

# Identification of matrix metalloproteinases inhibitors from methanol extract of *Peltophorum pterocarpum* leaves

Bhimrao V. Jaiwal<sup>1\*</sup>, Faiyaz K. Shaikh<sup>2</sup>, Sarwan W. Bradosty<sup>3</sup>, Ajit B. Patil<sup>1</sup>, Raghavendra L. S. Hallur<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Ghulam Nabi Azad Art, Commerce and Science College, Akola, Maharashtra, India.

<sup>2</sup>Centre for Biotechnology, Pravara Institutes of Medical Sciences (Deemed to be University), Loni, Ahmednagar, Maharashtra, India.

<sup>3</sup>Department of Community Health, College of Health Technology, Cihan University-Erbil, Kurdistan Region of Iraq.

## ARTICLE INFO

### Article history:

Received on: January 26, 2023

Accepted on: May 06, 2023

Available online: June 04, 2023

### Key words:

MMP inhibitors,  
*Peltophorum pterocarpum*,  
HR-LCMS,  
<sup>1</sup>H NMR,  
ChC,  
MMP-2,  
MMP-9.

## ABSTRACT

Natural matrix metalloproteinases (MMP) inhibitors are novel therapeutic interventions for the management of ailments associated with anomalous expressions of MMPs. Annoying side effects of existing synthetic chemical MMP inhibitors direct us toward natural sources. In this regard, here active principles of MeOH extract of *Peltophorum pterocarpum* leaves are reported to be a possible source. Five active principles with MMP inhibitory activity were purified by chromatography and identified using high-resolution liquid chromatography-mass spectrometry and proton nuclear magnetic resonance techniques. The identified compounds were quinapril, 27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol, ergosterol acetate, 6-exomrthylenesimvastatin, and flurandrenolide. *In vitro* solution assays were demonstrated that these compounds were effective in inhibition of *Clostridium histolyticum* collagenase, human recombinant MMP-2, and MMP-9. Unambiguously, quinapril exhibited the highest (98.86%) while flurandrenolide exhibited the lowest (65.36%) MMP-9 inhibition. Whereas, 27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol, and flurandrenolide showed 98.28% and 63.87% MMP-2 inhibition. Ergosterol acetate showed moderate inhibition toward both MMP-2 and MMP-9. Our results indicate that MeOH extract of *P. pterocarpum* leaves is enriched with compounds with MMP inhibitory potential and could be explored as therapeutic for the management of MMPs related disorders.

## 1. INTRODUCTION

Matrix metalloproteinases (MMPs) are proteolytic enzymes found to degrade extracellular matrix (ECM) proteins in the human body. They are Zn<sup>2+</sup> and Ca<sup>2+</sup> dependent endopeptidases comprising 25 different variants. Based on substrate specificity, MMPs are also named as gelatinases comprising MMP-2 and MMP-9, collagenases comprising MMP-1 and MMP-13, membrane MMPs, and stromelysins or MMP-3 [1]. These enzymes are synthesized as zymogens or proenzymes and found to activate on the extracellular membrane [2-4]. The MMPs in normal physiological conditions are involved in angiogenesis, embryogenesis, tissue repair, wound healing, and apoptosis [5,6].

Particularly, normal manifestation of gelatinases (MMP-2 and MMP-9) is considered vital for reproduction, growth, and development of human. The elevated level of these MMPs is found in several pathological conditions such as cardiovascular diseases, cancer, arthritis, pulmonary, and skin diseases [5,7,8]. Earlier studies indicated a correlation between the elevation of MMPs expression and the progression of cervical and

ovarian cancers [9-11]. MMP-2 and MMP-9 have been suggested to be linked with the development, invasion, and metastasis of colorectal cancer [12]. Inhibition of MMP-2 and MMP-9 (or gelatinase A and gelatinase B) is a probable way to suppress the growth of cancer and could be an attractive drug target for the treatment of cancer and other MMPs-related disorders [13-15]. Earlier plant-derived compounds with MMPs inhibitory activity have been found to suppress cancer growth without side effects [16-18]. Continuous efforts to identify such active principles from plants will provide natural drug candidates for the treatment of cancers and MMPs-related disorders.

*Peltophorum pterocarpum* (DC.) Baker ex. K. Heyne is an decorative plant with medicinal values. *P. pterocarpum* is distributed in the tropical region of the world. The bark of *P. pterocarpum* is used to treat dysentery, eye disease, muscular pains, toothache, muscular pains, and sores [19]. The flowers extract exhibits cardiogenic, antimicrobial, and antioxidative activity [20]. *P. pterocarpum* possesses analgesic and cytotoxic effects [21]. The plant extract is also used to treat insomnia and is found to induce good sleep [22]. The active principle compounds such as tannins, triterpenes, steroids, flavonoids, and glycoside were reported from *P. pterocarpum* [21,23]. Bergenin and C-glucoside isolated from *P. pterocarpum* were reported to have anti-inflammatory, anti-arthritis, anti-diabetic, anti-HIV, hepatoprotective, anti-arrhythmic, and neuroprotective activities [24-32].

