doi.org/10.36721/PJPS.2021.34.4.SUP.1561-1568.1

GC-MS profiling and *in vitro* antioxidant, cytotoxic and antimicrobial activities of *Trianthema triquetra* Rottl. ex Willed

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Abstract: *Trianthema triquetra* Rottl. ex Willed is being used as herbal remedy for chronic ulcer, wound healing, diabetes, skin and inflammatory diseases in India and Pakistan. Still, no scientific data is available about the therapeutic potential and phytochemistry of the plant. The aim of the current investigation is to perform GC-MS analysis, antioxidant (total phenolic and flavonoid content, DPPH assay), antimicrobial (disc diffusion assay) and cytotoxic (XTT and RBC's cellular membrane protection assay) studies. Whole plant material was dried and extracted with methanol to get crude methanolic extract and then it was fractionated with n-hexane, ethyl-acetate, chloroform, n-butanol and water. Results showed that n-butanol fraction exhibited a significant ($p<0.05$) antioxidant potential measured by DPPH assay ($IC_{50=}$63.35±0.13µg/mL) and also possess highest phenolic content (177±4.36mg/g GAE). Whereas, n-hexane fraction showed highest flavonoid content (14.67±1.53mg/g QE). Two major components (2, 4-Ditert-butyl-6-nitrophenol (26.79%) and Squalene (25.64%) were detected in GC-MS analysis of chloroform fraction, eluted from column chromatography. Moreover, chloroform fraction also exhibited antibacterial activity towards all the tested strains of bacteria and fungi. Significant ($p<0.05$) dose dependent inhibition response on cell growth against CCRF-CEM cell lines was exhibited by methanolic extract. Furthermore, hemolytic potential of methanolic extract was found to be in safe range (2.23%-6.37%). So, it can be inferred that *Trianthema triquetra* can be exploited as an alternative remedy for cancer, oxidative stress related disorders and in various skin diseases.

Keywords: *Trianthema triquetra*, GC-MS analysis, anticancer, antioxidant, antimicrobial.

INTRODUCTION

Several studies have suggested that most of the natural products of plant origin possess pronounced biological potential in terms of antioxidant, anti-inflammatory and anti-cancer activities (Rates, 2001). These findings have accentuated the search of novel plant-derived products to develop new drug molecules (Zengin *et al.*, 2016).

Oxidative stress is the leading cause of various diseases. Variety of compounds of plant origin have been found to decrease the risk of diabetes, cardiovascular and many neurodegenerative disorders (Pala and Gürkan, 2008; Arts and Hollman, 2005). Polyphenols from plants exert their effect by interfering the inflammatory process and cancer progression. Despite the advancements in surgical procedures and chemotherapeutic adjuvants, the toxicity of various chemo-radiation strategies to prevent cancer results in poor patient outcomes. This challenge requires discovering novel antitumor agents of natural origin with less toxicity while maintaining a high degree of efficacy (Sertel *et al*., 2011). Plant derived anticancer agents such as Vinblastine and Taxol are being used in cancer therapy (Khan *et al.,* 2019; Jaradat *et al*., 2016). During the process of drug designing, the evaluation of toxicity related to the bioactive molecules is a primary element. In this regard, stability of erythrocyte's membrane is an indication of the impact of several *in vitro* studies for the detection of cytotoxicity by various compounds (Da Silva *et al*., 2004).

The antioxidant potential of plant-based products is ascribed to the properties of phenolic compounds (Dai and Mumper, 2010). Resistance to antibiotics is a significant public health issue globally (Shakya *et al*., 2013; Gootz, 2010). Majority of currently used antibiotics are of natural origin. A number of compounds are produced by plants to protect themselves from microbial action (Riaz *et al*., 2012). Previous findings have shown that certain medicinal plants have antimicrobial aptitude and these plants are capable of being used as significant alternative source of antimicrobial agents against resistant micro-organism's strains (Kone *et al*., 2004).

Trianthema triquetra belongs to the family Aizoaceae which is called as ice plant family. This family consists of 127 genera and 1860 species (Kanwal *et al*., 2009). Aizoaceae is considered to be one of the most widespread and prolific families, but in terms of medicinal potential, it has been found to be least studied. A number of phytochemicals including essential oils, phenolic compounds, alkaloids, triterpenes, flavonoids, lignans, sterols and some miscellaneous compounds have been separated from various members of Aizoaceae (Van Der Watt and Pretorius, 2001).

