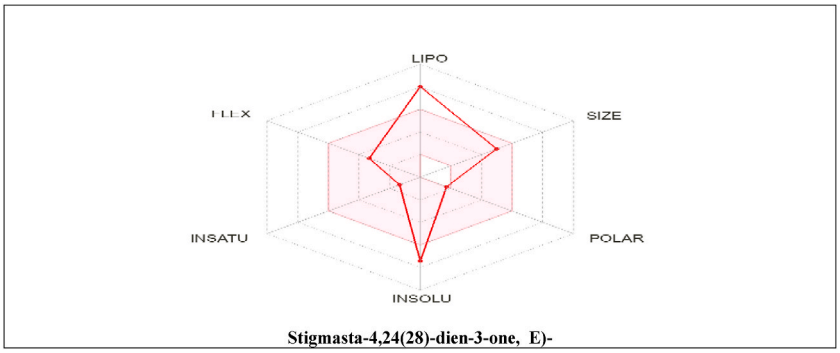

Fig. 7. (*continued*).

Table 11
Toxicity Evaluation of TPD extract of *T. peruviana.*

Sr#	Compound Name	Skin Sensization		carcinogencity		Eye Irritation		Respiratory Toxicity	
		Probability	Activity	Probability	Activity	Probability	Activity	Probability	Activity
1	Ergosta-5,7,9(11),22-tetraen-3-ol, (3. beta.,22E)-	0.1	ET	0.3	ET	0.1	ET	1.0	PT
2	Campesterol	0.1	ET	0.1	ET	0.1	ET	0.7	MT
3	Stigmasterol	0.1	ET	0.1	ET	0.1	ET	0.3	ET
4	Ursa-9(11),12-dien-3-ol	0.1	ET	0.1	ET	0.3	ET	1.0	PT
5	gamma.-Sitosterol	0.3	ET	0.1	ET	0.1	ET	0.7	MT
6	Stigmasta-5,24(28)-dien-3-ol, eta.,24Z)-	0.1	ET	0.3	ET	0.1	ET	0.5	MT
7	beta.-Amyrin	0.1	ET	0.1	ET	0.5	MT	1.0	PT
8	Lup-20(29)-en-3-one	0.1	ET	0.1	ET	0.3	ET	1.0	PT
9	24-Noroleana-3,12-diene	0.1	ET	0.1	ET	0.3	ET	1.0	PT
10	Stigmasta-4,24(28)-dien-3-one, E)-	0.1	ET	0.1	ET	0.1	ET	1.0	PT
11	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	0.1	ET	0.3	ET	0.3	ET	1.0	PT
12	Epilupeol; 20(29)-Lupen-3alpha-ol, acetate (isomer 1)	0.1	ET	0.1	ET	0.1	ET	0.7	MT
13	11-Oxo-.beta.-amyrin	0.1	ET	0.1	ET	0.7	MT	1.0	PT

ET; excellent toxicity, MT; moderate toxicity, PT; poor toxicity.

Table 12
Toxicity Evaluation of TPM extract of *T. peruviana.*

Sr#	Compound Name	Skin Sensization		carcinogencity		Eye Irritation		Respiratory Toxicity	
		Probability	Activity	Probability	Activity	Probability	Activity	Probability	Activity
1	Campesterol	0.3	ET	0.1	ET	0.1	ET	0.7	MT
2	Stigmasterol	0.1	ET	0.1	ET	0.1	ET	0.3	ET
3	Ursa-9(11),12-dien-3-ol	0.1	ET	0.1	ET	0.3	ET	1.0	PT
4	gamma.-Sitosterol	0.3	ET	0.1	ET	0.1	ET	0.7	MT
5	beta-Amyrin	0.1	ET	0.1	ET	0.5	MT	1.0	PT
6	Lupeol	0.3	ET	0.1	ET	0.1	ET	0.9	PT
7	alpha.-Amyronee	0.1	ET	0.1	ET	0.5	MT	1.0	PT
8	Olean-12-en-3-ol, acetate, (3.beta)	0.1	ET	0.1	ET	0.3	ET	1.0	PT
9	Urs-12-en-24oic acid,3-oxo-,methyl ester	0.1	ET	0.3	ET	0.3	ET	1.0	PT
10	Epilupeol; 20(29)-Lupen-3alpha-ol,acetate (isomer 1)	0.1	ET	0.1	ET	0.1	ET	0.7	MT
11	Lup-20(29)-en-3-ol, acetate,ta.)	0.1	ET	0.1	ET	0.1	ET	0.7	MT

ET; excellent toxicity, MT; moderate toxicity, PT; poor toxicity.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Huma Rao reports article publishing charges and writing assistance were provided by King Saud University. The authors declare that they have no conflict of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33151.

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