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PHARMACOLOGICAL EVALUATION OF *TAVERNIERA NUMMULARIA* DC.

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Abstract

The current study was focused on the phytochemical investigation and pharmacological activities of the various solvent fractions (methanolic, *n*-hexane, dichloromethane, ethyl acetate and water) of *Taverniera nummularia* (*T. nummularia*). The various solvent fractions were screened for phytochemicals, antibacterial assay, antifungal assay, DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, hydrogen peroxide scavenging assay and reactive oxygen species (ROS) detoxification effects on human blood lymphocytes under H_2O_2 induced stress. Methanolic extract of *T. nummularia* showed significantly higher antibacterial activity against *S. aureus* as compared to other fractions ($p<0.05$). The ethyl acetate, *n*-hexane and chloroform fractions exhibited significantly higher antibacterial activity against *P. aeruginosa*. The ranking of various fractions for antibacterial activity against *E. coli* was methanolic = ethyl acetate = aqueous > n-hexane = chloroform. Maximum antifungal activity against *Aspergillus fumigates* (59.25%), *Aspergillus flavus* (62.02%) and *Aspergillus niger* (64%) was shown by chloroform fraction. Antioxidant activity was higher for *n*-hexane, aqueous, methanolic and ethyl acetate fractions. The superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities of the human blood lymphocytes were significantly decreased under H_2O_2 induced oxidative stress ($p<0.05$). The activities of the antioxidant enzymes were significantly improved by the various leaf fractions of *T. nummularia* at 50 µg/ml under H_2O_2 stress; methanol and aqueous fractions being the most effective ones. The increase in antioxidant enzymes activities resulted in a significant decrease in the level of reactive oxygen species (ROS) and thiobarbituric acid (TBARS) content.

Key words: *Taverniera nummularia*, Antimicrobial activity, Antioxidant activity, Lymphocytes, Oxidative stress, Antioxidant enzymes.

Introduction

Plants are potential sources of antimicrobial agents such as about 60 to 90% of population in the developing countries use plant-derived medicine (Alviano & Alviano, 2009; Ullah *et al*., 2017). In traditional system of medication, crude plant extracts have been used by humans for treatment of pathogenic diseases since pre-historic times (Zhang *et al.*, 2006; Rehman *et al*., 2017). Various parts of plants such as leaves, stem, root, flowers, fruits and seeds are rich sources of phytochemicals with antimicrobial potential (Dorman & Deans, 2000;Talib & Mahasneh, 2010; Ahmad *et al*., 2016). Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses (Ruberto *et al*., 2000; Cruz *et al.*, 2007; Ahmed *et al*., 2016). Infectious diseases caused by pathogenic bacteria are common throughout the world. These bacterial species not only cause diseases in human but also in other economically important animal species such as livestock (Zhang *et al.*, 2006; Williamson *et al.*, 2008). The bacterial agents including *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus leuteas* and *Pseudomonas aeruginosa* cause various types of human infections (Cheesbrough, 1984; Peirano, 2008). Presently it has been reported that these bacterial species have shown resistance towards commonly available synthetic antibiotics (Eggleston *et al.*, 2010). Therefore, scientists are searching for potential antimicrobial agents of plant origin (Alviano & Alviano 2009; Bibi *et al.*, 2011; Tariq *et al*., 2016).

The human body faces challenges from negative effects of various agents with a potential of causing DNA damages. These agents may either come from food or the environment, viruses, ultra violet rays or oxidative agents (Gafrikova *et al.*, 2014). Hydrogen peroxide (H_2O_2) is produced in the cells during the process of oxidative metabolism. During normal metabolic process H_2O_2 is converted to water by peroxiredoxins, glutathione, peroxidases and catalases (Rhee *et al.*, 2005). However, when such mechanisms do not function properly, H_2O_2 reacts with transition metals and through the Fenton reaction produce hydroxyl radicals. This hydroxyl radical attacks the sugar component of the DNA resulting into single strand breaks of the DNA molecule (Gafrikova *et al.*, 2014). The antioxidants naturally occurring in human body functions as scavengers of hydroxyl radicals and reactive oxygen species and thus protect cell's biomolecules from adverse effects of oxidative damage (Sen *et al.*, 2010). Natural antioxidants can be obtained as dietary intake. The function of plant metabolites in scavenging ROS has attracted researchers such that many antioxidants have been isolated, characterized and purified from a number of plant species (Kalim *et al.*, 2010; Habiba *et al*., 2016).

In Pakistan, a diverse flora of medicinal plants is found naturally (*Bibi et al*., 2011; Kamal *et al*., 2016). *Taverniera nummularia* (Fabaceae) is a shrub (60-100 cm tall) and has trifoliate leaves. Flowering period is all the year round. Distribution of plant is in India and Pakistan. Roots have a sweet taste and are used as an alternative of

Glycyrrhiza glabra (Jamdhade *et al.,* 2015). Traditionally the plant is used in the treatment of digestive problems, antiulcer and anti-inflammatory (Prajapati & Patel, 2015).The present investigation was carried out to: (1) determine *In vitro* antimicrobial and antioxidant activity of the various solvent fractions of *T. nummularia* (2) determine role of *T. nummularia* in detoxification of ROS in human blood lymphocytes under H_2O_2 induced stress (3) to study prevalence of various phytochemicals in leaves of *T. nummularia*.