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Only a few species of the family Aizoaceae are phyto-pharmacologically reported. *Trianthema triquetra* is one of them. It is a desert plant and commonly known as Red Spinach and locally it is called as "Choti Ulwaiti" in Pakistan (Khurshid *et al*., 2019). Traditionally, it has been used as fodder, remedy for chronic ulcer, fever, healing of wounds, diabetes, skin and inflammatory diseases (Wariss *et al*., 2014; Ahmad *et al*., 2014). However, till to date no scientific reports are available to justify its traditional uses. Therefore, the current study was aimed to assess its antioxidant, antimicrobial and cytotoxic potential. Moreover, the eluted fraction from column chromatography of chloroform fraction of *Trianthema triquetra* was subjected to GC-MS analysis for the screening of bioactive chemical compounds.

MATERIALS AND METHODS

Collection of plant material

The whole plant was collected from Bahawalpur, Punjab, Pakistan in July 2017. The identification and authentication of the plant was done from Department of Botany, GC University, Lahore, Pakistan against the voucher number (GC. Herb.Bot.3445).

Preparation of plant extract and fractions

Plant was washed, dried and pulverized by using a mechanical mill. The plant powder (1000g) was extracted with methanol (2.5L) for seven days with periodic shaking. Afterwards, it was filtered and evaporated by removing the solvent in rotary evaporator to give solid masses. Crude methanolic extract of *Trianthema triquetra* (TTM) was shaken with (250mL) of water and partitioned with n-hexane (TTH), chloroform (TTC), ethyl-acetate (TTEA), n-butanol (TTB) and water (TTA). Extraction method was followed twice and obtained fractions were combined (Hossain *et al*., 2013).

In vitro antioxidant activities

Total phenolic content (TPC)

TPC of TTM extract and its fractions was determined by the Folin-Ciocalteu (FC) method with slight modification. The reaction mixture was prepared by combining (0.2mL of test solution+0.6mL of water+0.2mL of FC reagent). After 5 minutes, to the reaction mixture, 1mL of sodium carbonate was added and total volume (3mL) was made by adding distilled water and stayed in dark for 30 minutes. Absorbance of all the samples was taken at 765nm by using UV-Vis spectrophotometer. From the calibration curve TPC of each sample was determined and the results were expressed as mg of Gallic acid equivalent (GAE/g) of dry plant material (Song *et al*., 2020).

Total flavonoid content (TFC)

For the estimation of TFC of TTM extract and its fractions, Quercetin was taken as a standard drug. Samples were prepared by serial dilution in methanol (10-120µg/mL). Reaction mixture was prepared by (0.6mL of 2%aluminium chloride+0.6mL of test solution). The reaction mixture was incubated for 60 minutes at room temperature after mixing, then absorbance of all samples was taken by UV-Vis spectrophotometer at 420nm against blank. Concentration of TFC in all samples was measured by calibration curve and expressed as Quercetin equivalents (QE/g) of dried plant material (Elshibani *et al*., 2020).

DPPH radical scavenging assay

DPPH (1, 1-diphenyl-2-picryl hydrazyl) scavenging assay was followed for the determination of antioxidant activity of TTM extract and its fractions. 0.004% (w/v) DPPH solution was made in methanol. Ascorbic acid was taken as standard (positive control) drug. Different concentrations (10-100µg/mL) of plant samples and standards were also prepared in methanol. Each sample of plant/standard in a quantity of 2mL was added to the 2mL of DPPH solution and incubated in dark for 30 minutes. The absorbance was measured at 517nm by using UV-Vis spectrophotometer (Gaber *et al*., 2021). IC_{50} was calculated by the plotted graph between DPPH inhibition against various concentrations of plant samples and standard solutions. By using the following equation, antioxidant ability was calculated in terms of percentage inhibition of DPPH.

$$\text{Inhibition}\,(\%) = \left[\frac{A - B}{A}\right] \times 100$$

Where, A=Absorbance of control (DPPH + methanol), B=Absorbance of plant sample extract/fraction or standard.

Antimicrobial assay

Standard strains of bacteria *Escherichia coli* (ATCC 8739), *Psuedomonas aeruginosa* (ATCC 9027), *Staphylococcus epidermidis* (ATCC 12228), *Salmonella typhimurium* and *Staphylococcus aureus* (ATCC 6538) were used for the evaluation of antibacterial activity. Whereas, standard strains of *Saccharomyces cerevisiae* (ATCC 9763) and *Candida albicans* (ATCC 10231) were used for determination of antifungal activity.

