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Original article

# In vitro antioxidant and cytotoxic activities of polyherbal extracts from *Vetiveria zizanioides*, *Trichosanthes cucumerina*, and *Mollugo cerviana* on HeLa and MCF-7 cell lines

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ABSTRACT

Various metabolites exist in the medicinal plants have lot of potential to cure various diseases and disorders. Plants such as, *Vetiveria zizanioides*, *Trichosanthes cucumerina*, and *Mollugo cerviana* were collected from Western Ghats, Tamilnadu, India. Phytochemicals were extracted from these plants using various organic solvents and tested against Gram-positive and Gram-negative bacteria. The phytochemicals such as, carbohydrate, alkaloids, steroids, saponins, flavonoids and tannin were detected from these medicinal plants. Among the extracts, methanol showed potent activity and this solvent was used to extract polyherbal medicinal plants. Methanol extract of *V. zizanioides* was found to be highly active against *E. coli* (27 ± 2 mm), *P. mirabilis* (19 ± 3 mm) and *B. subtilis* (18 ± 2 mm). Ethyl acetate extract showed high activity against *E. coli* (24 ± 2 mm), *P. mirabilis* (22 ± 3 mm) and *B. subtilis* (20 ± 1 mm). These three plants were taken at 1:1:1 ratio and extracted with methanol at 1:10 ratio and synergistic activity was tested against bacterial pathogens. Synergistic activity of polyherbal extract was analyzed. The extracted crude herbal medicine was found to be effective against *Staphylococcus aureus, E. coli, Enterbacter* sp., *Pseudomonas aeruginosa, Bacillus subtilis* and *Proteus mirabilis*. The zone of inhibition was 33 ± 3 mm, 17 ± 2 mm, 22 ± 2 mm, 40 ± 2 mm, 33 ± 1 mm and 38 ± 2 mm zone of inhibition against *E. coli*, *S. aureus*, *P. aeruginosa*, *P. mirabilis*, *B. subtilis* and *Enterobacter* sp. Polyherbal extract was found to be highly effective against *P. mirabilis* and *Enterobacter* sp. MIC values of polyherbal extract ranged from 29 ± 2.5 μg/ml to 34 ± 2.5 μg/ml. MIC value was found to be less against *P. mirabilis* and was high against *S. aureus*. Antioxidant property varied between 49 ± 3% and 95.3 ± 2%. At 20 μg/ml antioxidant activity was reported as 49 ± 3% and it was increased at higher concentrations of polyherbal extract. Two cell lines (HeLa and MCF cell lines) were selected to analyze cytotoxic activity of polyherbal extract. The methanol extract of polyherbal fraction showed cytotoxicity against these two cell lines. The LC50 value was 467 ± 2.9 μg/ml against HeLa cell line and >800 μg/ml against MCF-7 cell lines. The polyherbal extract showed antibacterial, antioxidant and anticancer activities.



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## 1. Introduction

The microorganisms induced many diseases are highly responsible for about 50% mortality and are still a major concern to public health (WHO, 2002; Valsalam et al., 2019a). Synthetic antibacterial and antifungal drugs are very expensive and generally unavailable in under developed countries (Roopan et al., 2019). These synthetic

