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Original Research Article

Identification of novel anticancer terpenoids from *Prosopis juliflora* (Sw) DC (Leguminosae) pods

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Abstract

Purpose: *To identify a novel source of terpenoid anticancer compounds from P. juliflora (Sw.) DC. (Leguminosae) pods as a medicinal substitute for cancer medicines.*
Methods: *The pods were collected, dried and pulverized. The ethanol extract was prepared by maceration. Various phyto-constituents were detected in the extract by UV-VIS spectroscopy at a wavelength ranging from 200 - 800 nm. The molecular formula, chemical structure, and percent peak area of these phyto-constituents were determined by gas chromatography-mass spectrometry (GC-MS). attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used for functional group determination of bioactive compounds.*
Results: *P. juliflora pods were rich in tannins, carotenoid and terpenoids. Nineteen bioactive compounds were detected. Out of these, thirteen are here reported for the first time with four of them exhibiting anticancer activities, while two belong chemically to terpenoids. Furthermore, FTIR established characteristic peaks for the various biologically-active functional groups.*
Conclusion: *The results show that P. juliflora pods is a valuable source of anticancer, antitumor and chemoprotective compounds, especially terpenoids, that can potentially be developed as alternatives to current painful and costly cancer therapies.*

Keywords: *Prosopis juliflora (Sw.) DC., Anticancer terpenoids, Attenuated total reflectance-FTIR, Herbal medicine*

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INTRODUCTION

Cancer (tumor or neoplasm) is a broad word used for a large group of diseases that can affect any part of the human body. Unfortunately, in low- and middle-income countries especially Pakistan and India, 70 % of deaths occur from cancer [1]. In 2012, breast, lip, oral cavity, lung and liver cancers were increased including Hepatitis and Human Papilloma Virus (HPV) resulting into 25 % of cancer cases in Pakistan [2]. According to WHO, the overall economic impact of cancer on low income countries is increasing. The total annual economic cost of cancer in 2010 was estimated at approximately US$ 1.16 trillion [3,4].

Medicinal plants have been used orally and as

herbal infusions to treat various ailments and serious diseases [5]. *P. juliflora* is an ornamental traditional medicinal tree. It grows in arid and semi-arid regions of Indo-Pak region and has been used as a folk remedy for catarrh, cold, diarrhea, dysentery and excrescences [6]. In different studies it has been revealed that its fruits contain terpenoids, phenol, saponin and tannins with proven pharmacological activities [7,9].

Plant terpenoids are extensively used for their aromatic qualities and have played a role in traditional herbal remedies in India and China [10,11].

Since the discovery of terpenes, more than 150 years ago, scientists have identified and isolated more than 50,000 terpenoids. But the source of medicinal terpenoids, was mainly fungi. In the current study, a plant source was used for the first time, to identify anticancer terpenoids. Identification and confirmation of natural compounds is a crucial and vital step for anticancer drug discovery. GC-MS is a very powerful technique towards the specific detection and potential identification of compounds. For detailed screening of terpenoids, ultraviolet–visible spectroscopy [12], attenuated total internal Reflectance-Fourier transform infrared spectroscopy for functional group detection and GC-MS were used [13].

EXPERIMENTAL

Plant material

The pods of *P. juliflora* were picked from Jinnah Garden, Lahore, Pakistan during summer season, 2017 and identified by a taxonomist, Dr Zubaida Yousaf (Department of Botany, Lahore College for Women University). A voucher specimen (no. LCWU-15-128) was deposited in Prem Madam Herbarium, Department of Botany, Lahore College for Women University, Lahore, Pakistan.

P. juliflora pods extraction

P. juliflora pods were cut, shade dried, and powdered for further use. Two grams of powdered pods were extracted with 50 mL of ethanol with gentle stirring for 72 h, filtered and crude extract was collected.