Disc diffusion method

Antimicrobial potential of TTM extract and TTC fraction was assessed. 10% DMSO was used for the sample preparation. Discs of 6mm in diameter were loaded with different concentrations of TTM extract and TTC fraction (100µL/disc). Negative control disc was loaded with DMSO (100µL). Discs impregnated with Ciprofloxacin and Eberconazole (10µL/disc) served as a positive control for bacteria and fungi respectively. On the inoculated agar plates, all discs were placed aseptically. Plates were incubated at 37±0.1ºC for 24 hours for bacteria and 28±0.5ºC for 72 hours for fungi. After the incubation period, inhibition zones formed on media were measured. The test was performed in triplicates with control (Bhalodia and Shukla, 2011).

Table 1: DPPH scavenging assay of TTM extract and its fractions

Conc. (µg/mL)	%age DPPH Inhibition± SD						
	Ascorbic acid	TTM	TTH	TTC	TTEA	TTB	TTA
10	30.67±0.29	2.74±0.04	8.50±0.05	15.40±0.18	12.40±0.18	29.44±0.13	23.63±0.15
20	49.57±0.21	3.63±0.03	10.73±0.04	20.23±0.10	20.36±0.18	34.48±0.37	28.30±0.04
40	63.45±0.05	6.94±0.03	15.4±0.56	24.23±0.08	23.23±0.08	40.21±0.10	29.87±0.12
60	75.55±0.05	8.28±0.04	20.34±0.06	30.33±0.12	27.50±0.17	50.27±0.23	35.57±0.29
80	82.33±0.12	11.15±0.04	31.45±0.13	48.23±0.21	50.63±0.35	57.29±0.29	40.77±0.25
100	90.33±0.21	13.25±0.04	55.61±0.02	70.9±0.5	73.6±0.35	62.25±0.22	45.43±0.06
IC_{50}(µg/mL)	26.75±0.17[a]	411.66±1.42[b]	107.38±0.09[c]	78.08±0.47[d]	76.36±0.07[d]	63.35±0.13[e]	120.63±0.92[f]

Values representing same letter (a-f) in IC_{50} row have no significant difference ($p>0.05$) by Tukey's test at 95% confidence level

Table 2: Phytoconstituents detected through GC-MS analysis of TTC fraction

Compound Name	Retention Time	Molecular Formula	Molecular Weight	% Peak Area
Unidentified	10.000	-	-	1.58
2,4-Ditert-butyl-6-nitrophenol	13.677	$C_{14}H_{21}NO_3$	251	26.79
1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	13.958	$C_{16}H_{18}$	210	1.89
Nonadecane	14.567	$C_{19}H_{40}$	268	1.87
1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	19.395	$C_{24}H_{38}O_4$	390	4.47
Heneicosane	20.255	$C_{21}H_{44}$	296	2.23
Squalene	20.867	$C_{30}H_{50}$	410	25.64
Nonacosane	21.276	$C_{29}H_{60}$	408	9.33
Geranyl linalool	21.425	$C_{20}H_{34}O$	290	5.73
Tetratetracontane	21.804	$C_{44}H_{90}$	618	2.13
Tetracosane	22.395	$C_{24}H_{50}$	338	12.28
Pentatriacontane	23.044	$C_{35}H_{72}$	492	2.01
Hexatriacontane	23.791	$C_{36}H_{74}$	506	4.07

Table 3: Antimicrobial activity of TTM extract and TTC fraction against bacterial strains

Samples	Zones of inhibition (mm) ± SD				
	E. coli	*P. aeruginosa*	*S. typhimurium*	*S. aureus*	*S. epidermidis*
Negative Control	0±0[d]	0±0[f]	0±0[g]	0±0[f]	0±0[g]
Positive Control	26.98±0.98[a]	24.47±0.89[a]	43.04±1.18[a]	41.36±1.00[a]	42.12±1.43[a]
TTM 25%	7.19±0.71[b]	5.75±1.59[b]	6.53±1.50[b]	11.09±2.14[b]	9.95±1.07[b]
TTM 50%	9.53±1.38[b]	7.92±1.01[b,c]	14.99±1.06[c]	17.71±1.94[c]	13.78±1.10[c]
TTM 100%	11.05±1.78[b]	10.82±0.54[c,d]	18.92±0.48[d]	27.85±1.07[d]	20.86±1.03[d]
TTC 25%	10.35±1.40[b]	12.37±1.96[d]	21.42±0.52[d]	26.36±1.94[d]	19.98±1.86[d]
TTC 50%	18.43±1.96[c]	20.71±1.12[e]	26.00±1.45[e]	33.64±1.73[e]	29.46±0.60[e]
TTC 100%	25.88±2.13[a]	27.13±0.76[a]	37.15±0.74[f]	43.43±1.31[a]	34.79±0.28[f]