Materials and Methods

Collection and extraction: The plant material i.e., *Taverniera nummularia* D.C. was collected from district Bannu KP, Pakistan. The plant specimen was identified by one of us (SM) the plant specimen was assigned a voucher number (Tn-T-1) and deposited in the herbarium of Department of Botany, University of Science and Technology, Bannu, USTB.

After collection plant material was first washed with a running tap water followed by washing with distilled water. After drying under shade the dried material of *T. nummularia* (5 Kg) was converted to fine powder and dipped in methanol for seven days, and was subjected to filtration by a Whatman filter paper No.1. This protocol was also repeated for remaining residue. The filtrates were combined and methanol was evaporated under vacuum, using rotary evaporator (Buchi Rota vapor R-200) at 45°C.

Fractionation: Fractionation was carried out by suspending each extract in 250 ml distilled water separately and partitioning with different organic solvents (methanol, hexane, chloroform, ethylacetate) in order to increase polarity by using separating funnel. All the five fractions (methanol, hexane, chloroform, ethyl acetate and aqueous) were dried by evaporating respective solvent using rotary evaporator. All extracts were stored at 4°C till further analysis.

Antimicrobial activity

Antibacterial assay: The activity of extract against selected bacterial strains was carried out by agar well diffusion method (Leven *et al.*, 1979). For preparation of samples, 15mg/ml dimethyl sulfoxide (DMSO) solutions were constructed for each fraction of the plant. Similarly solutions of Roxithromycin and Cefixime-USP (1 mg/ml each) were used as positive control. Pure DMSO was incorporated as a negative control. Three bacterial strains viz. *Micrococcus luteus*, *Escherichia coli* and *Staphylococcus aureus* were cultured on solidified nutrient agar medium in petri plates separately. Wells were prepared in solidified agar nutrient medium in Petri dishes. Using a 0.1 ml pipette, 100 µl of the test sample of each fraction was put in the respective wells by micropipette. The Petri plates were incubated at 37ºC in an incubator for a period of 24 h. After completion of incubation period, zone of inhibition (mm) of all the extracts was measured.

Antifungal assay: The antifungal activity was determined by using famous agar tube dilution method as given by (Duraipandiyan & Ignacimuthu, 2009). The solutions of 12mg/ml of methanolic, chloroform, *n*-hexane, ethyl acetate and water of the plant were prepared in DMSO. Terbinafine (Positive control/ antifungal agent) at 1 mg/ml was also prepared in the DMSO. Negative control has only pure DMSO. Fungi was cultured on solidified Sabouraud dextrose agar in test tubes. The sample extracts of various fractions (67µl) having final concentration of 200 µg/ml was put in the test tubes individually. The test tubes were placed in an incubator at 28ºC for seven days. The linear growth of all the fungal strains in test tubes was measured in mm. The % inhibition in linear growth of fungi was determined by the formula given below:

$$\% \text{ Inhibition growth} = (dc\text{-}dt/dc) \times 100$$

Negative control group is represented by c whereas sample growth is given by t.

DPPH free radical scavenging activity: For antioxidant activity (DPPH free radical scavenging assay, the method of Bibi *et al*. (2011) was used. Pure DPPH (5 mg) was dissolved in methanol (100 ml) in a flask. The O.D of the solution was adjusted to 0.9 at 517 nm. A 100 µl of the various solvent fractions of the plant (250, 500, 1000 µg/ml in respective solvent) was mixed with 900 µl of the DPPH solution. The test tubes incubated in dark at room temperature for 25 minutes. The absorbance of all the samples was determined at 517 nm.

$$\text{Scavenging effect (\%)} = \frac{[(\text{Control absorbance-sample absorbance})]}{[(\text{Control absorbance})]} \times 100$$

Ascorbic acid was used as a positive control during the assay.

Hydrogen peroxide scavenging activity: Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different fractions were transferred into the test tubes and their volume was made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4). Stock solutions of the various solvent fractions were made in phosphate buffer to achieve the concentration of 50 µg/ml, 100 µg/ml, 250 µg/ml and 500 µg/ml. 0.6 ml of hydrogen peroxide solution was added to each solvent fraction in the tubes, tubes were subjected to spectrophotometer absorbance, and the hydrogen peroxide absorbance was made at 230 nm after an interval of 10 minutes as given by Ruch *et al.* (1989). Following equation was used for estimation of hydrogen peroxide scavenging activity.

$$H_2O_2 \text{ scavenging assay} = \frac{\text{Sample absorbance}}{\text{Absorbance of control}} \times 100$$

Cytotoxicity

Lymphocyte isolation: The blood samples were collected from volunteers (age 24-26 years old) for the isolation of blood lymphocytes. The blood samples (15 ml) were taken in sterile test tubes and diluted with an equal quantity of phosphate saline buffer having a pH of 7.4. The samples were centrifuged and the precipitate obtained was discarded. A 3 ml solution of ficoll-hypaque was added to 10 ml of blood by using a sterile pipette. After an hour of centrifugation (200 x *g*) lymphocytes collection was made above the ficoll-hypaque layer and added with a phosphate saline solution.

Trypan blue staining: Solution having lymphocytes was diluted by using cell culture medium (RPMI-1640, thermoscientific). The sample of lymphocytes (10 µl) was added to a 0.2% trypan blue stain (10 µl) and was loaded to a haemocytometer and observed under a light microscope. The dead (stained) and non-stained cells were counted for determination of the % of viable cells. It was found that 85% of cells per sample were alive.