P. juliflora extract analysis

Freshly prepared extract was exposed to standard methods of phytochemical analysis to detect the constituents, viz. terpenoids, flavonoids, alkaloids, phenolics and glycosides [14]. In our previous experiment, we have also investigated the qualitative and quantitative terpenoid contents with high anti-oxidative potential [9,15].

For UV-VIS analysis, the extract was centrifuged at 2000 rpm (15 min) and processed through micro filters. The sample was diluted to 1:4 with ethanol. The extract was scanned using wavelength ranging from 200 - 900 nm through Perkin Elmer Spectrophotometer and the characteristic peaks were noticed.

ATR- FTIR analyzed the phyto-constituent in extract. Infrared spectra were obtained with a Harrick Split-pea ATR microscope interfaced to a Perkin Elmer 2000 Fourier transform infrared spectrometer with a single bounce diamond ATR cell. Spectra over the 4000 - 1000 cm^{-1} range were recorded in % transmittance mode to fetch the characteristic peaks of the functional groups. The collected data are processed by optical user software.

The phytochemical constituents were determined by GC-MS analysis system (Clarus 500, Perkin Elmer, CT, USA) containing Agilent DB-5 (Thomas no. 2713R99) with specification (5 % diphenyl dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 µm, 5 cage). Constant flow of Helium gas was maintained at 1 mL/min. The oven temperature was maintained at 110 °C for 2 min and then increased to 280 °C in 9 min. Aliquots (2 µL) of ethanol extract were injected into the instrument, injector temperature was 250 °C. The MS detection took 36 min to screen the predominant constituents with computer-driven algorithm and using mass spectrum library (NIST version 2.0, 2005) [17]. Turbomass 5.2 software program was used in the analyzer.

RESULTS

Phytochemical profile

Qualitative phytochemical analysis of pods extract indicated the presence of terpenoids, falvonoids, phenols, quinine, saponins, glycosides and alkaloids.

UV-Vis spectra

The spectrum showed the peaks at 248.0, 306.0, 372.0, 452.0, 532.0, 658.0 nm with the absorption of 0.921, 0.912, 0.764, 1.030, 0.587 and 0.321 respectively. The results are presented in Figure 1 and Table 1.

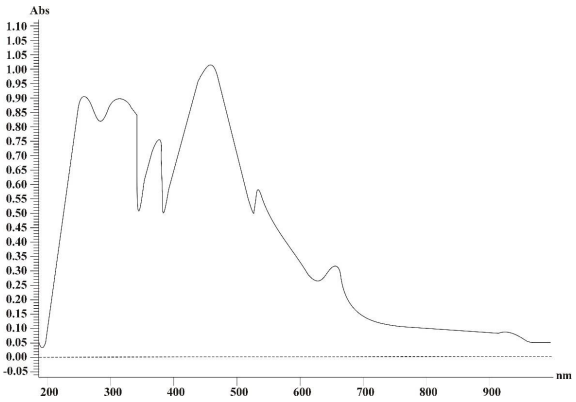


Figure 1: UV-VIS absorption spectra of ethanolic extract of *P. juliflora* pods

Table 1: UV-VIS peak values of ethanol extract of *P. juliflora pods*

Wavelength (nm)	Absorption	Compound
658.0	0.321	Nf
532.0	0.587	Tannins
452.0	1.030	Carotenoid*
372.0	0.764	Terpenoid
306.0	0.912	Terpenoid
248.0	0.921	Terpenoid

ATR-FTIR spectra

ATR-FTIR spectrum of *P. juliflora* pods extract was determined. The data interpreted from the infrared spectral pattern were given and detected as 8 peaks in Table 2. FTIR spectrum was used to identify functional group of active components present in extract [16]. ATR-FTIR spectrum analysis showed the presence of alkane (C - H), aldehyde (C - H), aromatic (C = C), secondary alcohol (C - O), amine (C - N), aromatic (C-H) with bands assignment located at 2975.29, 2943.42, 2832.51, 2349.34, 1449.57, 1087.87, 1024.22, 880.52 cm^{-1}, respectively (Figure 2).