Values representing same letter (a-g) in same column have no significant difference ($p>0.05$) by Tukey's test at 95% confidence level

Table 4: Antimicrobial activity of TTM extract and TTC fraction against fungal strains

Samples	Zones of inhibition (mm) ± SD	
	C. albicans	*S. cerevisiae*
Negative Control	0±0[a]	0±0[g]
Positive Control	26.79±1.17[b]	42.78±1.23[a]
TTM 25%	2.0±2.0[a]	11.45±1.50[b]
TTM 50%	3.33±3.06[a]	19.49±1.34[c]
TTM 100%	4.33±3.79[a]	28.96±1.06[d]
TTC 25%	1.33±1.15[a]	23.91±1.64[e]
TTC 50%	4.0±4.0[a]	32.71±1.25[f]
TTC 100%	4.33±4.51[a]	44.50±0.56[a]

Values representing same letter (a-g) in same column have no significant difference ($p>0.05$) by Tukey's test at 95% confidence level

Table 5: Cell proliferation and viability response of TTM extract by XTT assay

Samples	% age of cell viability*(CCRF-CEM) ± SD	% age of cell viability*(MDA-MB-231) ± SD
Control	100± 3.79[a]	100±1.83[a]
Vinblastine	0.578± 0.61[b]	18.271±2.59[b]
TTM 5µg/mL	88.527± 8.21[a]	81.816±4.28[c]
TTM 10µg/mL	86.544± 10.81[a]	82.532±2.01[c]
TTM 50µg/mL	30.317± 11.72[c]	74.164±4.22[d]

Values representing same letter (a-d) in same column have no significant difference (p>0.05) by Tukey's test at 95% confidence level

Gas chromatography/Mass spectrometry (GC/MS)

TTC fraction was subjected to column chromatography for the purification of compounds. The fraction eluted from the column chromatography at the mobile system (n-hexane:100%) was selected for GC-MS analysis, executed by a GC-MS (Schimadzu, Kyoto): QP2010), attached to Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, consisting of 100% Dimethyl polysiloxane, operating in electron impact mode at 70 eV, 100 KPa pressure and linear velocity of 47.2 cm/sec, carrier gas helium (99.99%) at 1mL/minute flow rate and 1µL injected sample volume was utilized at 10:1 split ratio. The sample injector temperature and ion source temperature was 200°C whereas the oven temperature was automated from 50°C (isothermal for 3 minutes), elevation of 15°C/minute to 320°C. Scan range was 30-800m/z with 0.5second scan interval. 24 minutes was total run time. Using library data, the compounds were detected from the GC-MS peaks (Rigano *et al*., 2020).

In vitro cytotoxicity studies

XTT assay

RPMI 1640 (Gibco®, Invitrogen, Darmstadt, Germany) culture media was used for the culturing of two human cell lines MDA-MB-231 and CCRF-CEM. The media was consisted of Streptomycin and Penicillin 1%, 2 mM L-glutamine (Sigma) and 10% fetal bovine serum (PAA Laboratories, Pasching, Austria). They were incubated at 37˚C in a 5% humidified CO_2 (Thermo Fisher Scientific Inc., Vienna, Austria). Cultures at approximately 80-90% confluency were routinely split in a ratio of 1:5 in a 25 cm culture flask. MDA-MB-231 and CCRF-CEM were cultured at a density of 5000 cells/well and 10,000 cells/well respectively, into every well of 96-well plates. After incubating the cells with TTM extract (5, 10 and 50µg/mL), XTT reagent (2, 3-bis-(methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was added, incubated again for 1.5 hours and readout by microplate reader (Hidex, Finland). Anticancer drug Vinblastine was taken as positive control. Viability of treatment was measured as a percentage of viability in comparison to untreated cells (Kretschmer *et al*., 2012).

