ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC EVALUATION OF VARIOUS EXTRACTS OF *IFLOGA SPICATA* (FORSSK.) SCH. BIP.

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***Abstract* – The current study reveals the pharmacological and phytochemical potential of different solvent fractions of *Ifloga spicata* (*I. spicata*). Antimicrobial and free radical scavenging activities, DPPH (1, 1-diphenyl-2-picrylhydrazyl), hydrogen peroxide scavenging assay, and cytotoxic effects of plant samples on lymphocytes stressed with hydrogen peroxide were performed. *Ifloga spicata* ethyl acetate fraction markedly showed higher antibacterial potential against *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) bacteria. While higher antifungal potential was shown by the chloroform fraction against all three different strain of *Aspergillus fumigatus* (ATCC 204305), *Aspergillus flavus* (ATCC 9643) and *Aspergillus niger (*ATCC 16404) as compared to others fractions of sample. The DPPH maximum antioxidant potential was noted for methanolic extract followed by ethyl acetate and chloroform. Similarly, hydrogen peroxide effect was significantly decreased by ethyl acetate fraction. In blood lymphocytes the increase in ROS and TBARS levels due to stress produced by H_2O_2 was also concentrated by different extracts of I. spicata. The decreased levels of CAT, SOD and POD were also restored to its normal by all extract of the plant.**

***Keywords* – Ifloga spicata, Antimicrobial, Reactive oxygen species, Human blood lymphocytes, Hydrogen peroxide.**

I. INTRODUCTION

Herbal medicine is ever raising demand of the modern era, because multiple drugs resistance has been developed against microbial disease due to irregular practice of the synthetic drugs. To avoid the side effect and microbial resistance of the synthetic drugs scientists are trying to use herbal medicine against microbial infections. Medicinal plants contain certain bioactive ingredients, which are used for various medicinal purposes. In the developing countries, approximately 60 to 90% peoples using medicinal plants to control of different diseases (Alviano and Alviano, 2009). The development of newly safe antimicrobial drug is one of the ever-raising demands. Pathogenic bacteria has adverse effect on human and livestock all around world (Williamson *et al.*, 2008). Therefore, to minimize the adverse effect of these pathogenic microbes' scientists are trying to identify and isolate antimicrobial agents of plant origin.

Antioxidants quench and stabilize free radicals. They protect cellular molecules from reactive oxygen species (Ayaz *et al*., 2014; Naz *et al*., 2020). When cells face oxidative stress due to attack of pathogens, entrance of toxic chemicals and pollutants antioxidants defense system is activated. This antioxidant defense system is composed of both enzymatic (antioxidant enzymes) and non-enzymatic (pehnols, flavonoids, etc) antioxidants. The antioxidant enzymes like hydrogen peroxidase and catalase cause the conversion of hydroperoxides and hydrogen peroxide to nonradical forms. Therefore, for the protection of cellular system from oxidative stress production of antioxidants is necessary (Barlow, 1990). Various kinds of synthetic compounds like gallic acid, butylated hydroxy anisole and butylated hydroxy toluene are extensively used as antioxidants. However, synthetic antioxidants are reported for their negative effects on living organisms (Kil *et al*., 2009). Therefore, natural antioxidants found in plants offer a plausible option for scavenging free radicals and protection of cellular components from oxidative stress (Ajaib *et al*., 2016; Naz *et al*., 2020)

Fruits, seeds, flower, leaves and stem possess various bioactive agents with antibacterial, phytochemicals and antioxidant potential (Talib and Mahasneh, 2010). During metabolism hydrogen peroxide is produced. In normal metabolism hydrogen peroxide is changed into water through peroxiredoxins, catalases and peroxidases (Rhee *et al*., 2005). But when hydrogen peroxide (H_2O_2) is not removed by cellular protective detoxification system, the transition elements can react to form hydroxyl radicals by Fenton reaction. These radicals then attack on DNA molecule sugar component and damages a single DNA molecule stand. To overcome the adverse effect of these agents medicinal plants are used for anticancer, anti-inflammatory and digestive problems (Prajapati and Patel,

2015). Naturally, there are different varieties of medicinal plants in Pakistan (Bibi, 2011). Based on ethno medicinal value *Ifloga spicata* is used in present study. *I. spicata* belonging to family Asteraceae, is an annual herb having height of 5-10 cm usually branched at the base. The stems and branches of the plant make dense globular to cylindrical inflorescence either at base or in the upper portion. It usually prefers to grow in desserts. *Ifloga spicata* is mostly distributed in Canary Island, southern Spain to N. Africa through Middle East to Afghanistan, Pakistan and India. Flowering period is February-September (Jamdhade *et al*., 2015). Studies have reported antiallergic, anti-leishmanial and anti-inflammatory properties of *I. spicata* (Osman *et al*., 2014; Shah *et al*., 2019). Therefore, the present investigation aimed on the determination of antimicrobial, antioxidant and cytotoxic potential of *I. spicata* extracts on human blood lymphocytes exposed to H_2O_2 oxidative stress.