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drugs cause various side effects. Emergence of resistance against various pathogenic bacteria due to unlimited use of drugs is important concern (Valsalam et al., 2019b). Infectious disease causes morbidity and mortality among the populations, mainly in under developed and developing countries. So, Pharma industries have also been initiated to formulate potent antibacterial drugs in recent times, mainly due to the constant emergence of resistance among pathogenic microorganisms. The drug resistance microorganisms have the ability to acquire and transmit resistance against presently available various antimicrobial drugs (Natarajan et al., 2005). Medicinal plants contain phytochemicals such as, tannins, terpenoids, alkaloids and flavonoids and these phytochemcials have various antimicrobial properties (Aron and Kenedy, 2008; Al-Dhabi and Arasu, 2016; Barathikannan et a., 2016; Cuong et al., 2017). Infectious disease caused by drug resistance microorganisms are the Global health care problem (Al-Dhabi et al., 2015; Elango et al., 2017). Many bacteria including, Proteus vulgaris, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus and Escherichia coli cause many infections. Emergence of multiple drug resistance and toxicity severely limit the application of various currently available antibacterial agents (Chitra et al., 2014; Elango et al., 2016a, Elango et al., 2016b). Methicillin-resistant Staphylococcus aureus (MRSA), MDR Mycobacterium tuberculosis and Vancomycin-resistant enterococci (VRE) and some Gram-negative bacteria are reported as the highly difficult healthcare-associated serioud infections to treat and control. The development of extended-spectrum β-lactamases (ESBLs) that specifically target Gram-negative organisms has resulted in serious infections that can be very difficult to treat leading to severe increased death rate and illness (WHO, 2002; Fowsiya et al., 2016). Multiple drug resistance demand to screen many medicinal plants for their antimicrobial potential, which are mainly due to the presence of secondary metabolites such as, alkaloids, tannins, flavonoids, phenolic compounds, resins, steroids, gums and fatty acids which are capable of producing various physiological action on body (Ahmad and Bey, 2001; Dahiya and Purkayastha, 2012; Glorybai et al., 2015; Park et al., 2016a; Park et al., 2016b). About 80% of world population using herbal medicine for the treatment of various infectious diseases, injuries and inflammations, etc. Most of the medicinal plants used in traditional medicine system are effectively proved and cheaper than modern medicine (Mann et al., 2008; Haritha et al., 2016; Helan et al., 2016; Ilavenil et al., 2017).

In Ayurvedic medicine system, the combinations of medicinal herbal plants have been used previously. For example, the combinations of long pepper with black pepper significantly enhances mucous-, and heat- reducing effects. Neem, ginger, cold herbs and bitter positively regulate any negative effects in the human body. Likewise, black pepper, cumin are applied to minimize weak digestion, whereas, the combination of turmeric and guduchi enhance immunity (Thyagarajan et al., 2002). There are two possible mechanism involved in synergism effects, pharmacokinetic and pharmacodynamics. Pharmacokinetic synergism involves absorption of herb, distribution of phytochemicals, metabolism of absorbed chemicals and removal (Parket al., 2017; Surendra et al., 2016a; Surendra et al., 2016b; Surendra et al., 2016c). In the other hand, pharmacodynamic synergism involves analysis of synergistic property when potent molecules with sample bioactivity are targeted to a similar physiological or receptor system. It is also believed that certain diseases cause multiple complications, leading to both invisible and visible symptoms. In these cases the combination of various herbs critically acts on various targets simultaneously to give maximum relief (Spinella, 2002; Gurusamy et al., 2019). Because of synergistic property, polyherbal provides more benefits not generally available in the formulation of single herbal medicine. It is more evident that good therapeutic property can be achieved with a formulation of a single multi-constituent formulation. For the formulation of poly-herbal medicine, medicinal plants with lower dose is highly preferable because to avoid side effects and to achieve required pharmacological activity. These kinds of poly-herbal formulations also avoid consuming more than one formulation at the time. All these positive effects resulted advantages of polyherbal formulation. Many medicinal plants have been directly used as the raw drug and these medicinal plants posses various therapeutic values. These medicinal plants are rich source of unique chemical substances with potent therapeutic effect. Plants contain many metabolites and are used as raw material for the production of new drugs. In developing countries and developed countries traditional medicine play very important role in primary healthcare system (Kannan et al., 2009). The plant based medicines are easily acceptable to the humans body than synthetic drugs. Hence, it is very important to utilize these natural medicines for providing good healthcare service to rural places.

## 2. Materials and methods

### 2.1. Chemicals

The solvents such as, methanol, ethyl acetate, acetone was purchased from Sigma, U.S.A. Mueller Hinton Agar and other culture media were obtained from Himedia, Mumbai, India. All other chemicals were analytical grade.

### 2.2. Medicinal plants

The medicinal plants such as, Vettiver (*Vetiveria zizanioides*), Peyputtal (*Trichosanthes cucumerina*), and Parpatakam (*Mollugo cerviana*) were collected from Western Ghats, Tamilnadu, India.