### \*Corresponding Author:

Bhimrao V. Jaiwal, Department of Biochemistry, Ghulam Nabi Azad Art, Commerce and Science College, Akola - 444 001, Maharashtra, India.  
E-mail: [jaiwalbv@gmail.com](mailto:jaiwalbv@gmail.com)

In the present study, an effort has been made for bioactivity-guided fractionation of methanol extract from leaves of *P. pterocarpum* leading to the purification and identification of active principle compounds with MMP inhibitory activity. Inhibitory assays against MMP-2 and MMP-9 (human recombinant proteins) were executed by *in vitro* solution assay using azocasein as substrate.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Human recombinant MMP-2 and MMP-9, azocasein, and trypsin inhibitor were purchased from Sigma Aldrich, trichloroacetic acid (TCA), silica-gel (mesh size 60–120), NaOH, Tris-buffer, X-ray films, calcium chloride, methanol, chloroform, hexane, and ethyl acetate were obtained from RANKEM, thin layer chromatography (TLC) plates (silica gel 60 F254) procured from Merck, Germany. Purification of compounds was done by high-performance liquid chromatography (HPLC) (Agilent Technologies, Eclipse XDB-C18). The chemicals and reagents used in this investigation were of analytical grade.

### 2.2. Purification of Bioactive Compounds from *P. pterocarpum* Leaves

*P. pterocarpum* leaves were collected in the campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad - 431 004 (MS), India. The leaves of *P. pterocarpum* were rinsed with distilled water twice and dried at room temperature. Using Mixer Grinder dried leaves (2.25 kg) leaves were crushed to fine powder. For preliminary fractionation, the powder was sequentially soaked in n-hexane, chloroform (CHCl<sub>3</sub>), ethyl acetate (EtOAc), and methanol (MeOH) in the ratio of 1:10 w/v. All extracts were incubated at room temperature (27–28°C) for 10–12 h with recurrent shaking.

The extracts were filtered by cellulosic filter papers and filtrates were further used as the crude source of MMPs inhibitors. On preliminary screening, only ethyl acetate and methanol extracts were found to have inhibitory activity against *Clostridium histolyticum* collagenase (ChC, sequence similarities to human MMPs) on X-ray film by dot blot assay. MeOH extract exhibited prominent activity and was used for further investigation.

MeOH was evaporated from MeOH extract to concentrate active principles and the final weight of precipitate was 125 g. Half of the MeOH precipitate was dissolved in 1.5 lit of MeOH: CHCl<sub>3</sub> (1:9 v/v) mixture for further fractionation, stirred vigorously, and filtered through Whatman filter paper. The solvents from the filtrate were evaporated at their boiling points and 10.4 g of gummy greenish precipitate was obtained. This precipitate was designated as A fraction and subjected to silica gel (mesh size 100–200) column chromatography by applying increasing order of linear gradient of MeOH in CHCl<sub>3</sub>. Eleven fractions (A1 to A11) were collected and the fractions A1, A2, and A3 were reported to have ChC inhibitory activity. Fraction A3 showing good ChC inhibitory activity (1.94 g) was subjected to silica gel column chromatography and 05 sub-fractions (A3 (1) to A3 (5)) were eluted and analyzed for ChC inhibitory activity. The sub-fraction A3 (4) showed high ChC inhibitory activity further applied to TLC analysis and 03 active principle compounds (compounds 1, 2, 3) were observed. These three active principle compounds were further purified by preparative TLC using a mobile phase MeOH: CHCl<sub>3</sub> (2: 8 v/v).

The remaining half of the MeOH extract (powder precipitate, which was not soluble in MeOH: CHCl<sub>3</sub> 1:9 v/v) was subjected to solid-liquid fractionation with rising order of linear gradient of MeOH in

dichloromethane (DCM) in the ratios 2:8 (B fraction), 4:6 (D fraction), 6:4 (E fraction), 8:2 (F fraction), and 10:0 (G fraction) v/v. On screening, fractions F and G showed ChC inhibition. The fraction F was selected for further purification of active principles and applied to silica gel (mesh size 60–120) column chromatography using a linear gradient of non-polar to polar solvents CHCl<sub>3</sub>, MeOH, and H<sub>2</sub>O. A total of seven (F1–F7) fractions were collected from the column analyzed for ChC inhibition and fractions F5, F6, and F7 were positive. These fractions were pooled together and subjected to preparative TCL and two active principle compounds (compounds 4 and 5) were purified.

All five purified compounds (compounds 1-3 of MeOH and compounds 4-5 MeOH: DCM) were further subjected to HPLC (Agilent Technologies, Eclipse XDB-C18) for the removal of small impurities. After purification, these bioactive compounds were identified by HPLC-MS and proton nuclear magnetic resonance (<sup>1</sup>H NMR).

### 2.3. Dot Blot Assay on X-ray Film

For preliminary screening of MMP inhibitory activity, a dot blot assay or spot test on gelatin-coated X-ray film was performed as described earlier [33,34]. The reaction mixture tubes were contain 5 µL 200 µg/mL ChC (prepared in 50 mM CaCl<sub>2</sub> containing 50 mM Tris-HCl buffer pH 7.5), 5 µL 100 mM NaCl, 5 µL buffer, 5 µL of the MeOH extract incubated at 37°C for 10 min. With this one tube was prepared without a MeOH extract and treated as a control. After incubation, tube contents were loaded on X-ray film separately and incubated at 37°C for 15 min. The resulting X-ray film was rinsed with distilled water and air dried. ChC and ChC inhibitors (MMP inhibitors) produced altered patterns of gelatin degradation depending on the potency of the inhibitor, which may be witnessed visually and the resulting X-ray film scanned at 300 dpi using an EPSON scanner.