Cell culturing and treatments: The samples having viable cells and diluted with cell culture medium were subjected to haemocytometer for count. The solutions having viable cells were further diluted to a concentration of 1×10^8 /ml and incubated either with pure cell culture medium or medium having added plant extracts at concentrations of 1, 10, 100 µg/ml respectively for 120 minutes at a temperature of 37ºC. The cultures of cells were subjected to centrifugation (200×g) for 10 minutes. The pellet having isolated cells was collected whereas supernatant was discarded. The pellet of cells was dispersed in a PBS (1×10^6) and preserved at -20ºC for further experimentation.

Biochemical analysis: The cells were taken and lysed by using ultra-sonication. The obtained lysate was utilized for the determination of activity of antioxidant enzymes and the content of reactive oxygen species.

Superoxide dismutase activity (SOD): For determination of activity of SOD, the procedure of Marklund & Marklund (1974) was followed by taking optical density of the supernatant at 470 nm by using aspectrophotometer.

The activity of SOD was taken as the amount of enzyme necessary to dismutase 50% of the superoxide radicals in one minute. The unit of SOD activity was expressed as mill i.e., mU/10^6 cells.

Catalase activity: A reagent of dichromate/acetic acid having potassium dichromate solution in acetic acid (5%) was prepared (1:3). A 0.2 M H_2O_2 was mixed with phosphate buffer (0.01 M). The method of Sinha (1972) was followed for the determination of activity of catalase. Reaction mixture was composed of 1 ml of phosphate buffer (0.01 M), 100µl of tissue homogenate and 400µl of H_2O_2 (2M). After completion of incubation period the reaction was stopped by the addition of dichromate-acetic acid reagent (2 ml). Measurement of absorbance was made at 530 nm and activity of catalase was measured as µM of H_2O_2 consumed/min/mg protein.

Peroxidase activity: Peroxidase activity was determined by the procedure of Carlberg & Mannervik (1975). A homogenate of 0.1 ml was prepared having 0.1 ml guaiacol, 0.3 ml H2O2 (40 mM) and 2.g ml phosphate buffer (50 mM). After a minute of incubation the change in color of mixture was measured at 470 nm using a spectrophotometer. The POD unit was measured as a change in the absorbance of 0.01 unit/minute.

TBARS: The estimation of TBARS was made by following the method of Li *et al.* (2010). The content of TBARS was estimated by absorption at 535 nm at an extinction coefficient of 156 mM/cm. The unit of TBARS content was nano-moles per 10^6 cells.

Reactive oxygen species (ROS): The content of reactive oxygen species was determined by using the method of Hayashi *et al.* (2007). A cell suspension (5 µl) or H_2O_2 (used as standard) was mixed with a buffer solution ofsodium acetate (pH 4.8) in a well plate. The mixture was subjected to incubation at a 37ºC for 5 minutes. A solution (100 ul) composed of DEPPD and $FeSO_4$ (mixed in ratio of 1:12) was put into the wells and incubated for 60 seconds at a 37ºC. Absorbance of the samples in wells was determined at 505 nm for three minutes at a time period of 5 seconds using a micro plate reader. The concentration of ROS in Unit/10^6 cells was reported.

Statistical analyses: The data of cytotoxicity were subjected to analysis of variance (two way ANOVA) whereas those of antimicrobial was analyzed by one way ANOVA. The mean values were compared by using least significant differences test (LSD).

Results

Antibacterial activity: We observed that methanolic extract of *T.nummularia* showed significantly higher antibacterial activity against *S. aureus* as compared to other fractions ($p<0.05$). Antibacterial activity of the ethyl acetate and chloroform fractions against *S. aureus* was statistically similar but significantly higher than aqueous and *n*-hexane fractions (Fig. 1).The ethyl acetate, *n*-hexane and chloroform fractions exhibited higher and statistically similar antibacterial activity against *P. aeruginosa*. The ranking of various fractions for antibacterial activity against *E. coli* was as: crude extract >methanolic = ethyl acetate = aqueous > n-hexane = chloroform.

Antifungal activity: The leaf fractions of *T. nummularia* showed antifungal activity against the tested fungal strains. Maximum antifungal activity against *Aspergillus fumigates* (59.25%), *Aspergillus flavus* (62.02%) and *Aspergillus niger* (64%) was shown by chloroform fraction. Next to the chloroform, methanolic fraction was more effective against *Aspergillus fumigates* (56.65%), *n*-hexane against *Aspergillus flavus* (48.5%) and ethyl acetate against *Aspergillus niger* (50.9%) (Fig. 2).

DPPH free radical scavenging activity: Antioxidant activity was measured as % reactive oxygen species scavenging activity of DPPH using ascorbic acid as a standard. Results showed that significantly higher antioxidant activity was recorded for methanolic extract followed by *n*-hexane and aqueous fractions at 1000µg/ml ($p<0.05$). At lowest concentration (250 µg/ml) the antioxidant activity was higher for methanolic extract (Fig. 3).