II. METHODS

The plant *I. spicata* was collected from Bannu District Khyber Pakhtunkhwa, Pakistan. Pant species was recognized by Dr. Faizan Ullah Khan Assistant professor Plant science Department Bannu University Pakistan. It was assigned with a voucher No (Is-I5) and was stored in Botany Department, Bannu University.

A. Extraction and Final Product Preparation

Plant material was first washed with tap water followed by distilled water to remove dust particles. The plant material was dried in shade for 10 days and converted into fine powder. A 5 kg powder of *I. spicata* was dissolved in 80 % aqueous methanol and was placed for 5 days. The methanolic solution of *I. spicata* was then filtered through Whatman filter paper and dried using rotary evaporator. To remove the fatty material the dried material was partitioned with *n*-hexane. After fatty material removal filtrate was mixed with water followed by chloroform, ethyl acetate in increasing order polarity to fractionate the remaining residue. All the five extracts were dried using rotary evaporator and stored under control condition.

B. Antimicrobial Activity

For antibacterial potential fraction (3 mg/ml DMSO) of each was checked against bacterial species *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 27853) and *E.coli* (ATCC 25922) by agar well known diffusion method. The bacterial strains were obtained from Department of Biotechnology University of Science and Technology Bannu. The antibiotics Cefixime and Roxithromycin (1 mg/ml DMSO) were taken into consideration as a positive control. A 100 µl of each extract or antibiotics was poured into separate wells made in agar plates inoculated with cultured bacteria. After incubation of plates at 37°C for 24 hours period, clear zones of inhibition surrounding the wells were measured in mm.

C. Antifungal Activity

For antifungal activity 67µl of the different extracts (12 mg/ml DMSO) were mixed with melted dextrose agar in test tubes. Test tubes were kept in a slating position and inoculated with fungi *Aspergillus flavus* (ATCC 9643), *Aspergillus niger* (ATCC 16404) and Aspergillus fumigatus (ATCC 204305) separately. The fungal strains were identified and identified by microbiologist Dr. Masroor Hussain in the Department of Biotechnology University of Science and Technology Bannu. Positive control had antifungal agent Terbinafine. After seven days of incubation at 28°C in each test tube percent inhibition in linear growth of fungi was determined.

$$\%\ inhibition\ growth\ =\ (dc - dt/dc)\ x100$$

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

C. DPPH Free Radical and Hydrogen Peroxide Scavenging Activities

The α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging assay was performed accordingly with that of Bibi *et al.* (2011). The different extracts of *I. spicata* were dissolved in respective solvents at various concentrations (250, 500 and 1000µg/ml). Then 100µl of the extracts were mixed with 3ml of DPPH solution and after incubating for 30 min the optical density of solutions was noted at 517 nm.

$$DPPH\ Scavenging\ (\%) = \frac{(Blank\ absorbance - sample\ absorbance)}{(Blank\ absorbance)}\ X\ 100.$$

D. Cytotoxic Activity

In the initial step lymphocytes were isolated from blood samples in a saline phosphate buffer (pH 7.4) solution collected from healthy persons with an average age of 25 years old. The samples of blood were rotated in a centrifuge machine and after discarding supernatant the pellets were added with ficoll-hypaque solution (3 ml). After rotating the samples again in a centrifuge machine (200 x g) lymphocytes were appeared above the ficoll-hypaque layer and were collected in a saline phosphate buffer solution. The isolated lymphocytes were then dilute with culturing medium (RPMI-1640, thermoscientific). A 10 µl of the culture medium was further added with trypan blue stain (0.2%) and taken to a haemocytometer equipped with a light microscope (Cole *et al*., 1988). After separating the stained (dead) and alive (non-stained) lymphocytes it was observed that more than 85% of the cells were in a living state. The culture media having lymphocytes was further added with more culture media (1×10^8 cells /ml) either pure or having 1, 10, 100 µg/ml of the extracts in separate containers and placed in an incubator at 37 ˚C for complete two hours. After centrifugation (200×g) for 15 minutes the pellet was collected and preserved in PBS (1×10^6) at -20 ˚C. The pellet having lymphocytes was used for biochemical evaluation. The antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were extracted and determined.