### 2.4. *In Vitro* Inhibition Assay of Bioactive Compounds against MMP-2 and MMP-9

*In vitro* inhibition of MMP-2 and MMP-9 by purified compounds (**compounds 1-3** of MeOH and **compounds 4-5** MeOH: DCM) was performed using protein substrate azocasein separately [34,35]. The MMP-2 and MMP-9 activation was performed by incubating Pro-MMP-2 and Pro-MMP-9 with 0.01 mg/mL trypsin distinctly at 37°C for 1 h. Proteolytic action of trypsin was ceased using 100 µg/mL trypsin inhibitor. In the reaction mixture, 20 µL (120 ng) activated MMP-2 and 20 µL (100 ng) activated MMP-9 (separately), 160 µL buffer (50 mM Tris-HCL pH 7.5, 1 mM CaCl<sub>2</sub>, 100 mM NaCl and 0.05% Brij-35) and 20 µL (100 µg/mL) purified compound were taken in 1.5 mL centrifuge tubes. After 10 min of incubation at 37°C, 50 µL azocasein (1% w/v) was added to the reaction mixture and incubated for 5 h at 37°C. After incubation, the reaction was stopped by adding 60 µL 5% TCA. TCA soluble peptides (supernatants) were collected by centrifugation at 10,000 rpm for 10 min. About 150 µL supernatant and an equal volume of 1 N NaOH were taken in a microtiter plate. The absorbance was recorded using a microtiter plate reader at 405 nm. Percent MMP-2 and MMP-9 inhibitions were calculated by given that the absorbance of control as 100% MMP-2 and MMP-9 activities separately.

### 2.5. Identification of Bioactive Compounds

Identification of compounds by high-resolution liquid chromatography-mass spectrometry (HR-LCMS) was carried out at SAIF, IIT Bombay Pawai (MS) India as described previously [36]. Specifically analysis was performed by liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-

QTOF-MS/MS) Agilent Technologies 6550 i-Funnel system equipped with a G4220B pump, G4226A autosampler and G1316C, and a diode array detector. The  $^1\text{H}$  NMR spectrum was taken on a Bruker AMX 500 ( $^1\text{H}$ , 500 MHz) spectrometer at Vishnu chemicals laboratories Pvt. Ltd. Hyderabad (MS) India. Metlin database was utilized to analyze structural confirmation.

## 2.6. Statistical Analysis

MMP inhibitor assays of active principle compounds were conducted with three replicate. Experimental data were analyzed using Microsoft excel 2010. All measured variables were analyzed by one-way analysis of variance (ANOVA) and the variances between the means were related at  $\alpha$  value 0.05. The results are presented in the form of bar diagram using mean value  $\pm$  standard error.

## 3. RESULTS AND DISCUSSION

MMPs sometimes known as matrixins are responsible for the degradation, repair, and remodeling of ECM components. Although, elevated levels of MMPs are observed in various pathological conditions such as cardiovascular diseases, cancer, arthritis, pulmonary disease, and skin diseases. MMP-2 and MMP-9 are predominant matrixins expressions found to be elevated in various kinds of cancer [12]. The exogenous inhibitors of MMP-2 and MMP-9 of natural origin are drug candidates for the treatment of cancer and other MMPs-related disorders [15]. In this perspective, numerous compounds, namely, flavonoids, alkaloids, and phenolic compounds from plants have shown a promising ability to inhibit MMPs [16-18]. These natural compounds are found to be effective to control MMP-related disorders whereas chemically synthesized inhibitors such as marimastat, doxycycline, and trocade exhibit unwanted side effects [12,37,38]. In the present investigation, we are proposing MeOH extract of *P. pterocarpum* leaves as a possible source of bioactive compounds with MMP-2 and MMP-9 inhibitory potentials.

### 3.1. Purification of Active Principle Compounds

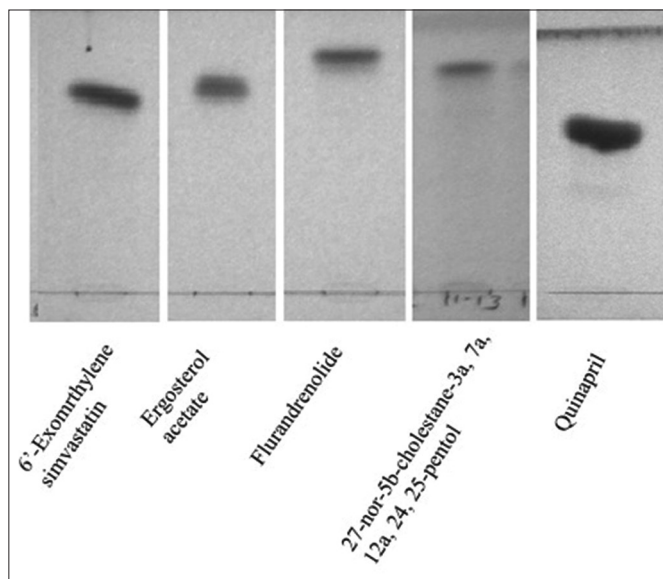
Active principle compounds from MeOH extract of *P. pterocarpum* leaves were purified based on bioactivity assay guided fractionation using silica gel column and TLC. The potential of MMP inhibition was checked at every stage of purification. The fraction with good inhibition toward bacterial ChC was selected. The bacterial ChC shows functions and amino acid sequence similarities with human MMPs [39]. The purity of isolated compounds was assessed by TLC, a single spot appears on the chromatogram when observed [Figure 1].