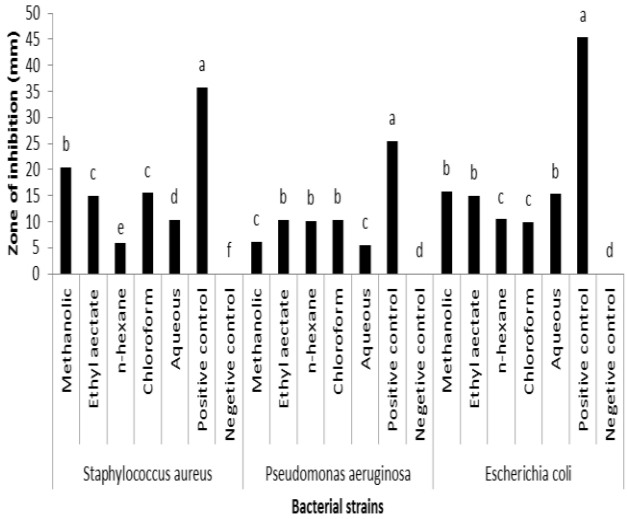

Fig. 1. Antibacterial activity of the various fractions of *Taverniera nummularia* against *Staphylococcus aureus* (Least Significant difference: 1.449), *Pseudomonas aeruginosa* (Least Significant difference: 0.648) and *Escherichia coli* (Least Significant difference: 1.053). Means sharing common English letters are statistically similar.

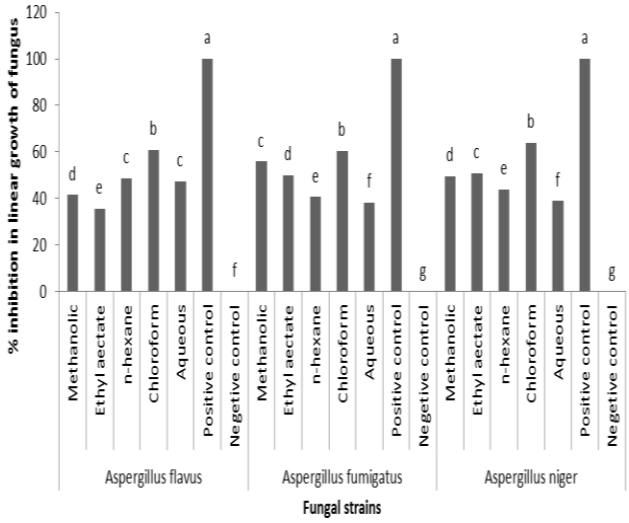

Fig. 2. Antifungal activity of the various fractions of *Taverniera nummularia* against *Aspergillus flavus* (Least Significant difference: 2.749), *Aspergillus fumigates* (Least Significant difference: 1.883) and *Aspergillus niger* (Least Significant difference: 0.702). Means sharing common English letters are statistically similar.

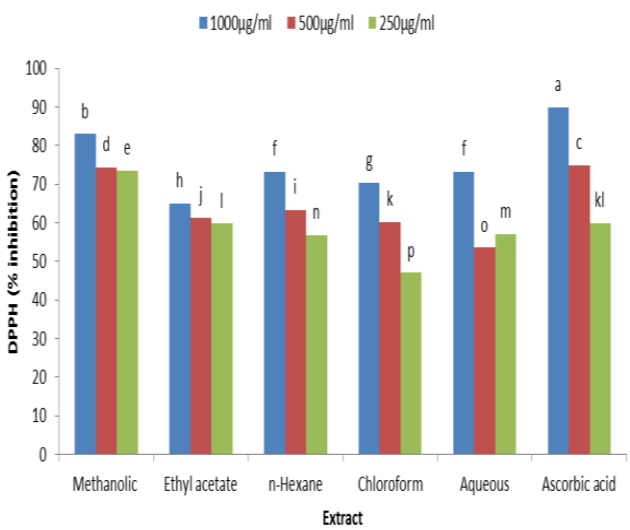

Fig. 3. Antioxidant activity (DPPH method) of *Taverniera nummularia* (Least Significant difference: 0.273). Means sharing common English letters are statistically similar.

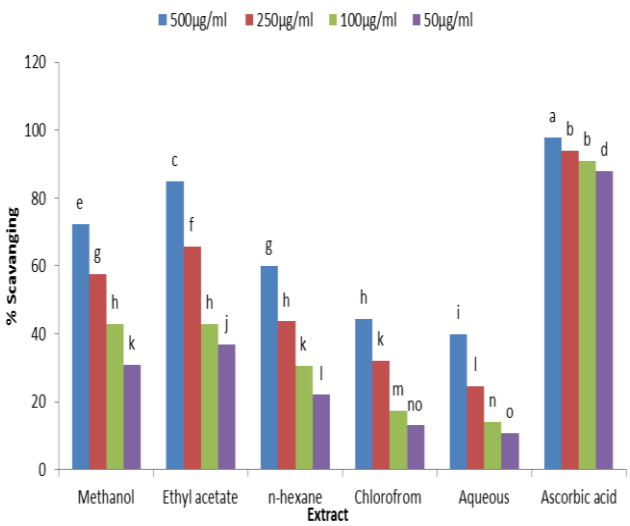

Fig. 4. Antioxidant activity (H_2O_2 scavenging method) of *Taverniera nummularia* (Least Significant difference: 2.850). Means sharing common English letters are statistically similar.