The method of Marklund and Marklund (1974) was followd for SOD determination by determining optical density of enzyme extract using a spectrophotometer at 470 nm. The superoxide dismutase activity unit was

demonstrated as mU/10^6 cells.

For catalase action dichromate/acetic acid reagent (1:3) was initially prepared (Sinha, 1972). The H_2O_2 (0.2 M) was dissolved in 0.01 M phosphate buffer. Reaction mixture was comprised of 0.01 M phosphate buffer, tissue homogenate (100µl) and 2M $H2O_2$ (400µl). After adding 2 ml dichromate/acetic acid reagent samples were incubated and analyzed for absorbance at 530 nm. The catalase activity was measured µM of H_2O_2 consumed/min/mg protein.

Using method of Carlberg and Mannervik,1975 peroxidase activity was meassured. Homogenete (0.1 ml) was set containing guaiacol (100 µl), H_2O_2 (300 µl) and 50 mM phosphate buffer. Change in color after incubation of samples for 60 seconds optical density was scaled at 470 nm. Estimation of TBARS was made according to Li et al.,2010 by meassuring absorbance of samples at 535 nm.

The content of ROS was estimated according to procedure of Hayashi *et al.* (2007). Cell suspension (5 µl) or pure H_2O_2 (taken as standard) was poured in a well plate containing sodium acetate buffer (pH 4.7). Mixtures of solutions were incubated at 37˚C for 5 minutes and then added with DEPPD and $FeSO_4$ (mixed in ratio of 1:12) solution (100 µl) and again incubated at 37 C^o for one minute. Samples absorbance was measured at 505 nm for a duration of three minutes at an interval f 3 minutes. The ROS concentration was reported as Units/10^6 cells.

E. Statistical Analysis

Using two-way ANOWA data of cytotoxicity were analyzed. The data of anti-microbial activity were analyzed by ONE WAY ANOWA and least significant differences test.

III. RESULTS

This study revealed that *I. spicata* has greater antibacterial potential ($p < 0.05$) against *P. aeruginosa*. The aqueous fraction (Fig. 1) having low antibacterial effect on *S. aureus* as matched to other solvent fractions. Methanolic fraction of *I. spicata* have significantly higher antibacterial activity against *E.coli* and *P. aeruginosa*. Ethyl acetate and aqueous fractions of *I. spicata* also slowed significantly higher antibacterial activity against *E. coli*. While Hexane, chloroform and fractions have low antibacterial activity. The antibacterial potential of ethyl acetate on *E. coli* was higher than methanolic and aqueous fractions.

Several fractions of *I. spicata* revealed significant antifungal activity against various fungus strains including *A. fumigatus*, *A. flavus* and *A. niger*. In Fig. 2 the Chloroform fraction showed higher zone of inhibition against several fungal strains. Against *A. flavus* the chloroform fraction was more effective than other fractions showing 62 % decrease in fungus growth. The methanolic and chloroform fractions showed 59 % and 57% decrease in liner growth of *A. fumigatus* respectively. The chloroform fraction showed maximum antifungal potential against *A. niger* (60 %). The standards for antifungal potential was created on percent inhibition in linear growth of fungi. According to the scale compounds showing 70% growth of inhibition were considered with a significant antifungal activity. Those compounds sowing 60-70 % inhibition of fungal growth were consider as good,50-60% inhibition activity was moderate, whereas compounds showing less than 50% inhibition of fungal growth were taken as non-significant. Chloroform fraction of *I. spicata* has good antifungal activity against all the strains of fungi. While ethyl acetate, n-hexane and aqueous fraction have low and non-significant antifungal activity against tested fungal strains. Antimicrobial activity has been reported for plant extracts (Vermaak *et al.*, 2009). Plants contain various kinds of biologically active compounds which possess bactericidal and fungicidal properties.