### 3.2. Structural Properties of Active Principle Compounds

The structures of all purified active principle compounds of methanolic extract of *P. pterocarpum* leaves are depicted in Figure 2. The structural features assessed by HR-LCMS and  $^1\text{H}$  NMR are summarized.

#### 3.2.1. Compound 1

*6-Exomrthylenesimvastatin* was obtained as a whitish colored compound with the molecular formula ( $\text{C}_{25}\text{H}_{36}\text{O}_5$ ) as deduced by HR-LCMS with a matching spectrum to the  $^1\text{H}$  NMR. Positive-ion ESI MFE  $m/z$  416  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (DMSO) Bruker-300MHz,  $\delta$  (ppm): 6.96 (s, 2H), 5.89 (s, 2H), 4.96 (d,  $J = 10.5\text{Hz}$ , 2H), 3.99 (t,  $J = 9.6$ , 2H), 3.85 (d,  $J = 10.2\text{Hz}$ , 2H), 3.76 (s, 6H), 3.66 (t,  $J = 8.7\text{Hz}$ , 3H), 3.50–3.55 (m, 4H), 3.39–3.46 (m, 7H), 3.22 (t,  $J = 9.3$  and  $2.4\text{Hz}$ , 4H), 1.65 (s, 2H).



**Figure 1:** Demonstration of bioactive compounds of methanol extract of *Peltophorum pterocarpum* leaves on thin layer chromatography plate.

#### 3.2.2. Compound 2

*Ergosterol acetate* was obtained as whitish colored compound and its molecular formula ( $\text{C}_{32}\text{H}_{50}\text{O}_2$ ) was determined by HR-LCMS with a matching spectrum to the  $^1\text{H}$  NMR. Positive-ion ESI MFE  $m/z$  466.74  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (DMSO) Bruker-300 MHz,  $\delta$  (ppm): 6.63–6.73 (m, 1H), 5.81–5.83 (m, 1H), 5.72 (s, 1H), 4.09–4.20 (m, 1H), 3.72–3.89 (m, 6H), 3.66 (d,  $J = 10.8\text{Hz}$ , 3H), 3.48–3.57 (m, 3H), 3.43 (s, 3H), 2.80–3.19 (m, 5H), 1.84–2.19 (m, 5H), 1.60 (s, 3H), 1.23 (s, 5H), 1.13–1.17 (m, 2H), 1.00–1.11 (m, 6H), 0.93 (s, 2H), 0.87–0.91 (m, 3H).

#### 3.2.3. Compound 3

*Flurandrenolide* was obtained as whitish colored compound and a molecular formula was assigned as ( $\text{C}_{24}\text{H}_{33}\text{FO}_6$ ) by HR-LCMS with a matching spectrum to the  $^1\text{H}$  NMR. Positive-ion ESI MFE  $m/z$  436.23  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (DMSO) Bruker-300MHz,  $\delta$  (ppm): 3.73–3.76 (m, 6H), 3.17 (s, 5H), 2.54 (s, 4H), 2.01 (s, 1H), 1.83 (d,  $J = 7.5\text{Hz}$ , 2H), 1.56 (s, 3H), 1.23 (s, 4H), 1.15 (t,  $J = 7.2\text{Hz}$ , 2H), 1.00–1.06 (m, 2H), 0.90–0.93 (m, 1H), 0.85–0.86 (m, 2H), 0.75 (s, 1H).

#### 3.2.4. Compound 4

*27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol*: was obtained as whitish colored compound and its molecular formula ( $\text{C}_{26}\text{H}_{46}\text{O}_5$ ) was determined by HR-LCMS with a matching spectrum to the  $^1\text{H}$  NMR. Positive-ion ESI MFE  $m/z$  338.6  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (DMSO) Bruker-300MHz,  $\delta$  (ppm): 4.38–4.75 (m, 12H), 4.12 (s, 1H), 3.63 (s, 4H), 3.51–3.56 (m, 3H), 3.37–3.44 (m, 20H), 3.16 (s, 3H), 2.97–3.03 (t,  $J = 9.3\text{Hz}$ , 2H), 1.23 (s, 1H).

#### 3.2.5. Compound 5

*Quinapril* was obtained as whitish colored compound its molecular formula ( $\text{C}_{25}\text{H}_{30}\text{N}_2\text{O}_5$ ) was determined by HR-LCMS with a matching spectrum to the  $^1\text{H}$  NMR. Positive-ion ESI MFE  $m/z$  438.5  $[\text{M} + \text{H}]^+$ , 325.09  $[\text{M} + \text{Na}]^+$ .  $^1\text{H}$  NMR (DMSO) Bruker-300MHz,  $\delta$  (ppm): 8.40–9.30 (m, 5H), 7.97 (s, 1H), 6.77 (s, 3H), 6.34 (s, 2H), 5.50–5.90 (m, 2H), 4.36–4.73 (m, 6H), 3.63 (s, 3H), 3.44 (s, 4H), 3.03 (t,  $J = 9.3$  Hz, 2H), 1.91 (s, 1H), 1.23 (s, 1H).