Hydrogen peroxide scavenging activity: H_2O_2 scavenging activity is concentration dependent. All the solvent fractions were found to have significant scavenging ability (Fig. 4). At higher concentration of the extracts (500 µg/ml) maximum scavenging activity was shown for ethyl acetate fraction (85%). Whereas lowest for aqueous (40%). H_2O_2 scavenging activity of the various solvent fractions of *Taverniera nummularia* were found to be in the decreasing order of E/Acetate >Methanolic extract > n-hexane > chloroform and aqueous.

Effect of *T. nummularia* on antioxidant enzymes activity of lymphocytes: The SOD, CAT and POD activities of lymphocytes were significantly decreased under H_2O_2 induced oxidative stress as compared to control ($p<0.05$). Under unstressed conditions the various leaf fractions of *T. nummularia* at 50 µg/ml significantly increased antioxidant enzymes activity than control. The extracts at all the concentrations minimized adverse effects of H_2O_2 stress on antioxidant enzymes activity. Among the various fractions, methanol and aqueous fraction was significantly more effective (Tables 1-3).

Effect of *T. nummularia* on ROS content of lymphocytes: The H_2O_2 stress resulted in elevated level of ROS in lymphocytes as compared to control (Table 4). The increase in ROS due to H_2O_2 treatment was significantly reduced by various fractions of *T. nummularia*. Most effective concentrations of the various fractions were 5ug/ml and 50 ug/ml. The methanol and ethyl acetate fraction was significantly more effective in reducing the level of ROS ($p>0.05$).

Table 1. Effect of *Taverniera nummularia* on SOD activity (milli Units / 10^6 cells) of lymphocytes under H_2O_2 induced oxidative stress.

Treatment	Fractions					Mean
	Methanol	Ethyl acetate	*n*-hexane	Chloroform	Aqueous	
Control	9.244 ± 1.05^{de}	9.244 ± 1.05^{de}	9.244 ± 1.05^{de}	9.244 ± 1.05^{de}	9.244 ± 1.05^{de}	9.244 ± 1.05^{b}
H_2O_2 (100µM)	4.036 ± 0.31^{k}	4.036 ± 0.31^{k}	4.036 ± 0.31^{k}	4.036 ± 0.31^{k}	4.036 ± 0.31^{k}	4.036 ± 0.31^{e}
Extract 0.5 µg/ml	$10.648 \pm 3.14^{a-d}$	$10.124 \pm 1.98^{a-e}$	9.152 ± 2.10^{e}	9.110 ± 1.69^{ef}	$9.610 \pm 2.13^{b-e}$	9.729 ± 2.21^{ab}
Extract 5 µg/ml	$10.284 \pm 1.97^{a-e}$	$9.552 \pm 2.18^{c-e}$	$10.156 \pm 3.19^{a-e}$	$9.870 \pm 1.26^{b-e}$	11.082 ± 3.16^{ab}	10.189 ± 2.36^{a}
Extract 50 µg/ml	$10.749 \pm 2.16^{a-c}$	$10.478 \pm 1.79^{a-e}$	$9.658 \pm 2.17^{b-e}$	7.668 ± 1.95^{fg}	11.484 ± 2.10^{a}	10.007 ± 2.03^{a}
H_2O_2 (100µM) + extract 0.5 µg/ml	5.297 ± 0.98^{k}	$5.016 \pm 0.41^{i-k}$	4.492 ± 0.50^{jk}	$4.896 \pm 0.21^{i-k}$	$6.302 \pm 0.71^{g-i}$	5.201 ± 0.56^{d}
H_2O_2 (100µM) + extract 5 µg/ml	7.367 ± 0.31^{gh}	6.094 ± 0.95^{hi}	$5.330 \pm 0.24^{i-k}$	4.172 ± 0.37^{k}	5.770 ± 0.29^{ij}	5.747 ± 0.43^{cd}
H_2O_2 (100µM) + extract 50 µg/ml	7.547 ± 0.29^{gh}	$5.262 \pm 0.38^{i-k}$	$5.112 \pm 0.20^{i-k}$	$4.966 \pm 0.29^{i-k}$	7.635 ± 1.00^{fg}	6.104 ± 0.43^{c}
Mean	8.147 ± 040^{a}	7.476 ± 0.41^{b}	7.148 ± 0.44^{bc}	6.745 ± 0.12^{c}	8.145 ± 0.38^{a}	

± Represents value of standard error. Means sharing common English letters are statistically similar. (Treatment: Least significant difference 0.662, Fractions: Least significant difference 0.523, Treatment x Fraction: Least significant difference 1.481)

Table 2. Effect of *Taverniera nummularia* on CAT activity (milli Units / 10^6 cells) of lymphocytes under H_2O_2 induced oxidative stress.