The DPPH percent inhibition method using different concentration to assess free radical neutralizing effect of *I spicata* .The results obtained showed that higher DPPH percent inhibition was shown by methanolic extract at all

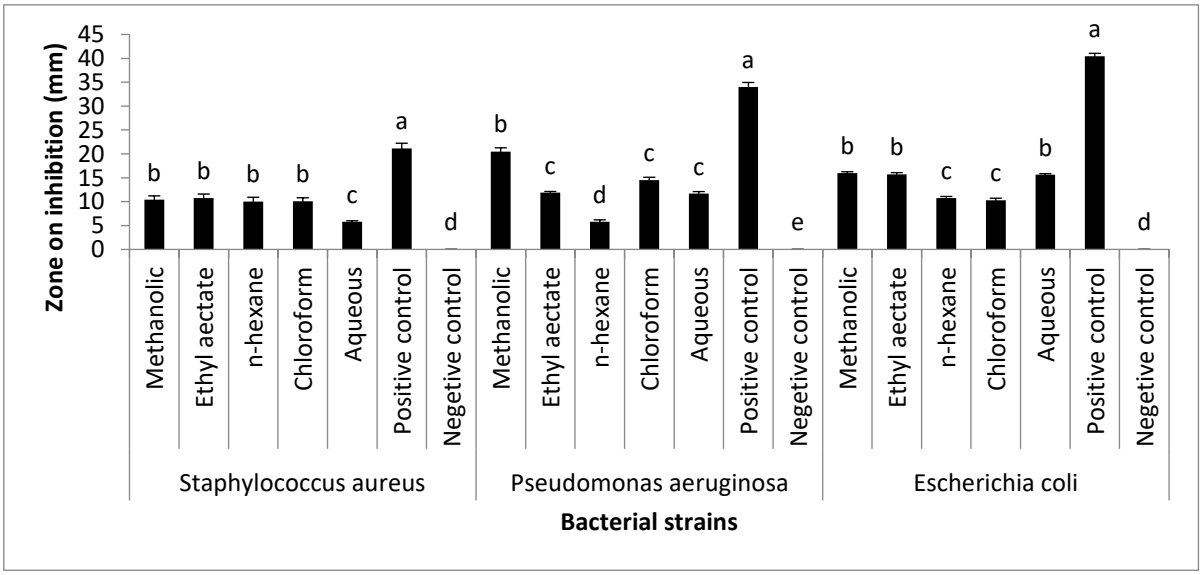

Fig 1. Antibacterial potential of the solvent fractions of *I. spicata* against *S. aureus* (LSD: 4.309), *P. aeruginosa* (LSD: 0.815) and *E. coli* (lea LSD: 3.257). Means with similar English letters do not differ significantly. LSD: least significant difference.

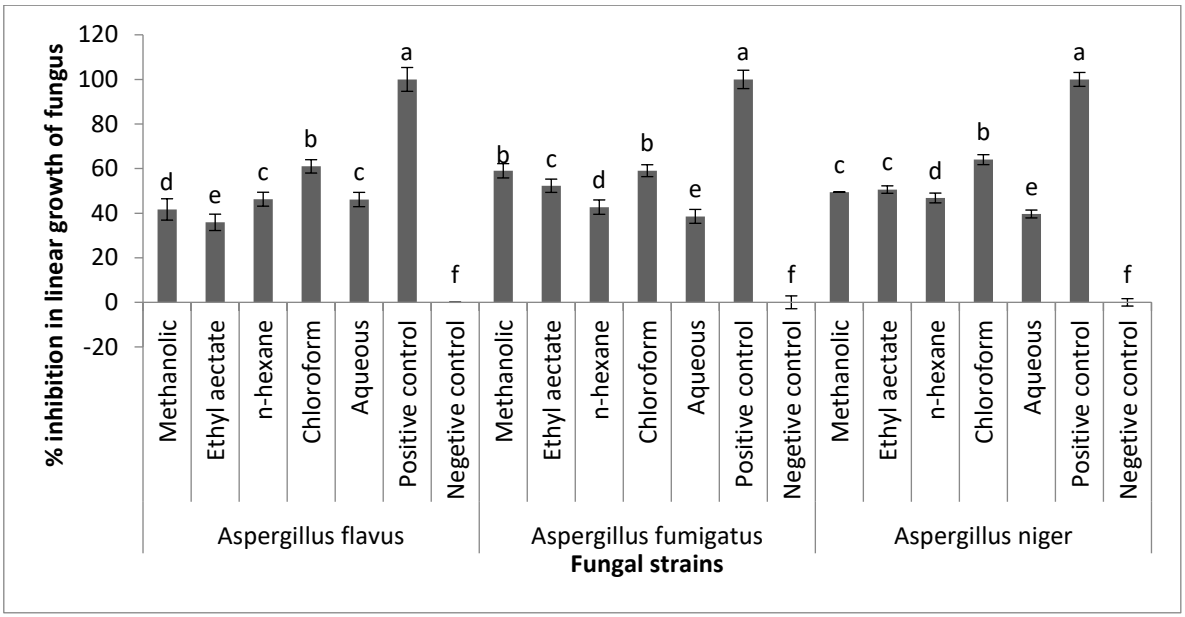

Fig 2. % inhibition in linear growth of fungi by different solvent fraction of *I. spicata* against *A. flavus* (LSD: 4.015), *A. fumigatus* (LSD: 2.17) and *A. niger* (LSD: 1.595). Means with similar English letters do not differ significantly. LSD: Least significant difference.