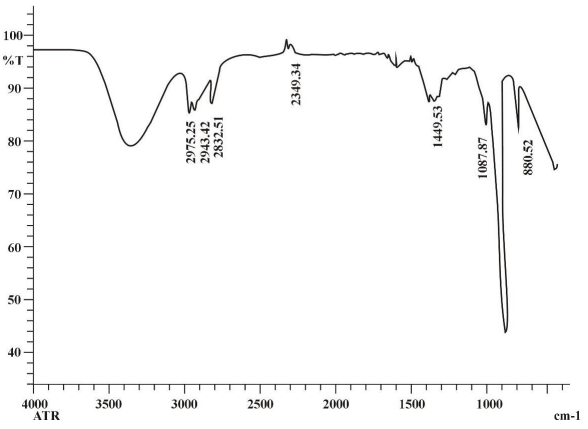


Figure 2: FTIR spectra of ethanol extract of *P. juliflora* pods with peak values and functional groups

Table 2: FTIR spectral peak values and functional groups obtained from ethanol extract of *P. juliflora* pods

Peak value	Type of vibration	Bond/functional group
880.52	bending	C-H/Aromatic
1024.22	stretching	C-N /Amine
1087.87	stretching	C-O /Secondary alcohol
1449.57	bending	C=C/Aromatic
2349.34	stretching	O=C=O
2832.51	stretching	C-H/ Aldehyde
2943.42	stretching	C-H/ Alkane
2975.29	stretching	C-H/ Alkane

GC-MS data

Interpretation of GC-MS was conducted by comparing spectrum, SI (similarity index), RT (retention time) and RI (retention indices) of the unknown component with database of NIST with 62,000 patterns and Scifinder having more than 250,000 patterns of compounds. The percentage relative amounts of all components were calculated by comparing it average peak areas to the total areas. The biological activity of the compound was based on Duke's Phytochemical and ethnobotanical Databases by Jim Duke (Agricultural Research Service/USDA) [17]. The name, molecular weight and structure of chemical motifs of the test materials were presented in Tables (3,4,5) and Figure 3.

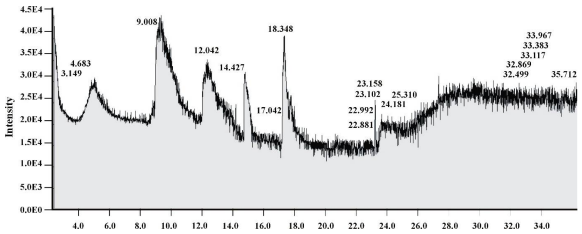


Figure 3: Chromatogram of ethanol extract of *P. juliflora* pods

DISCUSSION

Pakistan being an agricultural land is a precious source of medicinal as well as ornamental plants. A huge population of Pakistan cannot afford costly imported anticancer synthetic medicines. Terpenoids will not only reduce economic burden but also provide local medication with low side effects.

Spectroscopic methods are becoming a powerful analytical tool for secondary metabolite profiling as well as the qualitative and quantitative fingerprinting of bioactive compounds.

Table 3: Identified bioactive compounds of ethanol extract of *P. juliflora* by GC-MS (*contd*)

