Supplement

Phytochemical Profile of *Zygophyllum paulayanum* Extracts and their Promising Biological Activities

Mohan Lal^{1*}, Sachin Salunkhe², Amit Kumar², Dipankar Sutradhar¹

¹ School of Advanced Sciences and Languages, VIT Bhopal University, Madhya Pradesh, 466114, India

² R&D, OmniActive Health Technologies, International Biotechnology Park, Hinjewadi, Phase-II, Pune,

Maharashtra-411 057, India

* Corresponding author, e-mail: mohan.lal2019@vitbhopal.ac.in

Table S1 Extraction parameters, yield and phytochemicals*								
Easter at a second	Extraction	0/ 37: 11		Contents	(%w/w) of phyto	chemicals		
Extract name	temp (°C)	70 Tield	Polyphenols	Flavonoids	Tannins	Saponins	Alkaloids	
Extraction at ambient temp (25-30 °C)								
n-Hexane extract	25-30	1.92 ± 0.18	1.77 ± 0.44	1.45 ± 0.16	0.00	0.00	0.10 ± 0.04	
Ethyl acetate extract	25-30	2.42 ± 0.16	13.06 ± 0.49	1.73 ± 0.19	0.00	0.00	2.17 ± 0.18	
Ethanol extract	25-30	7.36 ± 0.15	14.87 ± 0.58	1.71 ± 0.06	2.13 ± 0.18	11.15 ± 0.43	14.32 ± 0.30	
Water extract	25-30	12.60 ± 0.25	5.30 ± 0.47	1.31 ± 0.17	0.78 ± 0.17	12.21 ± 0.27	1.13 ± 0.30	
Extraction at hot condition (60-65 °C)								
n-Hexane extract	60-65	2.34 ± 0.05	1.34 ± 0.26	1.23 ± 0.06	0.00	0.00	0.02 ± 0.02	
Ethyl acetate extract	60-65	3.00 ± 0.17	22.15 ± 1.85	2.93 ± 0.09	0.00	0.00	2.17 ± 0.22	
Ethanol extract	60-65	13.28 ± 0.12	16.18 ± 0.06	4.02 ± 0.05	2.18 ± 0.22	8.68 ± 0.24	12.45 ± 0.50	
Water extract	60-65	16.80 ± 0.56	7.51 ± 0.87	1.82 ± 0.21	0.78 ± 0.12	9.62 ± 0.15	1.13 ± 0.27	

*The highest yield was observed with hot extraction compare to ambient temperature. Among the extracts, hot ethanol provided the maximum phytochemicals including flavonoids, tannins, and alkaloids where's the hot ethyl acetate only contains polyphenol and saponins in water.

Table S2 Phytochemical evaluation of fractions*

Samula noma	% Yield of fractions	Contents (%w/w) of phytochemicals					
		Polyphenols	Flavonoids	Tannins	Saponins	Alkaloids	
n-Hexane fraction	6.38 ± 0.51	0.00	0.00	0.00	0.00	0.10 ± 0.05	
Ethyl acetate fraction	5.02 ± 0.39	22.14 ± 0.18	3.13 ± 0.21	0.00	0.00	2.13 ± 0.22	
Ethanol fraction	40.96 ± 1.03	9.44 ± 0.61	5.82 ± 0.04	4.95 ± 0.28	2.10 ± 0.32	18.50 ± 0.84	
Water fraction	47.62 ± 1.80	4.32 ± 0.23	0.89 ± 0.04	0.44 ± 0.09	16.10 ± 0.14	3.70 ± 0.26	

*Among the fractions, the ethanol fraction was found to be the richest in phytochemicals such as flavonoids, tannins, and alkaloids, whereas the ethyl acetate fraction was richest in polyphenols, and the water fraction was richest in saponins.

Table S3 Fatty acid content (%w/w) of n-hexane fraction*								
Extract name	Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Lenolieic acid	Linolenic acid	TDFA**
n-Hexane fraction	0.62	0.44	6.73	1.10	3.11	10.44	2.27	24.71

*The non-polar bioactives were found in hexane fraction which were identified as seven fatty acids.

**TDFA- Total detected fatty acids.

Table S4 Phytoestrogenic activity* Sample name Concentration (µg/mL) % Viability Standard deviation 1.00 194.64 52.02 n-Hexane fraction 0.01 72.32 32.71 1.00 209.34 12.86 Ethyl acetate fraction 0.01 161.76 32.58 1.00 126.3 33.55 Ethanol fraction 0.01 55.1 35.88 1.00 121.54 22.14 Water fraction 0.01 103.03 15.89 Positive control (β-estradiol) 1 nm 147.32 19.36 Negative Control ---101.94 2.12

*This study examined the impacts of plant extracts as phytoestrogens on MCF-7 cell line. Cell line was penetrated with extract samples along with positive and negative controls, in order to exhibit the phytoestrogenic activity. All fractions display positive response in which the n-hexane and ethyl acetate fractions showed maximum phytoestrogenic activity.

Table S5 Antioxidar	t activities of ethanol	extract and fractions*
---------------------	-------------------------	------------------------

Sample name	ame Remaining DPPH (calculated according to Eq. 8)**					
Concentration (µg/mL)	$5.00\pm SD$	$15.00\pm\text{SD}$	$25.00\pm\text{SD}$	$50.00\pm\text{SD}$	$100.00\pm\text{SD}$	
n-Hexane fraction	11.22 ± 1.51	33.00 ± 0.57	42.24 ± 0.57	48.18 ± 1.51	50.50 ± 1.98	
Ethyl acetate fraction	35.31 ± 1.14	50.83 ± 1.51	69.64 ± 2.06	78.55 ± 1.51	85.15 ± 0.99	
Ethanol fraction	54.13 ± 0.57	66.67 ± 1.51	76.57 ± 1.51	87.13 ± 0.99	92.08 ± 0.99	
Water fraction	5.28 ± 1.14	14.52 ± 1.51	23.10 ± 1.14	36.30 ± 0.57	31.02 ± 1.51	
Mixed tocopherols-70%	96.04 ± 0.00	96.04 ± 0.00	96.70 ± 0.57	98.02 ± 0.00	98.35 ± 0.57	

*The antioxidant activity of all fractions was evaluated using the DPPH assay, with mixed-tocopherol (70%) used as a positive control. The ethanolic fraction was found to have highest antioxidant among all fractions.

**Where Abs, is the absorbance of the control and Abs, is the absorbance in the presence of test compounds.

	Table	S6 Anti-inflamm	natory activity*
--	-------	------------------------	------------------

		· · · · · · · · · · · · · · · · · · ·	
Sample name	%Inhi	bition (calculated according to	Eq. 9)
Concentration (µg/mL)	250 μg/mL	500 μg/mL	1000 µg/mL
n-Hexane fraction	55.62 ± 0.34	56.78 ± 0.39	58.95 ± 0.57
Ethyl acetate fraction	53.60 ± 0.90	55.96 ± 0.63	58.61 ± 0.36
Ethanol fraction	53.67 ± 0.90	57.42 ± 0.30	58.99 ± 2.14
Water fraction	56.70 ± 0.43	56.82 ± 0.84	59.78 ± 1.01
Diclofenac sodium	93.65 ± 0.58	93.60 ± 0.61	$94.42\pm0.1.01$

*All fractions were tested for anti-inflammatory activity against diclofenac sodium as positive control. All the fractions were gave positive response in which the water fraction was found to be slightly better.