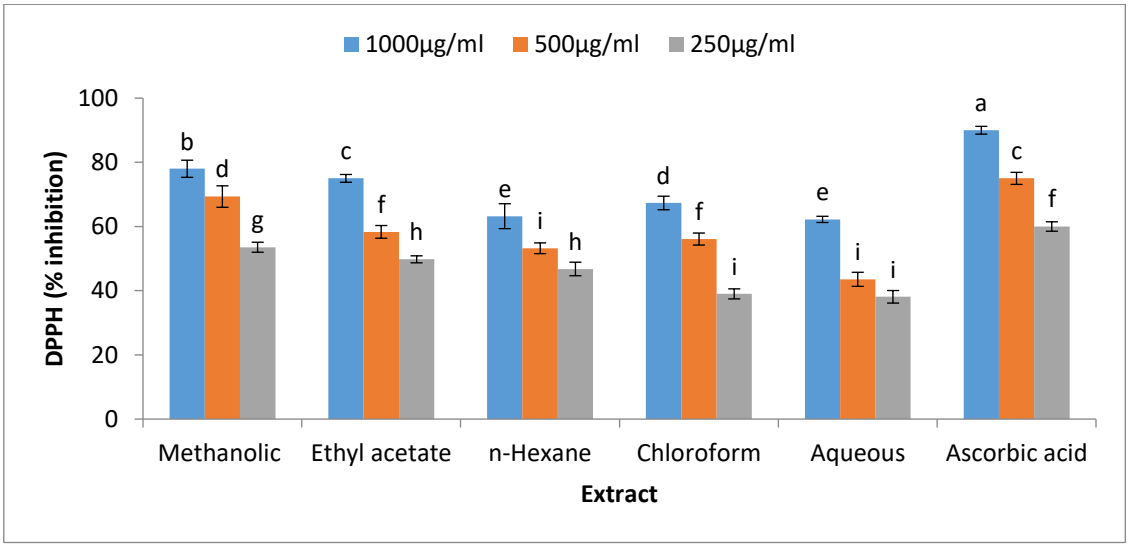

Fig3. Antioxidant potential (DPPH method) of *I. spicata* (LSD: 0.293). Means with similar English letters do not differ significantly. LSD: Least significant difference.

three different concentrations. Next higher inhibition effect was presented by ethyl acetate and chloroform. The highest DPPH percent inhibition was recorded at 1000 µg/ml of the various solvent extracts as shown in Fig. 3.

Hydrogen Peroxide percent inhibition method was used in order to evaluate the effect of *I. spicata*. In present study four different concentrations of *I. spicata* extracts were used. The obtained results presented higher H_2O_2 percent inhibition by ethyl acetate extract at all four different concentrations. Next higher inhibition effect was presented by methanolic fraction tracked by *n*-hexane and chloroform. Natural compounds of plant origin have been explored for antioxidative properties by different authors (Duthie *et al*., 2000; Dudonne *et al*., 2009). Our studies established that I. spicata could be a potential source of natural antioxidants for human consumption.

The ROS level was higher in H_2O_2 treated lymphocytes as compared to those in control group (Table 1 and Fig. 4). One group of lymphocytes which was not treated with H_2O_2 and supplemented with various solvent fractions of *I. spicata* has no alteration in ROS level. The rise in ROS level by H_2O_2 stress was considerably recovered by *I. spicata* extracts at 5 µg /ml and 50µg/ml methanolic and chloroform fractions were more potent in the recovery effect.