RBC's cellular membrane protection activity

To determine the cytotoxic effects of the plant on the mechanical strength of RBC's cell membrane, hemolytic activity of the TTM extract was evaluated. Fresh human blood (3mL) was taken in an EDTA vial and centrifuged for 5 minutes at 800rpm. After centrifugation, the clear supernatant was carefully decanted. The sedimented pellets were washed thrice with 5mL of chilled PBS at pH 7.4. Afterwards, cells were suspended in chilled PBS (20mL) followed by counting on a hemocytometer. For each test, the count of erythrocytes was maintained at 7.068×10^8 cells/mL. 180µL of diluted RBC's suspension was added to the reaction mixture which contained 20µL of plant samples as well. Triton X-100 (0.1%) served as positive control whereas PBS was taken as negative control. For 35 minutes, all samples were incubated at 37ºC. All tubes were put in ice-cold PBS for 5 minutes after incubation, and centrifuged at 1500rpm. The supernatant (100µL) was diluted with 900µL of chilled PBS in eppendorf tubes. Each sample including positive and negative control (200µL) was transferred into 96 well micro-titer plates. At 576nm, absorbance of all samples was measured. By the following formula % hemolysis was calculated (Zubair *et al*., 2017).

$$\%\ \text{Haemolysis}\left[\frac{\text{Abs (absorbance of sample)}}{\text{Abs (absorbance of control)}}\right]\times 100$$

STATISTICAL ANALYSIS

All values were expressed as the arithmetical mean ±SD. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied for the determination of statistically significant differences. P≤0.05 was considered to represent statistically significant results.

RESULTS

Extraction and percentage yields of plant extract and fractions

The percentage yield of TTM was 14.38% while the yields of TTH, TTC, TTEA, TTA and TTB fractions were 7.98, 6.30, 5.20, 6.00 and 4.30 % respectively.

Antioxidant activities

TPC of TTM extract and its fractions was calculated using the linear regression equation of gallic acid (y=0.0103x+0.1884). The highest phenolic content was found in TTB fraction (177±4.36mg/g GAE) while the

least was observed in TTA fraction (19.33±3.79mg/g GAE). TPC of TTM extract and its fractions were found to decrease in the following order: TTB>TTC>TTH> TTEA>TTM>TTA. Similarly TFC was calculated using linear regression equation of Quercetin (y=0.0116x+ 0.2591). Highest TFC was found in TTH fraction (14.67±1.53mg/g QE) while the least was observed in TTEA fraction (4.67± 0.58mg/g QE). TFC of TTM extract and its fractions was found to decrease in the following order: TTH>TTM>TTC>TTB>TTA>TTEA. The results are also shown in (fig. 1). IC_{50} values of TTM extract and its fractions were in the range of (63.35±0.07-411.66±1.62µg/mL). TTB fraction exhibited lowest IC_{50} value (63.35±0.13µg/mL) followed by TTEA fraction (76.36±0.07µg/mL), TTC fraction (78.08±0.47µg/mL), TTH fraction (107.38±0.09µg/mL), TTA fraction (120.63±0.92µg/mL) and TTM extract (411.66±1.42 µg/mL). TTB fraction exhibited a significant ($p<0.05$) antioxidant potential. Results are shown in table 1.

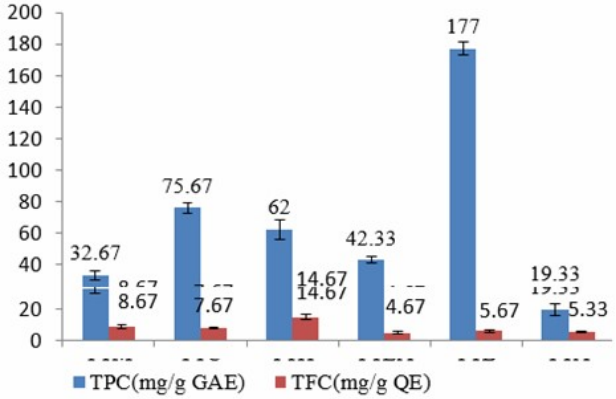

Fig. 1: TPC and TFC of TTM extract and its fractions

Antimicrobial activity and GC-MS analysis

TTM extract and TTC fraction showed remarkable antimicrobial activities against 5 bacterial and 2 fungal strains. Inhibition zones of TTM extract against bacterial and fungal strains were measured in the range of (5.75±1.59-27.85±1.07mm) and (2.0±2.0-28.96±1.06mm) respectively. Whereas, inhibition zones of TTC fraction against bacterial and fungal strains ranged from (10.35±1.40-43.43±1.31mm) and (1.33±1.15-44.50±0.56mm) respectively. The maximum and the most significant inhibition (43.43±1.31mm, $p<0.05$) and (44.50±0.5mm, $p<0.05$) against *S. aureus* and *S. cerevisiae* respectively was observed by TTC fraction. The results are shown in table 3 & 4.