### 2.3. Plant extract preparation

The collected medicinal plants were washed with tap water, air dried and powdered. About 10 gm powder was extracted with methanol, ethyl acetate, acetone and water (hot water extraction) and the efficacy was tested against bacteria.

### 2.4. Phytochemical screening

Phytochemcial components of medicinal plants were analyzed by standard method. Total carbohydrate, alkaloids, steroids, saponins, flavonoids and tannins assayed. All experiments were performed in triplicates and the mean ± standard deviation was considered for analysis (Sofowora, 1993; Trease and Evans, 2002; Bhat and Al-daihan, 2014).

### 2.5. Bacterial cultures and maintenance

The bacteria such as, *Staphylococcus aureus, E. coli, Enterobacter* sp., *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus mirabilis* were used. The selected bacterial strains were sub cultured on nutrient agar slants and maintained at 4 °C. These bacteria were further cultured in nutrient broth for 18 h prior to antibacterial assay.

### 2.6. Antibacterial screening

The antimicrobial activity of medicinal plants extracted with solvent and water was determined according to National Committee for Clinical Laboratory Standards by disc diffusion method (NCLLS, 1993; Rajkumari et al., 2019). The inoculum concentration was determined as $10^6$ cfu/ml and it was spread on Meuller Hinton

Agar plates with the use of sterile swab dipped with selected bacterial suspension. Then, 6 mm disc was made and loaded with 25 μl plant extract (20 μg/ml) and it was allowed to diffuse at 2–8 °C for 4 h and incubated for 24 h at 37 °C. Antibiotic standard (10 μg) was used as the positive control. After 24 h, the zone of inhibition (mm) was measured. Three different experiments were performed and the results (zone of inhibition) were expressed as mean ± standard deviation.

### 2.7. Extraction of polyherbal medicine and evaluation of synergistic effect

Methanol extract showed the presence of various phytochemicals and also found to be effective against various tested bacterial pathogens. Hence, methanol was selected to extract polyherbal compounds until otherwise stated. About five grams of selected three plant powder was weighed, to this 150 ml methanol was added and kept on an rotary shaker at 150 rpm. The solvent was evaporated and diluted the precipitate appropriately and used for further analysis. The polyherbal extract was tested against the selected bacteria as described earlier. It was used for the analysis of MIC, antioxidant and anticancer activity analysis.

### 2.8. Minimum inhibitory concentration (MIC)

The methanol extract was used to test the minimum inhibitory concentration (MIC) for tested bacterial sample. MIC of the selected polyherbal extract was tested by broth dilution assay where plant extract (100 mg/ml) was resuspended in DMSO and dilutions were made upto 5 mg/ml. All dilution was seeded with selected bacterial suspension ($1 \times 10^5$ cfu/ml) and incubated at 37° for 24 h. After 24 h, the growth of the culture was monitored using a spectrophotometer (600 nm) against blank. Experiments were performed in triplicate analysis.

### 2.9. Antioxidant assay (DPPH free radical scavenging assay)

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was performed to analyze the antioxidant potential of polyherbal methanol extract. In this assay, the sample was added with 0.1 M methanolic DPPH at different concentrations (20, 40, 60, 80 and 100 μg). It was mixed well for 20 min and incubated at 37 °C and the absorbance was measured at 517 nm. Blank experiment was run without plant extract. Lower absorbance of the sample indicated high degree of free radical scavenging activity. Finally, the free radical scavenging activity (%) was calculated.

### 2.10. Assessment of in-vitro anti-inflammatory activity inhibition of albumin denaturation

To analyze anti-inflammatory activity inhibition effect, 0.4 ml Bovine serum albumin (3%, w/v) and different concentration of methanol extract (20, 40, 60, 80 and 100 μg/ml) were added. 0.1 N Hydrochloric acid was used to reduce the pH value to 6.3. The samples were incubated for 30 min at 37 °C and kept on a water bath for 2 min. The reaction mixture was cooled and phosphate buffer saline (PBS) (pH 6.3) was added and the absorbance was read at 416 nm against reagent blank. The anti-inflammatory drug, diclofenac was used as the standard drug. Experiment was performed in triplicate analysis and the percentage inhibition of protein denaturation was calculated.