In present study the increase in the level of antioxidant enzymes SOD, CAT, POD due to hydrogen peroxide stress was significantly normalized by various solvent extracts of *I. spicata*. The most significant decrease in level of antioxidant enzymes was shown by *I. spicata* at concentration of 50 µg/ml. Tables 2-4 shown that aqueous and methanolic fraction of the *I.*

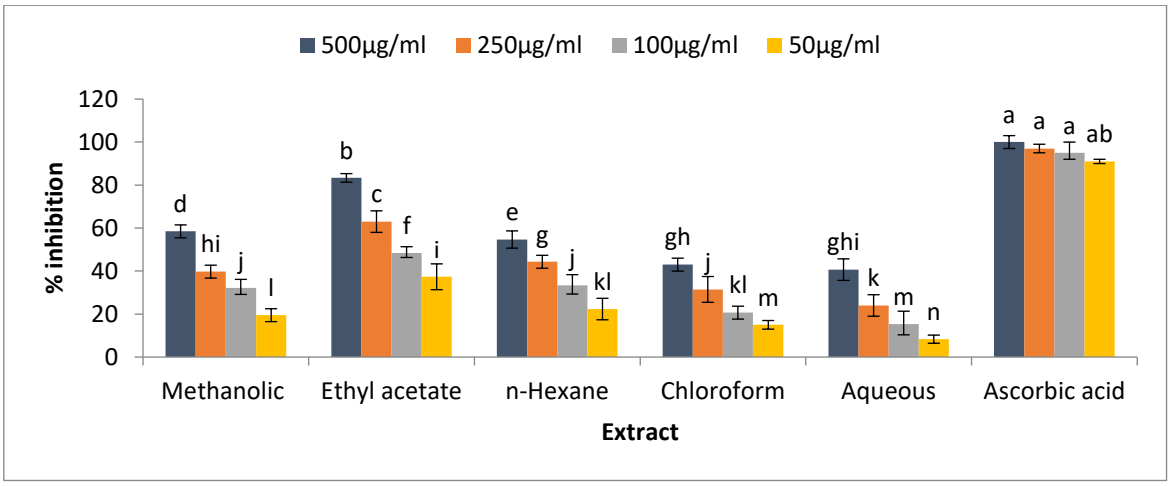

Fig. 4. Antioxidant potential (H_2O_2 scavenging method) of *I. spicata* (LSD: 3.779). Means with similar English letters do not differ significantly. LSD: Least significant difference.

Table 1. *I. spicata* recovery effect on ROS content of human blood lymphocytes

Treatment	*I. spicata* different solvent fractions					Mean
	E1	E2	E3	E4	E5	
Control	0.1340 g-j	0.1340 g-j	0.1340 j	0.1340 g-j	0.1340 g-j	0.1340 de
H_2O_2 (100µM)	0.2840 a	0.2840 a	0.2840 a	0.2840 a	0.2840 1a	0.2840 a
Dose 0.5 µg/ml	0.1322 g-j	0.1664 e-h	0.1532 f-i	0.1298 g-j	0.1540 f-i	0.1471 d
Dose 5 µg/ml	0.1300 g-j	0.1418 e-j	0.1226 h-j	0.1268 g-j	0.1250 g-j	0.1292 de
Dose 50 µg/ml	0.1000 J	0.1312 g-j	0.1246 g-j	0.1078 i-j	0.1216 h-i	0.1170 e
T1 (100µM)+ Dose 0.5 µg/ml	0.2642 ab	0.2778 ab	0.2568 ab	0.2528 ab	0.2448 a-d	0.2593 b
T2 (100µM)+ Dose 5 µg/ml	0.1726 e-g	0.2546 ab	0.2364 a-d	0.2013 d-e	0.2338 b-d	0.2197 c
T3 (100µM)+ Dose 50 µg/ml	0.1622 e-h	0.2694 ab	0.2510 a-c	0.2030 c-e	0.2394 a-d	0.2250 c
Mean	0.1724 c	0.2074 a	0.1953 ab	0.1799 b-c	0.1921 ab	

± denotes standard error. Means with similar English letters do not differ significantly. (Treatment: LSD: 0.0218, Fractions: LSD: 0.0173, Treatment x Fraction: LSD: 0.0489). LSD: Least significant difference.
Methanol = E1, Ethyl acetate = E2, *n*-hexane=E3, Chloroform=E4, Aqueous= E5
T1, T2, T3 denotes various H_2O_2 concentration while *I. spicata* various extracts concentrations are denotes by doses.