### 3.3. ChC Inhibition by Active Principle Compounds

Purified active principle compounds were analyzed for ChC inhibition by dot blot assay on X-ray film. The principle of this method is that when we put ChC (or proteolytic enzymes) on gelatin-coated X-ray film, a transparent blue plastic sheet gets exposed indicating gelatin hydrolysis. This confirms proteinase activity in the control whereas in presence of an inhibitor a dark spot of unhydrolyzed gelatin appeared against the background [40]. We have visually confirmed that all purified active principle compounds exhibited ChC inhibition [Figure 3].

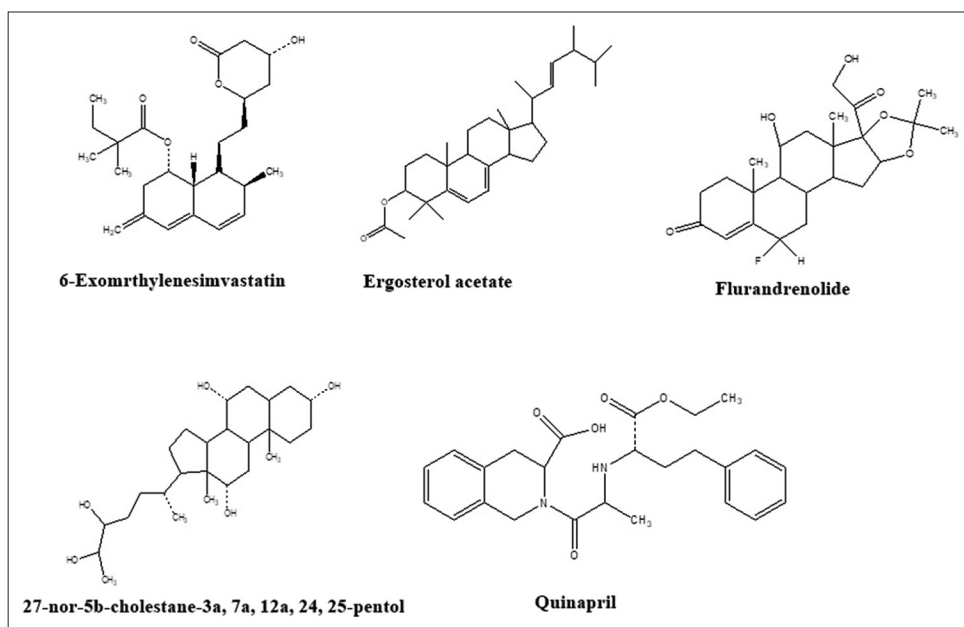
### 3.4. MMP-2 and MMP-9 Inhibition by Active Principle Compounds

Proteolytic activity of MMP-2 and MMP-9 and their inhibition by active principle compounds was assessed by *in vitro* solution assays. The one-way ANOVA analysis showed that all purified active principle compounds exhibited significant ( $P < 0.001$ ) inhibitory activities against MMP-2 and MMP-9 [Table 1]. We have observed that quinapril is a potent inhibitor of MMP-2 and MMP-9 displaying  $98.21\% \pm 4.3\%$  and  $98.86\% \pm 3.2\%$  inhibition, respectively. Flurandrenolide exhibited the lowest inhibitory potential against MMP-2 and MMP-

9. The 27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol exhibited the highest inhibitory potential (98.28%) while flurandrenolide exhibited the lowest inhibitory potential (63.87%) against MMP-2 [Figure 4]. Inhibitory potentials of all compounds against MMP-2 were observed in order of quinapril ( $98.21\% \approx 27$ -nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol ( $98.28\%$ ) > ergosterol acetate ( $80.68\%$ ) > 6-exomrthylenesimvastatin ( $70.38\%$ ) > flurandrenolide ( $63.87\%$ ) at  $100 \mu\text{g/mL}$ .

Inhibitory potentials of all compounds against MMP-9 were in order of quinapril ( $98.86\%$ ) > 27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol ( $83.85\%$ ) > ergosterol acetate ( $79.58\%$ ) > 6-exomrthylenesimvastatin ( $72.37\%$ ) > flurandrenolide ( $65.36\%$ ) at  $100 \mu\text{g/mL}$  [Figure 5].

Quinapril is an angiotensin-converting enzyme inhibitor and for the 1<sup>st</sup> time purified from *P. pterocarpum* leaves [41]. Ergosterol acetate, flurandrenolide, and 27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol are steroidal types of compounds. 6'-Exomrthylenesimvastatin is a statin-like compound. Earlier, it has been reported that the statins are capable to reduce the concentration of MMP-9 in the aortic wall of abdominal aortic aneurysm patients [42]. Simvastatin is a bioactive compound and exhibits anti-inflammatory and anti-atherosclerotic activities in carrageenan-induced female mice [43].