### 2.11. Anticancer activity of medicinal plants

#### 2.11.1. Cell lines

HeLa and MCF-7 cells were cultured using Minimum Essential Medium (MEM) containing nonessential amino acids, Earle's salt with 2 mM L-glutamate, 1 mM sodium pyruvate and 5% heat-inactivated fetal calf serum. The selected cell lines were grown in 95% air and 5% $CO_2$ and incubated at 37 °C.

#### 2.11.2. Cytotoxicity assay (MTT Assay)

MTT assay was performed as described earlier by Carmichael et al. (1987). Briefly, HeLa and MCF-7 cells were aseptically seeded at $1 \times 10^5$ cells/mL in a microtiter plate in MEM medium. Then it was incubated for overnight and polyherbal extract was added in cell lines. The cells were treated with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltratrazolium bromide (MTT). After 4 h of incubation, cell culture medium was aspirated from the microtiter plates. Then the formazan crystal from the microtiter plates were dissolved in DMSO and the plate was subjected to analysis at 570 nm using a 96 well microplate reader. The cytotoxic effect (%) of polyherbal extract was calculated.

## 3. Results and discussion

Polyherbal formulations (PHF) have potential response to treat various diseases. In polyherbal system the effect was found to be high due to the availability of various phytochemical components (Nayak et al., 2018; Salama and Marraiki, 2010). PHF have various therapeutic applications. Most of these formulations were found to be highly effective at low dose and highly safe even at high dose. For example, a PHF, "Diakyur" has been used to treat diabetes and even at 12800 mg/kg p.o. did not show any toxic effect. These finding revealed that the formulated herbal medicine is highly safe. PHF shows antioxidant and hypoglycaemic activity at 1600 mg/kg doses. PHFs have very less side effects than allopathic drugs. The modern allopathic drugs have various side effects, including, vomiting, insomnia, dry mouth, fatigue, seizures, diarrhea, confusion, impotency, organ toxicities, hair loss and also death. In some cases, patients advised with various anti-inflammatory drugs from non-steroidal drugs for the treatment of rheumatoid arthritis (RA) may highly experience mainly renal and gastrointestinal side effects, including fluid and salt retention, gastric ulceration and dyspepsia. Treatment with RA patients with polyherbal medicine treatment did not show any harmful side effects (Krishna, 2011). Considering the impact of poly-herbal medicine, the present study was performed to analyze antibacterial and antioxidant properties of poly-herbal extract.

### 3.1. Phytochemical components of some medicinal plants

The phytochemicals such as, carbohydrate, alkaloids, steroids, saponins, flavonoids and tannin were detected from the medicinal plants, *V. zizanioides*, *T. cucumerina* and *M. cerviana*. Methanol extract showed large number of phytochemicals than ethyl acetate, acetone and water extract (Table 1). Many medicinal plants were used to analyze the active components possessing antimicrobial properties. Ates and Turgay (2003) used methanol and ethanol to extract phytochemicals from the medicinal plants and reported the presence of tannins, terpenoids, essential oil, flavonoids and alkaloids. Ethanol has the capacity to yield more number of phytochemicals. Kannan et al. (2009) reported phytochemicals such as, phenols, saponins, terpenoids, alkaloids and flavonoids showing antimicrobial activity from the ethanolic extract of *Vetiveria zizanioides*. The phytochemical such as, carbohydrate, alkaloids, steroids, saponins, flavonoids and tannin have been detected. These