GC-MS analysis of eluted fraction of TTC fraction by column chromatography revealed the identification of 13 components. The major constituents (>5) were found to be 2, 4-Ditert-butyl-6-nitrophenol (26.79%), Squalene (25.64%), Tetracosane (12.28%), Nonacosane (9.33%), and Geranyl linalol (5.73%). GC-MS spectrum has been showed in fig. 2.

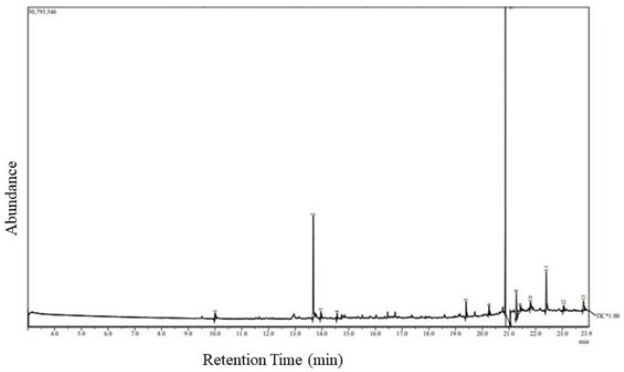

Fig. 2: GC-MS chromatogram of eluted fraction from TTC fraction by column chromatography at (n-hexane: 100%) solvent system

Cytotoxic assays

TTM extract at different concentrations (5µg/mL, 10µg/mL and 50µg/mL) showed very little and non-significant cytotoxicity against MDA-MB-231cell lines. But a significant ($p<0.05$), dose-dependent inhibitory effect (30.317±11.72%) on cell growth against CCRF-CEM cell lines at 50µg/mL was observed. The results in table 5 showed that the CCRF-CEM cell lines are sensitive to TTM extract at higher concentration as compared to MDA-MB-231 cell lines. Furthermore, toxic effect of TTM extract at different doses (125µg/mL, 250µg/mL, 500µg/mL and 1000µg/mL) against human red blood cells (RBC's) was evaluated. TTM presented the hemolytic effect ranged from (2.23%-6.37%). The hemolytic activity of TTM extract at all tested doses was found to be within a safe range, i.e., below 10 % (fig. 3).

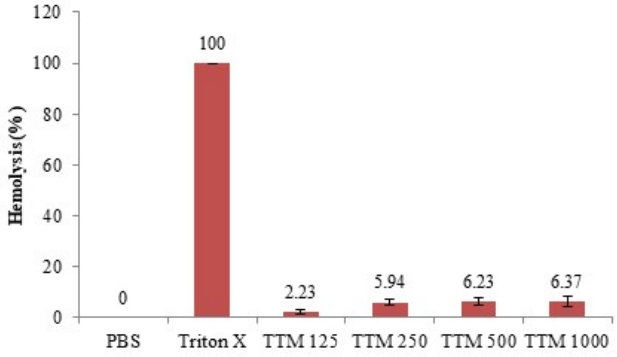

Fig. 3: Hemolytic effect of TTM extract on human erythrocytes

DISCUSSION

Various bioactive components are present in plants and the successful estimation of these components depends upon extracting solvents (Al-Nemari *et al*., 2020). In the current study, six different solvents were utilized for the extraction of phyto-constituents and the highest percentage yield was gained by methanol (14.38%).