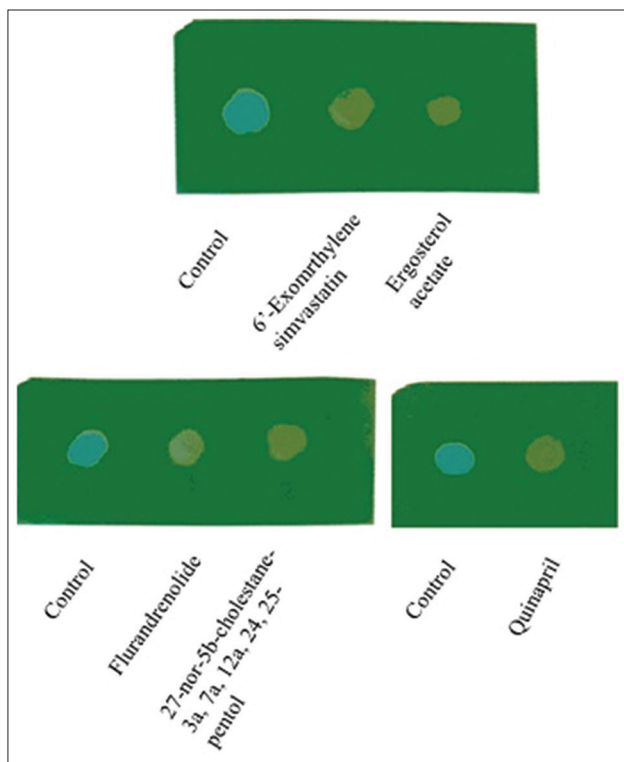
**Figure 2:** Structures of bioactive compounds of methanol extract of *Peltophorum pterocarpum* leaves.

**Table 1:** One-way ANOVA of the percent inhibition of MMP2 and MMP9.

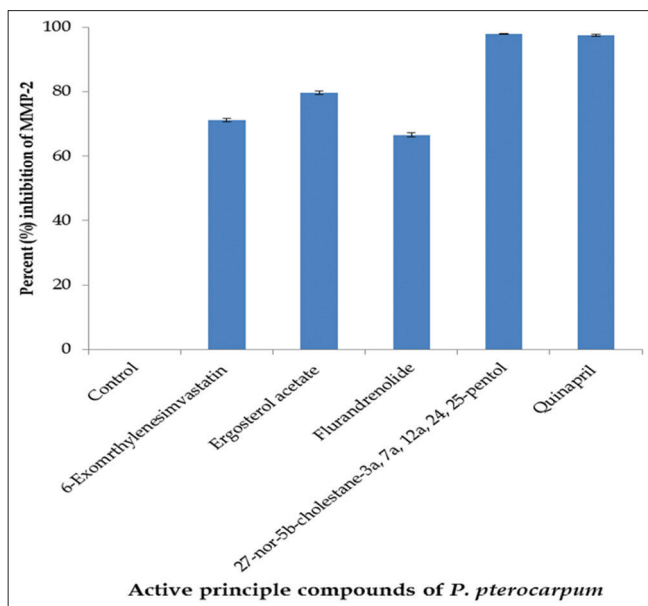
Active principle compounds	n	MMP2				MMP 9			
		Average	SE	F	P	Average	SE	F	P
Control	3	0	0	6974	<0.001	0	0	8374	<0.001
6-Exomrthylenesimvastatin	3	71.11	0.54			72.08	0.39		
Ergosterol acetate	3	79.63	0.53			78.90	0.43		
Flurandrenolide	3	66.59	0.61			66.20	0.53		
27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol	3	97.96	0.19			82.95	0.27		
Quinapril	3	97.55	0.35			96.02	0.34		

ANOVA: Analysis of variance, SE: Standard error.



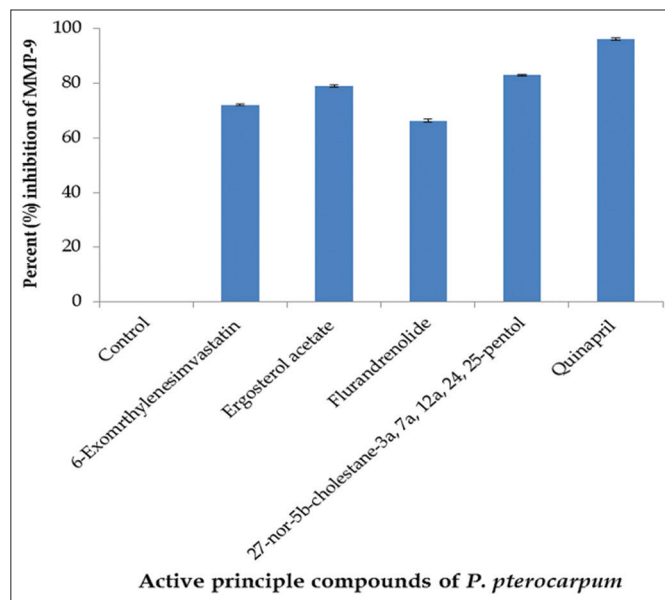


**Figure 3:** Assessment of bioactive compounds of methanol extract of *Peltophorum pterocarpum* leaves for ChC inhibition by dot-blot assay.



**Figure 4:** Assessment of bioactive compounds of methanol extract of *Peltophorum pterocarpum* leaves for MMP-2 inhibition by *in vitro* solution assay.

In nutshell, our results indicated that purified active principle compounds of MeOH extract of *P. pterocarpum* leaves display inhibitory activity against ChC, MMP-2, and MMP-9 could be investigated further in the future for designing or formulating natural drugs for cancer and MMP-related disorders.



**Figure 5:** Assessment of bioactive compounds of methanol extract of *Peltophorum pterocarpum* leaves for MMP-9 inhibition by *in vitro* solution assay.