Treatment	Fractions					Mean
	Methanol	Ethyl acetate	*n*-hexane	Chloroform	Aqueous	
Control	$2.810 \pm 0.13^{b-e}$	$2.810 \pm 0.13^{b-e}$	$2.810 \pm 0.13^{b-e}$	$2.810 \pm 0.13^{b-e}$	$2.810 \pm 0.13^{b-e}$	2.810 ± 0.13^{a}
H_2O_2 (100µM)	0.946 ± 0.17^{n}	0.946 ± 0.17^{n}	0.946 ± 0.17^{n}	0.946 ± 0.17^{n}	0.946 ± 0.17^{n}	0.946 ± 0.17^{c}
Extract 0.5 µg/ml	3.356 ± 0.21^{ab}	$2.750 \pm 0.20^{b-e}$	$2.356 \pm 0.19^{e-h}$	$2.690 \pm 0.15^{b-e}$	$2.707 \pm 0.18^{b-e}$	2.772 ± 0.19^{a}
Extract 5 µg/ml	$3.230 \pm 0.24^{a-c}$	$2.550 \pm 0.18^{d-f}$	$2.618 \pm 0.13^{c-e}$	$2.886 \pm 0.10^{b-e}$	$2.876 \pm 0.12^{b-e}$	2.832 ± 0.15^{a}
Extract 50 µg/ml	3.698 ± 0.19^{a}	$2.534 \pm 0.31^{d-f}$	$3.126 \pm 0.54^{a-d}$	$2.716 \pm 0.40^{b-e}$	$2.942 \pm 0.37^{b-e}$	3.003 ± 0.36^{a}
H_2O_2 (100µM) + extract 0.5 µg/ml	$1.902 \pm 0.11^{f-j}$	0.988 ± 0.02^{n}	0.932 ± 0.18^{n}	$1.448 \pm 0.12^{j-n}$	$1.672 \pm 0.26^{i-m}$	1.388 ± 0.14^{b}
H_2O_2 (100µM) + extract 5 µg/ml	$2.460 \pm 0.10^{d-g}$	1.009 ± 0.31^{mn}	1.052 ± 0.28^{mn}	$1.290 \pm 0.10^{j-n}$	$1.724 \pm 0.30^{h-l}$	1.507 ± 0.22^{b}
H_2O_2 (100µM) + extract 50 µg/ml	$2.316 \pm 0.14^{e-i}$	$1.084 \pm 0.29^{l-n}$	$1.188 \pm 0.28^{k-n}$	$1.292 \pm 0.18^{j-n}$	$1.842 \pm 0.20^{g-k}$	1.544 ± 0.22^{b}
Mean	2.590 ± 0.40^{a}	1.834 ± 0.47^{c}	1.879 ± 0.39^{c}	2.0097 ± 0.58^{bc}	2.1899 ± 0.39^{b}	

± Represents value of standard error. Means sharing common English letters are statistically similar. (Treatment: Least significant difference 0.299, Fractions: Least significant difference 0.236, Treatment x Fraction: Least significant difference 0.670)

Table 3. Effect of *Taverniera nummularia* on POD activity (nmol / 10^6cells) of lymphocytes under H_2O_2 induced oxidative stress.

Treatment	Fractions					Mean
	Methanol	Ethyl acetate	*n*-hexane	Chloroform	Aqueous	
Control	4.892 ± 0.73^{ab}	4.892 ± 0.73^{ab}	4.892 ± 0.73^{ab}	4.892 ± 0.73^{ab}	4.892 ± 0.73^{ab}	4.892 ± 0.73^{ab}
H_2O_2 (100µM)	2.272 ± 0.18^{e}	2.272 ± 0.18^{E}	2.272 ± 0.18^{e}	2.272 ± 0.18^{E}	2.272 ± 0.18^{e}	2.272 ± 0.18^{d}
Extract 0.5 µg/ml	$4.622 \pm 0.27^{A-C}$	3.258 ± 0.14^{de}	4.973 ± 0.18^{ab}	$5.671 \pm 0.35a$	5.217 ± 0.28^{a}	4.748 ± 0.24^{b}
Extract 5 µg/ml	$4.650 \pm 0.16^{a-c}$	5.049 ± 0.29^{ab}	$4.689 \pm 0.13^{a-c}$	5.160 ± 0.20^{a}	5.538 ± 0.34^{a}	5.017 ± 0.22^{ab}
Extract 50 µg/ml	2.272 ± 0.27^{e}	5.312 ± 0.27^{a}	5.136 ± 0.19^{A}	5.250 ± 0.42^{a}	5.814 ± 0.29^{a}	5.434 ± 0.29^{a}
H_2O_2 (100µM) + extract 0.5 µg/ml	2.733 ± 0.10^{de}	2.399 ± 0.17^{e}	2.197 ± 0.10^{e}	2.370 ± 0.11^{e}	2.222 ± 0.18^{e}	2.384 ± 0.26^{cd}
H_2O_2 (100µM) + extract 5 µg/ml	2.624 ± 0.09^{de}	3.226 ± 0.22^{de}	2.568 ± 0.16^{de}	2.939 ± 0.13^{de}	$3.364 \pm 0.11^{c-e}$	2.944 ± 0.14^{c}
H_2O_2 (100µM) + extract 50 µg/ml	2.804 ± 0.11^{de}	2.694 ± 0.18^{de}	2.699 ± 0.21^{de}	2.411 ± 0.20^{e}	$3.782 \pm 0.19^{b-d}$	2.878 ± 0.18^{c}
Mean	3.782 ± 0.38^{ab}	3.638 ± 0.25^{b}	3.678 ± 0.17^{ab}	3.871 ± 0.32^{ab}	4.137 ± 0.58^{a}	

± Represents value of standard error. Means sharing common English letters are statistically similar. (Treatment: Least significant difference 0.592, Fractions: Least significant difference 0.468, Treatment x Fraction: Least significant difference 1.325)

Table 4. Effect of *Taverniera nummularia* on ROS content (Units / 10^6 cells) of lymphocytes under H_2O_2 induced oxidative stress.