**Table 1**
Phytochemicals of some medicinal plants extracted with various organic solvents.

| Plants | Phytochemicals | Methanol | Ethyl acetate | Acetone | Water |
|---|---|---|---|---|---|
| | Carbohydrate | + | + | + | + |
| | Alkaloids | + | + | – | + |
| *V. zizanioides* | Steriods | + | + | + | + |
| | Saponins | + | + | – | + |
| | Flavonoids | + | + | + | + |
| | Tannin | + | – | + | – |
| | Carbohydrate | + | + | + | + |
| | Alkaloids | + | – | + | – |
| | Steriods | – | + | + | + |
| *T. cucumerina* | Saponins | + | – | – | – |
| | Flavonoids | + | + | – | + |
| | Tannin | + | – | – | – |
| | Carbohydrate | + | + | + | + |
| | Alkaloids | + | – | – | – |
| *M. cerviana* | Steriods | + | + | – | + |
| | Saponins | + | – | + | – |
| | Flavonoids | + | – | + | – |
| | Tannin | + | + | + | + |

secondary metabolites are useful in cell growth, body building and replacement. These also have antibacterial, anti-inflammatory, immune stimulant, antiviral and detoxification activities (Mensah et al., 2008; Karimi et al., 2011).

### 3.2. Antibacterial activity of medicinal plants

The medicinal plants showed activity against various Gram-positive and Gram-negative bacteria. The antagonistic activity (zone of inhibition in mm) of medicinal plants (*V. zizanioides*, *T. cucumerina* and *M. cerviana* were described in Table 2. Methanol extract of *V. zizanioides* was found to be highly active against *E. coli* (27 ± 2 mm), *P. mirabilis* (19 ± 3 mm) and *B. subtilis* (18 ± 2 mm). Ethyl acetate extract showed high activity against *E. coli* (24 ± 2 mm), *P. mirabilis* (22 ± 3 mm) and *B. subtilis* (20 ± 1 mm). Acetone and water extract showed considerable activity against *P. mirabilis* and *B. subtilis*. The antimicrobial property of medicinal plants varied widely and was depend based on bacterial strains tested. The selected Gram-positive bacterial pathogens were highly susceptible to the polyherbal extract when compared to bacteria from Gram-negative group. The difference in cell wall structure cause significant changes in the permeability of the drug (Zhao et al., 2001). Tannins from the medicinal plants disintegrate bacterial cells and affect bacterial cell wall (Venkataswamy et al., 2010). Also, tannins from the medicinal plants are used to treat ulcerated tissues and inflamed tissues (Akinpelu and Onakoya, 2006). In medicinal plants potential bioactive compounds are accumulated as secondary metabolites and this amount varies according to seasons, plant parts, growth phases and climatic conditions (Jain and Tiwari, 2012; Mensah et al., 2008). The antimicrobial property of the whole plant of T. cucumirina has been described previously by Kage et al. (2009). It has been effective against the bacterial pathogens such as, Staphyloccus aureus, Klebsiella pneumoniae and Escherichia coli. The antibacterial activity of T. cucumirina has been tested against Staphyloccus aureus and Escherichia coli (Arawwawala et al., 2011). Recently, Sharma et al. (2014) reported the effect of methanol of polyherbal medicine against bacterial species. The formulated polyherbal medicines showed less activity against C. albicans and high activity was reported against S. mutans.

### 3.3. Synergistic antibacterial activity of polyherbal extract

Fig. 1 shows synergistic activity of polyherbal extract (methanol extract) against selected bacteria. The zone of inhibition was 33 ± 3 mm, 17 ± 2 mm, 22 ± 2 mm, 40 ± 2 mm, 33 ± 1 mm and 38 ± 2 mm against *E. coli*, *S. aureus*, *P. aeruginosa*, *P. mirabilis*, *B. subtilis* and *Enterobacter* sp. Polyherbal extract was found to be highly effective against *P. mirabilis* and *Enterobacter* sp. Previously reported antimicrobial potential of flavonoids from the medicinal