#### 4. CONCLUSIONS

This research aimed to identify active principle compounds of MeOH extract of *P. pterocarpum* leaves with inhibitory potential against ChC and human recombinant MMP-2 and MMP-9. Five bioactive compounds were purified based on bioactivity assay-guided fractionation using silica gel column and TLC. These compounds were identified by HR-LCMS and <sup>1</sup>H NMR as quinapril, 27-nor-5b-cholestane-3a, 7a, 12a, 24, 25-pentol ergosterol acetate, 6-exomrthylene simvastatin, and flurandrenolide. All five active principle compounds showed inhibition against ChC, human recombinant MMP-2, and MMP-9 assessed by dot-blot assay and *in vitro* solution assays. The identified compounds from *P. pterocarpum* leaves could be investigated further in the future for designing or formulating natural drugs for the treatment of cancer and MMPs-relevant disorders.

#### 5. ACKNOWLEDGMENTS

The authors are grateful to IIT-Bombay, Mumbai (MS) India, and Vishnu chemicals Laboratory Pvt. Ltd. Hyderabad, India, for the analysis of compounds by HRLCMS and NMR.

#### 6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

#### 7. FUNDING

There are no funding sources to disclose.

## 8. CONFLICTS OF INTEREST

We declare that there is no conflicts of interest.

## 9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## 10. DATA AVAILABILITY

All data are available in the manuscript.

## 11. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

## REFERENCES

- Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: The good, the bad, and the ugly. *Circ Res* 2002;90:251-62.
- Birkedal-Hansen H. Proteolytic remodeling of extracellular matrix. *Curr Opin Cell Biol* 1995;7:728-35.
- Stetler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor cell interactions with the extracellular matrix during invasion and metastasis. *Annu Rev Cell Biol* 1993;9:541-73.
- Chakrabarti S, Patel KD. Matrix metalloproteinase-2 (MMP-2) and MMP-9 in pulmonary pathology. *Exp Lung Res* 2005;31:599-621.
- Dollery CM, McEwan JR, Henney AM. Matrix metalloproteinases and cardiovascular disease. *Circ Res* 1995;77:863-8.
- Zeng F, Cen S, Tang Z, Kang X. Elevated matrix metalloproteinase-9 expression may contribute to the pathogenesis of bladder cancer. *Oncol Lett* 2016;11:2213-22.
- Liotta LA, Tryggvason K, Garbisa A, Hart I, Foltz CM, Shafie S. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 1980;284:67-8.
- Fidler IJ. Molecular biology of cancer: Invasion and metastasis. In: De Vita VT, Hellman S, Rosenberg SA, editors. *Cancer: Principles and Practice of Oncology*. 5<sup>th</sup> ed. Philadelphia: Lippincott-Raven; 1997.
- Zhou C, Yao JF, Chen XD. Expression of matrix metalloproteinase-2, 9 and their inhibitor-TIMP 1, 2 in human squamous cell carcinoma of uterine cervix. *Ai Zhen* 2002;21:735-9.
- Zhu L, Zheng X, Du Y, Xing Y, Xu K, Cui L. Matrix metalloproteinase-7 may serve as a novel biomarker for cervical cancer. *Oncotargets Ther* 2018;11:4207-20.
- Lopata A, Agresta F, Quinn MA, Smith C, Ostor AG, Salamonsen LA. Detection of endometrial cancer by determination of matrix metalloproteinases in the uterine cavity. *Gynecol Oncol* 2003;90:318-24.
- Mook OR, Frederiks WM, Van Noorden CJ. The role of gelatinases in colorectal cancer progression and metastasis. *Biochim Biophys Acta* 2004;1705:69-89.
- Puricelli L, Dell'Aica I, Sartor L, Garbisa S, Caniato R. Preliminary evaluation of inhibition of matrix-metalloprotease MMP-2 and MMP-9 by *Passiflora edulis* and *P. foetida* aqueous extracts. *Fitoterapia* 2003;74:302-4.
- Mehlen P, Puisieux A. Metastasis: A question of life or death. *Nat Rev Cancer* 2006;6:449-58.
- Vartak DG, Gemeinhart RA. Matrix metalloproteases: Underutilized targets for drug delivery. *J Drug Target* 2007;15:1-20.
- Ferdinando M. Natural bio-drugs as matrix metalloproteinase inhibitors: New perspectives on the horizon? *Recent Pat Anticancer Drug Discov* 2006;1:91-103.
- Chowdhury A, Wei B, Noonan S, Stetler-Stevenson W. Preclinical development of recombinant human tissue inhibitor of metalloprotease-2 as a novel biologic therapy for cancer. *FASEB J* 2015;29:945-16.
- Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodriguez JF, *et al.* The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci* 2020;21:9739.
- Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement Altern Med* 2006;17:6-35.
- Bairi R, Vijaya C, Ramu A. Evaluation of cardiotoxic activity of *P. pterocarpum*. *J Phytopharmacol* 2011;2:1-6.
- Islam MS, Ali S, Rahman M, Islam R, Ali A, Azad AK, *et al.* Antidiabetic, cytotoxic activities and phytochemical screening of *Peltophorum pterocarpum* (DC.). K. Heyne root. *J Med Plants Res* 2011;5:3745-50.
- Nathan VK, Antonisamy JM, Gnanaraj WE, Subramanian KM. Phytochemical and bio-efficacy studies on methanolic flower extracts of *Peltophorum pterocarpum* (DC.) Baker ex Heyne. *Asian Pac J Trop Biomed* 2012;2:S641-5.
- Jain SC, Pancholi B, Jain R. *Peltophorum pterocarpum* (DC.) Baker ex. K. Heyne flowers: Antimicrobial and antioxidant efficacies. *Res J Med Plant* 2011;5:274-80.
- Swarnalakhshi T, Sethuraman MG, Sulochana N, Arivudainambi RA. Notice on the antiinflammatory activity of bergenin. *Curr Sci* 1984;53:917.
- De Oliveira CM, Nonato FR, De Lima FO, Couto RD, David JP, David JM, *et al.* Antinociceptive properties of bergenin. *J Nat Prod* 2011;74:2062-8.
- Chauhan R, Ruby K, Dwivedi J. Himalayan bergenia: A comprehensive review. *Int J Pharm Sci Rev Res* 2012;14:139-41.
- Kumar R, Patel DK, Prasad SK, Laloo D, Krishnamurthy S, Hemalatha S. Type 2 antidiabetic activity of bergenin from the roots of *Caesalpinia digyna*. *Rottler Fitoterapia* 2012;83:395-401.
- Piacente S, Pizzi C, De TN, Mahmood N. Constituents of *Ardisia japonica* and their *in vitro* anti-HIV activity. *J Nat Prod* 1996;59:565-9.
- Pu HL, Huang X, Zhao JH. Bergenin is the antiarrhythmic principle of *Fluggea virosa*. *Planta Med* 2002;68:372-4.
- Kim HS, Lim HK, Chung MW, Kim YC. Antihepatotoxic activity of bergenin, the major constituent of *Mallotus japonicus*, on carbon tetrachloride-intoxicated hepatocytes. *J Ethnopharmacol* 2000;69:79-83.
- Takahashi H, Kosaka M, Watanabe Y, Nakade K, Fukuyama Y. Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. *Bioorg Med Chem* 2003;11:1781-8.
- Maduka HC, Okoye ZS, Eje A. The influence of *Sacoglottis gabonensis* stem bark extract and its isolate bergenin, Nigerian alcoholic beverage additives, on the metabolic and haematological side effects of 2,4-dinitrophenyl hydrazine-induced tissue damage. *Vascul Pharmacol* 2002;39:317-24.
- Pichare MM, Kachole MS. Detection of electrophoretically separated protease inhibitors using X-ray film. *J Biochem Biophys Methods* 1994;28:215-24.
- Jaiwal BV, Shaikh FK, Patil AB, Hallur RL. Identification of three coagulins as MMP inhibitors from *Withania Coagulans* dunal fruits. *Biomedicine* 2022;42:929-37.
- Collette T, Bellehumeur C, Kats R, Maheux R, Mailloux J, Villeneuve M, *et al.* Evidence for an increased release of proteolytic activity by the eutopic endometrial tissue in women with endometriosis and for involvement of matrix metalloproteinase-9. *Hum Reprod* 2004;19:1257-64.
- Tekale SS, Jaiwal BV, Padul MV. Identification of metabolites from an active fraction of *Cajanus cajan* seeds by high resolution mass spectrometry. *Food Chem* 2016;211:763-9.
- Nelson AR, Fingleton B, Rotherberg ML, Matrisian LM. Matrix metalloproteinases: Biologic activity and clinical implications.