Treatment	Fractions					Mean
	Methanol	Ethyl acetate	*n*-hexane	Chloroform	Aqueous	
Control	$0.134 \pm 0.02^{g-j}$	$0.134 \pm 0.02^{g-j}$	$0.134 \pm 0.02^{g-j}$	$0.134 \pm 0.02^{g-j}$	$0.134 \pm 0.02^{g-j}$	0.134 ± 0.02^{de}
H_2O_2 (100µM)	0.284 ± 0.04^{a}	0.284 ± 0.04^{a}	0.284 ± 0.04^{a}	0.284 ± 0.04^{a}	0.284 ± 0.04^{a}	0.284 ± 0.04^{A}
Extract 0.5 µg/ml	$0.132 \pm 0.01^{g-j}$	$0.166 \pm 0.01^{e-h}$	$0.153 \pm 0.04^{f-i}$	$0.129 \pm 0.01^{g-j}$	$0.154 \pm 0.02^{f-i}$	0.147 ± 0.12^{d}
Extract 5 µg/ml	$0.130 \pm 0.06^{g-j}$	$0.142 \pm 0.07^{g-j}$	$0.123 \pm 0.02^{h-j}$	$0.127 \pm 0.02^{g-j}$	$0.125 \pm 0.04^{g-j}$	0.129 ± 0.04^{de}
Extract 50 µg/ml	0.100 ± 0.01^{j}	$0.131 \pm 0.03^{g-j}$	$0.125 \pm 0.01^{g-j}$	0.108 ± 0.06^{ij}	$0.122 \pm 0.05^{H-J}$	0.117 ± 0.03^{e}
H_2O_2 (100µM) + extract 0.5 µg/ml	0.264 ± 0.08^{ab}	0.278 ± 0.01^{ab}	0.257 ± 0.06^{AB}	0.253 ± 0.05^{ab}	$0.245 \pm 0.01^{a-d}$	0.259 ± 0.04^{b}
H_2O_2 (100µM) + extract 5 µg/ml	$0.173 \pm 0.01^{e-g}$	0.255 ± 0.04^{ab}	$0.236 \pm 0.04^{a-d}$	$0.201 \pm 0.02^{d-f}$	$0.234 \pm 0.01^{b-d}$	0.220 ± 0.03^{c}
H_2O_2 (100µM) + extract 50 µg/ml	$0.162 \pm 0.01^{e-h}$	0.269 ± 0.02^{ab}	$0.251 \pm 0.01^{a-c}$	$0.203 \pm 0.05^{c-e}$	$0.239 \pm 0.04^{a-d}$	0.225 ± 0.03^{c}
Mean	0.172 ± 0.02^{c}	0.207 ± 0.01^{a}	0.195 ± 0.03^{ab}	0.179 ± 0.02^{bc}	0.192 ± 0.01^{ab}	

± Represents value of standard error. Means sharing common English letters are statistically similar. (Treatment: Least significant difference 0.021, Fractions: Least significant difference 0.017, Treatment x Fraction: Least significant difference 0.048)

Table 5. Effect of *Taverniera nummularia* on TBARS (nano-moles/ 10^6 cells) of lymphocytes under H_2O_2 induced oxidative stress.

Treatment	Fractions					Mean
	Methanol	Ethyl acetate	*n*-hexane	Chloroform	Aqueous	
Control	$0.696 \pm 0.09^{f-j}$	$0.696 \pm 0.09^{f-j}$	$0.696 \pm 0.09^{f-j}$	$0.696 \pm 0.09^{f-j}$	$0.696 \pm 0.09^{f-j}$	0.696 D
H_2O_2 (100µM)	1.014 ± 0.17^{a}	1.014 ± 0.17^{a}	1.014 ± 0.17^{a}	1.014 ± 0.17^{a}	1.014 ± 0.17^{a}	1.014 A
Extract 0.5 µg/ml	$0.535 \pm 0.06^{k-m}$	$0.631 \pm 0.07^{h-k}$	$0.652 \pm 0.04^{h-k}$	$0.624 \pm 0.10^{h-l}$	$0.652 \pm 0.07^{h-k}$	0.619 ± 0.07^{e}
Extract 5 µg/ml	0.484 ± 0.08^{lm}	$0.609 \pm 0.04^{i-l}$	$0.683 \pm 0.07^{g-j}$	$0.651 \pm 0.09^{h-k}$	$0.589 \pm 0.02^{i-m}$	0.603 ± 0.06^{e}
Extract 50 µg/ml	0.465 ± 0.09^{m}	$0.587 \pm 0.04^{i-m}$	$0.568 \pm 0.03^{j-m}$	$0.641 \pm 0.09^{h-k}$	$0.558 \pm 0.02^{J-M}$	0.564 ± 0.05^{e}
H_2O_2 (100µM) + extract 0.5 µg/ml	$0.903 \pm 0.03^{a-c}$	$0.907 \pm 0.09^{a-c}$	0.994 ± 0.05^{ab}	$0.874 \pm 0.08^{b-d}$	$0.756 \pm 0.08^{d-h}$	0.887 ± 0.12^{b}
H_2O_2 (100µM) + extract 5 µg/ml	$0.831 \pm 0.05^{c-f}$	$0.910 \pm 0.04^{a-c}$	$0.883 \pm 0.07^{a-d}$	$0.849 \pm 0.10^{c-e}$	$0.754 \pm 0.01^{D-H}$	0.845 ± 0.05^{bc}
H_2O_2 (100µM) + extract 50 µg/ml	$0.72 \pm 0.09^{e-i}$	$0.844 \pm 0.05^{c-e}$	$0.813 \pm 0.09^{c-g}$	$0.950 \pm 0.08^{a-c}$	$0.718 \pm 0.05^{e-i}$	0.810 ± 0.07^{c}
Mean	0.707 ± 0.06^{b}	0.7749 ± 0.03^{a}	0.788 ± 0.02^{a}	0.787 ± 0.06^{a}	0.717 ± 0.046^{b}	