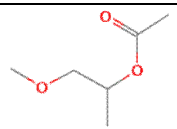
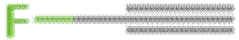
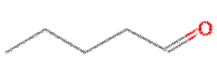
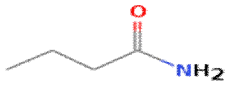
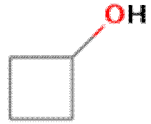
Compound name	Molecular formula	Chemical structure	Molecular weight	Peak area (%)	Retention Time	Terpenoid Y/N	Therapeutic activity
▪1-methoxy-2-propyl acetate	$C_6H_{12}O_3$		132.157	0.23	3.149	▫Y	Nf
▪Ethyne, fluoro-	C_2HF		44.027	0.57	4.683	N	Nf
Pentanal (n-Pentanal)	$C_5H_{10}O$		86.132	5.41	9.008	N	*Antitumor (Nasopharynx), Neurogenic, Increase natural killer cell activity, Nephroprotective, Neuroprotective, GABA-nergic
Butyramide (n-Butylamide)	C_4H_9NO		87.120	0.39	12.042	N	*Antitumor (Nasopharynx), Neurogenic, Increase natural killer cell activity, Nephroprotective, Neuroprotective, GABA-nergic
▪Cyclobutanol	C_4H_8O		72.105	1.61	14.427	N	Nf
▪1-Methyldecylamine	$C_{11}H_{25}N$		171.322	1.02	17.042	N	Nf
n-Hexadecanoic acid (Glycon P-45)	$C_{16}H_{32}O_2$		256.424	5.20	18.348	▫Y	* Anticancer (Pancreas, Pharynx, Prostate), Anticarcinomic (Pancreas, Prostate), Antitumor (Breast, Lung, Prostate, Pancreas), Antidote Poison Gas, DNA-Protective, P21-Inducer, P450-1A-Inducer, P450-2B-Inducer, Antitumor-Promoter, Cancer-Preventive (Esophagus), Provide Silicon, Provide Vit D, K and Zinc, Ulcer Protective

Table 4: Identified bioactive compounds of ethanol extract of *P. juliflora* by GC-MS (*contd*)

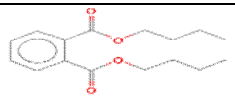
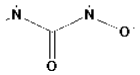
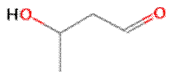
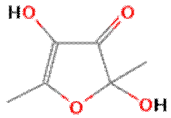
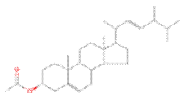
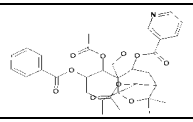
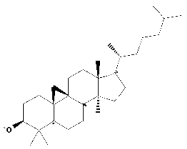
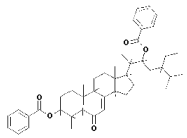
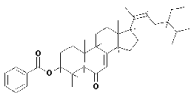
Compound name	Molecular formula	Chemical structure	Molecular weight	Peak area (%)	Retention Time	Terpeniod Y/N	Therapeutic activity
▪▪Dibutyl phthalate (Genoplast B)	$C_{16}H_{22}O_4$		278.343	0.65	22.881	▫Y	*Anticancer (Breast, Bladder), Antitumor (Brain, Breast, Lung, Prostate, Bladder), Anticarcinomic (Breast), Fat Burning, Hormone Balancing, Increase Vit K, D, Zinc and calcium Bioavailability
Hydroxyurea	$CH_4N_2O_2$		76.055	0.15	22.992	N	**_In Vivo_ Anticancer Drug Screen, Anti-proliferative against P388 leukemic cell, Inhibition of human CA9
▪3-Hydroxybutanal	$C_4H_8O_2$		88.105	0.12	23.102	N	Nf
▪Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl]cyclopropyl]methyl]-, methyl ester	$C_{22}H_{38}O_2$		334.535	0.16	23.158	N	Nf
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$		144.125	0.06	24.181	N	*Anti-HIV-Integrase, Antidote (Heavy Metals, Hydrazine, Hypoglycin-A), Hepatoprotective, Hormone Balancing, Improve Cerebral Hypoxia

Table 5: Identified bioactive compounds of ethanol extract of *P. juliflora* by GC-MS (*contd*)