- J Clin Oncol 2000;18:1135-49.
38. Provenzano M, Andreucci M, Garofalo C, Faga T, Michael A, Ielapi N, *et al.* The Association of matrix metalloproteinases with chronic kidney disease and peripheral vascular disease: A light at the end of the tunnel? *Biomolecules* 2020;10:154.
  39. Eckhard U, Huesgen PF, Brandstetter H, Overall CM. Proteomic protease specificity profiling of clostridial collagenases reveals their intrinsic nature as dedicated degraders of collagen. *J Proteomics* 2014;100:102-14.
  40. Shaikh FK, Gadge PP, Shinde AA, Padul AA, Kachole MS. Characterization of the *AlTI13* protein from Indian siris (*Albizia lebbek*) that inhibit the growth of cotton bollworm (*Helicoverpa armigera*). *J Asia Pac Entomol* 2014;17:319-25.
  41. Gupta RK, Kjeldsen SE, Krause L, Kneisley J, Posvar E, Weder AB, *et al.* Hemodynamic effects of quinapril, a novel angiotensin-converting enzyme inhibitor. *Clin Pharmacol Ther* 1990;48:41-9.
  42. Evans J, Powell JT, Schwalbe E, Loftus IM, Thompson MM. Simvastatin attenuates the activity of matrix metalloproteinase-9 in aneurysmal aortic tissue. *Eur J Vasc Endovasc Surg* 2007;34:302-3.
  43. Carl PS, Charlotte AB, Melba H, Steven M, Heide H, Sushma P, *et al.* Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. *Arterioscler Thromb Vasc Biol* 2001;21:115-21.

**How to cite this article:**

Jaiwal BV, Shaikh FK, Bradosty SW, Patil AB, Hallur RLS. Identification of matrix metalloproteinases inhibitors from methanol extract of *Peltophorum pterocarpum* leaves. *J App Biol Biotech.* 2023;11(4):92-98. DOI: 10.7324/JABB.2023.109398