The proportion of total phenolic and flavonoid contents of a plant is an important indicator of its therapeutic potential (Moreira *et al*., 2008). Phenolic compounds have tendency to transfer hydrogen atom to free radicals (Zin *et al*., 2006). They possess the structural properties of free radical scavengers that enable them to act as potential antioxidants (Jayathilakan *et al*., 2007). Whereas, flavonoids are synthesized by plants and are known to have antioxidant, anti-inflammatory and antimicrobial properties (Kalogeropoulos *et al*., 2009). The results of present study suggested that *Trianthema triquetra* encompasses a significant amount of phenolic and flavonoid components. Estimation of TPC and TFC of TTM extract and its fractions are first time reported in this study. TTB fraction was found to contain the highest phenolic whereas TTH fraction was observed with the highest flavonoid content. DPPH assay was performed for the determination of antioxidant potential of the plant. DPPH is a stable, free radical with maximum absorption in methanol at 517nm. If DPPH finds proton donating substances such as antioxidants, the absorbance at 517nm disappears due to the scavenging of the radicals (Jayasinghe *et al*., 2003). Reduction in the number of DPPH is proportional to the number of available hydroxyl groups. In our study, significant correlation was seen between the phenolic contents and DPPH assay of the plant. Amongst all the fractions and extract, TTB fraction was found to have significant ($P<0.05$) antioxidant activity with the least IC_{50} value and the highest TPC. In another study, DPPH assay was performed on four different extracts of *Trianthema triquetra* e.g., methanol, chloroform, ethyl-acetate and petroleum-ether while the methanolic extract was found to have the highest antioxidant potential with the lowest IC_{50} (220µg/mL) (Indumathy and Ajithadas, 2017). These findings suggests the polar components might be responsible for the antioxidant potential of *Trianthema triquetra* which are consistent with our study e.g. the antioxidant potential was found in polar plant fraction e.g., TTB.

Previous studies have reported that the volatile components of the plants such as terpenes, terpenoids, phenol derived aliphatic and aromatic compounds are bactericidal, virucidal and fungicidal (Akthar *et al*., 2014). Disc diffusion method was employed for the determination of antimicrobial activity of TTM extract and TTC fraction against bacterial and fungal strains. Results indicated that TTC fraction was effective against both gram-positive as well as gram-negative bacteria. However, gram-negative bacteria were found comparatively less sensitive to TTM extract. The difference in the sensitivity between gram-positive and gram-negative bacteria is due to the morphological differences between micro-organism (Bereksi *et al*., 2018). Amongst fungal strains, promising results were obtained against *S. cerevisiae,* however, *C. albicans* was found to be resistant against all the tested samples.

Major compounds identified in GC-MS analysis of eluted fraction by column chromatography of TTC fraction at mobile system (n-hexane: 100%) exhibits various pharmacological properties. According to literature, 2, 4-Ditert-butyl-6-nitrophenol is a phenolic compound having antioxidant and xanthine-oxidase inhibition activity already reported whereas squalene is a hydrocarbon and triterpene, it is reported as antioxidant, antibacterial, antifungal, anticancer, cardioprotective, detoxifying and pharmaceutical agent (Lozano-Grande *et al*., 2018). Another major compound detected was Tetracosane which has been reported as an antioxidant, antibacterial, cytotoxic, anti-inflammatory and also effective in various skin disorders (Banakar and Jayaraj, 2018; Devakumar *et al*., 2017). Compounds detected with minor quantities are also mentioned in table 2. So far, this is the first report on the identification of these compounds in this plant.

A broad variety of phenolic substances especially those found in medicinal plants have been documented to exhibit anti-cancer activities (Wang and Bachrach, 2002). TTM extract was evaluated for cytotoxic activity by XTT assay, using two human cancer cell lines. XTT assay has been intended for spectrophotometric quantification of cell growth and viability is based on the cleavage of yellow tetrazolium salt to create orange formazan dye by metabolically active cells (Chanda and Nagani, 2013). Significant ($p<0.05$) dose dependent inhibition response on cell growth against CCRF-CEM cell lines was exhibited by TTM extract which might be due to the presence of polyphenolic constituents of the plant. Whereas, non-significant ($p>0.05$) activity of TTM extract was observed against MDA-MB-231 cell lines. Further studies are suggested for the identification of compounds responsible for anti-cancer potential. TTM extract was also screened for RBC's cellular membrane protection assay. TTM extract was found to be safe towards human erythrocytes at all the tested doses. However, the activity increases with the increasing dose. To the best of our knowledge, this is the first study on safety and cytotoxic profile of *Trianthema triquetra.*

CONCLUSION

The present study offered scientific evidence for the antioxidant, cytotoxic and antimicrobial potential of *Trianthema triquetra*. It also provides supportive data for the future research that may contribute to its role in cancer, oxidative stress related disorders and antimicrobial remedies. Hemolytic activity concluded that *Trianthema triquetra* is harmless towards human erythrocytes and thus possess potential to be used in pharmaceuticals.

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