Compound name	Molecular formula	Chemical structure	Molecular weight	Peak area (%)	Retention Time	Terpeniod Y/N	Therapeutic activity
▪▪Methyl 5-(2-undecylcyclopropyl) Pentanoate	$C_{20}H_{38}O_2$		310.514	0.07	25.310	N	*Methyl-Donor
▪Ergosterol, acetate	$C_{30}H_{46}O_2$		438.685	0.06	32.499	N	Nf
▪Maymyrsine	$C_{32}H_{37}NO_{10}$		595.645	0.03	32.869	N	Nf
▪▪Cycloartanol	$C_{30}H_{52}O$		428.745	0.04	33.117	N	**Antiviral
▪Carpesterol Benzoate	$C_{44}H_{58}O_5$		666.943	0.05	33.383	N	Nf
Carpesterol Dehydrate	$C_{37}H_{52}O_3$		544.821	0.02	33.967	N	Nf
▪▪9-(2-oxiranyl)-1-nonanol	$C_{11}H_{22}O_2$		186.295	0.04	35.712	▫Y	Nf

*▪Novel Compound, ▪▪Novel Source, RT – Retention Time, Nf – Not found, Y/N – Yes/No, ▫Dr. Hochmuth scientific consulting database-Terpenoids Library (2008-2012), *Dr. Duke's ethanobotanical databases **Pubchem Open Chemistry Database*

The qualitative UV-Vis spectroscopy of ethanol extract of *P. juliflora* displayed characteristic peaks of major bioactive compounds like terpenoid, carotenoids and tannins. The results obtained were confirmed by comparing the absorption values and wavelength with already published research data [18]. These results showed that the pods are rich source of terpenoids [15]. Previously, (-) mesquitol was isolated from *P. juliflora*, heartwood [19]. The antibacterial properties of alkaloid from *P. juliflora* were also determined [6]. There seems a connection between terpenoids and antimicrobial activity, hence, we used pods for the first time to identify anticancer terpenoids and fortunately, we are successful. It will bring a revolution in cancer treatment with less side effects and low cost. Many scientists proposed that terpenoids are natural products for cancer therapy [20].

Previously, we revealed that *P. juliflora* pods had 25 % of total terpenoid contents [15], and these may be major contributing factors for the highest antioxidant potential of pods by DPPH free radical scavenging activity (92 %) with IC_{50} (0.045 ± 0.005) [9].

For further confirmation of bioactive compounds, these findings must be supplemented with other analytical techniques like FTIR and GC-MS, for proper extract characterization and finger-printing of metabolites. The FTIR spectrum identifies the functional group of the active components on the basis of peak value in infrared radiation region. The functional groups were identified by comparing the wavelength of the phytochemicals with infrared spectroscopy absorption Table published online by UC Davis ChemWiki and OChemonline. The results confirmed the presence of phenol, cyclohexane, alkene and aromatic compounds [18].

Functional groups play in the activity of secondary metabolites, their kinetics, and overall role in chemical activity and therapeutic functioning of compounds [21].

GC-MS analysis identified 19 bioactive compounds comprising 5 anticancer compounds with 2 novel anticancer compounds and 2 anticancer terpenoids from the ethanol extract of pods of *P. juliflora* (Figure, 3). Furthermore, for the first time, in the pods a total of 13 novel compounds, 2 anticancer compounds have been identified i.e. dibutyl phthalate and hydroxyurea. Dibutyl phthalate (Genoplast B), magnificent potential compound against breast and bladder cancer. Similarly, hydroxyurea (0.15 %) has been used in *in vitro* anticancer drug screening. Although alkaloids were identified from *P. juliflora*, but they are used under different sources like heartwood and leaves [6,19].

Previously, these terpenoids have been synthetically produced or obtained from microbes like fungi, bacteria and marine organisms [22].

CONCLUSION

The findings of this study indicate that *P. juliflora* pods are a novel source of anticancer, chemo-preventive and medicinally important bioactive compounds, especially terpenoid. Thus, further investigations, including preclinical studies are recommended on cancer animal model.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shazia Kanwal Malik conceived and designed the study. Shazia Kanwal Malik, Masood Ahmad, Farah Khan collected and analyzed the data. Shazia Kanwal Malik, Masood Ahmad, Farah Khan wrote the manuscript.

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