Table 2. *I. spicata* recovery effect on (CAT) antioxidant enzymes activity of human blood lymphocytes

Treatment	*I. spicata* different solvent fractions					Mean
	E1	E2	E3	E4	E5	
Control	2.8100 b-e	2.8100 b-e	2.8100 e v	2.8100 b-e	2.8100 b-e	2.8100 1a
H_2O_2 (100µM)	0.9460 n	0.9460 n	0.9460 2n	0.9460 n	0.9460 n	0.9460 3c
Dose 0.5 µg/ml	3.3560 ab	2.7500 b-e	2.3560 e-h	2.6900 b-e	2.7072 b-e	2.7718 5a
Dose 5 µg/ml	3.2300 abc	2.5500 d-f	2.6180 c-e	2.8858 b-e	2.8760 b-e	2.8320 5a
Dose 50 µg/ml	3.6980 a	2.5340 d-e	3.1260 a-d	2.7158 b-e	2.9420 b-e	3.0032 3a
T1 (100µM)+Dose 0.5 µg/ml	1.9020 f-j	0.9880 n	0.9320 n	1.4480 j-m	1.6720 i-m	1.3884 2b
T 2 (100µM)+Dose 5 µg/ml	2.4600 d-g	1.0088 mn	1.0520 nm	1.2900 j-m	1.7240 h-l	1.5070 4b
T3 (100µM)+Dose 50 µg/ml	2.3160 e-i	1.0840 mn	1.1880 k-n	1.2920 j-m	1.8422 j-k	1.5444 1b
Mean	2.5898 a	1.8339 c	1.8785 1c	2.0097 bc	2.1899 1b	

± denotes standard error Means with similar English letters do not differ significantly. (Treatment: LSD: 0.2996, Fractions: LSD: 0.2369, Treatment x Fraction: LSD: 0.6700). LSD: Least significant difference.
Methanol = E1, Ethyl acetate = E2, *n*-hexane=E3, Chloroform=E4, Aqueous= E5
T1, T2, T3 denotes various H_2O_2 concentrations, while *I. spicata various extracts concentration are represented by doses.*

spicata showed more potent recovery as compared other fractions.

The elevated level of TBARS was considerably recovered by different fractions of *I. spicata*. The most potent recovery effect shown at concentration of 5ug/ml and 50ug/ml. Table 5 shown that the methanolic fraction fallowed by aqueous fraction potently recovered TBARS action as matched to other fractions.

IV. CONCLUSIONS

Different solvent fractions of *I. spicata* showed significant antimicrobial potential against different bacterial and fungi strains. The hydrogen peroxide and DPPH free radical uptake potential of all the extract was significant. Although greater inhibitory potential effect was shown by methanolic solvent of *I. spicata*. The elevated level of TBARS and the decrease in content of COD, CAT, POD was also recovered by several fractionsof *I. spicata* effectually secure the lipid peroxidation membrane of lymphocytes. Our studies report that different organic solvents fractions of *I. spicata* showed bal ancing effects on the antioxidant defense system of human lymphocytes. This study also showed that

Table 3. *I. spicata* recovery effect on (POD) antioxidant enzymes activity of human blood lymphocytes

Treatment	*I. spicata* different solvent fractions					Mean
	E1	E2	E3	E4	E5	
Control	4.8920 ab	4.8920 ab	4.8920 ab	4.8920 ab	4.8920 ab	4.8920 b
H_2O_2 (100µM)	2.8280 ab	2.8280 de	2.8280 de	2.8280 de	2.8280 de	2.8280 cd
Dose 0.5 µg/ml	5.0260 ab	3.6460 cd	5.8888 a	5.8888 a	5.2174 ab	5.1334 ab
Dose 5 µg/ml	4.9400 a	5.1744 ab	5.0534 ab	5.1600 ab	5.5380 a	5.1732 a b
Dose 50 µg/ml	6.0382 a	5.5900 a	5.3374 a	5.2900 ab	5.8140 a	5.6139a
T1 (100µM)+ Dose 0.5 µg/ml	2.7326 de	2.3994 e	2.3888 e	2.3702 e	2.2180 e	2.4218d
T2 (100µM)+ Dose 5 µg/ml	2.6240 de	3.2262 c-e	2.5680 de	2.9398 c-e	3.3640 c-e	2.9444 cd
T3 (100µM)+ Dose 50 µg/ml	3.2520 c-e	2.4460 de	2.6990 de	2.4926 de	4.0960 bc	2.9971 c
Mean	4.0416 ab	3.7752 b	3.9569 ab	3.9827 ab	4.2459 a	