± Represents value of standard error. Means sharing common English letters are statistically similar. (Treatment: Least significant difference 0.062, Fractions: Least significant difference 0.049, Treatment x Fraction: Least significant difference 0.139)

Table 6. Qualitative analysis of various fractions.

Fractions	Sterols	Anthocynidins	Cardiac glycosides	Tannins	Saponins	Terpenoids	Coumarins	Anthraquinones
Crude methanolic extract	-	-	+	+	+	-	-	-

- Indicates absence whereas+ Represents presence of bioactive compound

Effect of *T. nummularia* on TBARS of lymphocytes: The lymphocytes treated with H_2O_2 exhibited higher TBARS activity (nano-moles per 106 cells) over control group. Under unstressed conditions various fractions of *T. nummularia* did not significantly affect TBARS as compared to control (Table 5). The increase in TBARS activity due to H_2O_2 treatment was significantly reduced by various fractions at all the concentrations tested. The most effective concentrations were 5 ug/ml and 50 ug/ml. Among the various fractions, methanol and ethyl acetate fraction was significantly more effective in reducing TBARS content under oxidative stress ($p<0.05$).

Phytochemicals: The crude extract was analyzed for the presence of biologically activity compounds and showed the presence of Cardiac glycosides, tannins and saponins (Table 6).

Discussion

The use of herbal medicines in the treatment of pathogenic diseases has been increased in recent years due to their effectiveness, sustainability and local availability (Haq *et al.*, 2016; Ikram *et al.*, 2015). The antimicrobial potential of plants isdue to the presence of various kinds of phytochemicals (Nascimento *et al.*, 2000; Gibbons, 2004; Begum *et al.*, 2015).The disadvantages of the presently available drugs have propelled the discovery of novel pharmaco therapeutic agents from plants (Cordell, 1993). *T. nummularia* leaf was a rich source of phytochemicals such as cardiac glycosides, tannins and saponins with a potent antibacterial and antifungal activity. The antimicrobial activities of natural compounds are helpful in the discovery of new and novel antibiotic compounds which can be used in the control of pathogenic diseases of both animals and plants (Sandhu &Heinrich, 2005).

The various solvent fractions of *T. nummularia* exhibited antioxidant activity measured as DPPH and H_2O_2 scavenging method**.** The antioxidant activity of methanolic, *n*-hexane and aqueous fractions was higher. Previous studies have shown that oxidative stress is associated with pathogenesis and various chronic diseases and therefore antioxidant behavior is one of the most commonly determined biological activities for biologically active compounds (Duthie *et al.,* 2000). The *T. nummularia* exhibited antioxidant activity and therefore must be tested for cytotoxic and anticancer activities because compounds having a good antioxidant activity are potentially anticancer agents (Dudonne *et al.,* 2009).

The removal of reactive oxygen species by natural compounds is correlated with their antioxidant activity (Ames *et al.,* 1993). During present studies the methanolic and aqueous fractions of *T. nummularia* were highly effective to increase activity of antioxidant enzymes and which resulted in lower ROS content. Similarly both the methanol and aqueous fractions exhibited higher antioxidant activity. Water and methanol are polar solvents and are used to extract polar compounds. Both of these solvents have low toxicity and can be used with success in animal models (Al-Terehi *et al.,* 2012). This study established that phytochemicals present in methanol and aqueous extracts are rich sources of antioxidants with a potential to detoxify ROS generated as a result of oxidative stress. Previous studies of (Al-Terehi, 2012) have shown that *Glycyrrihza glabra* root extract was highly effective to reduce oxidative stress in experimental animals. Plant extracts contain phenolics and flavonoids which have antioxidant activity because of their potential of donor proton to free radicals. Moreover, they have protective effects on antioxidant enzymes because they prevent oxidation of proteins by free radicals (Dimitrios, 2006). These reactive oxygen species cause

damages to DNA resulting into mutation which leads to cancer (Gafrikova *et al.,* 2014). It was established that *T. nummularia* minimized adverse effects of H_2O_2 induced oxidative stress on human blood lymphocytes.

Conclusion

The various solvent fractions of *T. nummularia* exhibited antibacterial (*S. aureus*, *P. aeruginosa*, *E. coli*) and antifungal (*Aspergillus fumigatus*, *Aspergillus flavous*, *Aspergillus niger*) activity. The DPPH free radical scavenging activity was higher at 500 µg/ ml for methanolic extract. The decrease in TBARS content exhibited that various fractions of the *T. nummularia* effectively protected membrane lipid peroxidation of the lymphocytes.

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