± denotes standard error. Means with similar English letters do not differ significantly. (Treatment: LSD: 0.4350, Fractions: LSD: 0.4350, Treatment x Fraction: LSD: 1.2305). LSD: Least significant difference
Methanol = E1, Ethyl acetate = E2, *n*-hexane=E3, Chloroform=E4, Aqueous= E5
T1, T2, T3 denotes various H_2O_2 concentration while *I. spicata various extracts concentration are represented by doses.*

Table 4. *I. spicata* recovery effect on (SOD) antioxidant enzymes activity of human blood lymphocytes

Treatment	*I. spicata* different solvent fractions					Mean
	E1	E2	E3	E4	E5	
Control	9.244de	9.324 e	9.244 ± 0.01de	9.244 e	9.244 de	9.244 b
H_2O_2 (100µM)	4.036k	4.106 k	4.036± 0.03k	4.036 k	4.036 k	4.036e
Dose 0.5 µg/ml	10.723a-d	10.214a-e	9.212e	9.210 ef	9.610 e	9.729 ab
Dose 5 µg/ml	10.334a-e	9.432 cde	10.215a-e	9.770b-e	11.082a	10.189 a
Dose 50 µg/ml	10.749abc	10.548 a-e	9.558 b-e	7.768fg	11.484 a	10.007 a
T1 (100µM)+ Dose 0.5 µg/ml	5.327 ijk	5.116 ijk	4.542 k	4.986 i-k	6.302 g-i	5.201 d
T2 (100µM)+ Dose 5 µg/ml	7.437 gh	6.094 hi	5.230 -k	4.212 k	5.770 ij	5.747 cd
T3 (100µM)+ Dose 50 µg/ml	7.447 gh	5.322 ijk	5.212 i-k	4.896 i-k	7.635 fg	6.104 1c
Mean	8.1466 a	7.4757 b	7.2145bc	6.7453 c	8.1454 a	

± denotes standard error. Means with similar English letters don't differ significantly. (Treatment: LSD: 0.5239, Fractions: LSD: 0.6627, Treatment x Fraction: LSD: 1.4818). LSD: Least significant difference.
Methanol = E1, Ethyl acetate = E2, *n*-hexane=E3, Chloroform=E4, Aqueous= E5
T1, T2, T3 denotes various H_2O_2 concentration while *I. spicata various extracts concentration are represented by doses.*

Table 5. *I. spicata* recovery effect on (TBARS) contents of human blood lymphocytes

Treatment	*I. spicata* different solvent fractions					Mean
	E1	E2	E3	E4	E5	
Control	0.6960 f-j	0.6960 f-j	0.6960 f-j	0.6960 f-j	0.6960 f-j	0.6960 a
H_2O_2 (100µM)	1.0144 a	1.0144 a	1.0144 a	1.0144 a	1.0144 a	1.0144 a
Dose 0.5 µg/ml	0.5352 k-m	0.6310 h-k	0.6522 h-k	0.6236h-l	0.6518 h	0.6188 e
Dose 5 µg/ml	0.4840 2lm	0.6088 i-	0.6834 g-j	0.6506 h-k	0.5888 -m	0.6031 e
Dose50 µg/ml	0.4652 m	0.5874 i-m	0.5680 j-m	0.6410 h-k	0.5580 jm	0.5639 e
T1 (100µM)+ Dose 0.5 µg/ml	0.9034 abc	0.9074 abc	0.9938 ab	0.8742 bcd	0.7564 d-h	0.8870 b
T2 (100µM)+ Dose 5 µg/ml	0.8306 c-f	0.9102 abc	0.8832 abcd	0.8492 cde	0.7540 h	0.8454 bc
T3 (100µM)+ Dose 50 µg/ml	0.7260 e-i	0.8444 cde	0.8130 c-g	0.9500 abc	0.7178 i	0.8102 c
Mean	0.7068 b	0.7749 4a	0.7880 a	0.7874 a	0.7171 b	

± denotes standard error. Means with similar English letters do not differ significantly. (Treatment: LSD: 0.0625, Fractions: LSD: 0.0494, Treatment x Fraction: LSD: 0.0625).
Methanol = E1, Ethyl acetate = E2, *n*-hexane=E3, Chloroform=E4, Aqueous= E5
T1, T2, T3 denotes various H_2O_2 concentration while *I. spicata various extracts concentration are represented by doses.*

lymphocytes treated with *I. spicata* extracts performed better under oxidative stress in terms of better scavenging of free radicals. Studies of various authors like Ayyanar and Subash-Babu, (2012) and Naz et al., (2020) have reported that plant extracts contain natural antioxidants having potential of stabilization of reactive oxygen species and other free radicals.

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