**Table 2**
Antibacterial activity of medicinal plants against bacterial pathogens.

| Plants | Bacteria | Methanol | Ethyl acetate | Acetone | Water |
|---|---|---|---|---|---|
| | | | Zone of inhibition (mm) | | |
| | *E. coli* | 27 ± 2 | 24 ± 2 | 16 ± 1 | 14 ± 3 |
| | *S. aureus* | 12 ± 1 | 14 ± 2 | 14 ± 2 | 10 ± 2 |
| *V. zizanioides* | *P. aeruginosa* | 14 ± 0 | 12 ± 1 | 10 ± 0 | 13 ± 3 |
| | *P. mirabilis* | 19 ± 3 | 22 ± 3 | 20 ± 1 | 21 ± 1 |
| | *B. subtilis* | 18 ± 2 | 20 ± 1 | 19 ± 2 | 21 ± 2 |
| | *Enterobacter* sp. | 13 ± 1 | 18 ± 2 | 12 ± 1 | 20 ± 1 |
| | *E. coli* | 17 ± 1 | 16 ± 2 | 17± | 20 ± 0 |
| | *S. aureus* | 16 ± 0 | 15 ± 1 | 16 ± 0 | 12 ± 2 |
| | *P. aeruginosa* | 12 ± 1 | 14 ± 0 | 15 ± 1 | 12 ± 2 |
| *T. cucumerina* | *P. mirabilis* | 31 ± 2 | 30 ± 2 | 12 ± 2 | 10 ± 3 |
| | *B. subtilis* | 18 ± 2 | 17 ± 2 | 19 ± 0 | 12 ± 2 |
| | *Enterobacter* sp. | 26 ± 2 | 21 ± 0 | 20 ± 2 | 18 ± 1 |
| | *E. coli* | 19 ± 2 | 12 ± 0 | 13 ± 0 | 16 ± 0 |
| | *S. aureus* | 17 ± 3 | 19 ± 2 | 22 ± 2 | 18 ± 1 |
| *M. cerviana* | *P. aeruginosa* | 16 ± 3 | 17 ± 2 | 13 ± 1 | 18 ± 2 |
| | *P. mirabilis* | 13 ± 1 | 16 ± 3 | 21 ± 3 | 13 ± 2 |
| | *B. subtilis* | 28 ± 2 | 16 ± 1 | 17 ± 1 | 19 ± 1 |
| | *Enterobacter* sp. | 27 ± 0 | 20 ± 0 | 25 ± 0 | 26 ± 1 |

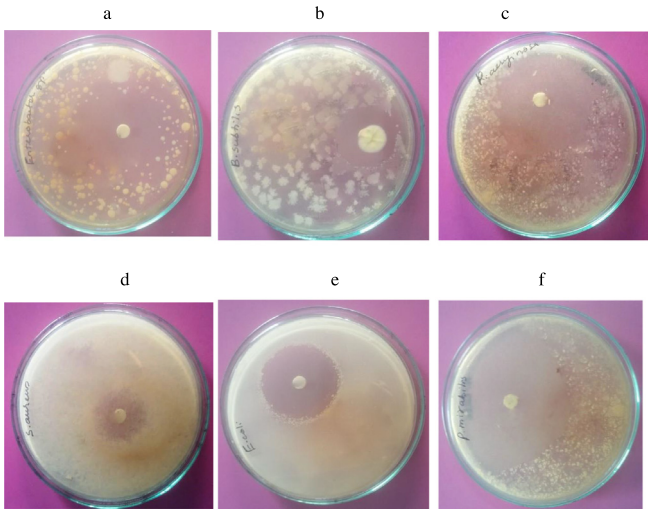


**Fig. 1.** Synergistic activity of polyherbal extract against bacterial pathogens. Antibacterial activity is expressed as zone of inhibition (mm) (a: *Enterobacter* sp; b: *B. subtilis*; c: *P. aeruginosa*; d: *S. aureus*; e: *E. coli*; f: *P. mirabilis*). 20 μl polyherbal extract was loaded and incubated for 24 h and the zone of inhibition was observed.

plants. Flavonoids showed antimicrobial properties against Gram-positive and Gram-negative bacteria. These flavonoids make a complex with soluble proteins or extracellular protein and finally complex with cell wall of bacteria; also lipophilic flavonoids significantly disrupt microbial membrane of the cells. These tannins from medicinal plant origin inactivate microbial adhesion enzymes and also inhibit the transport of various proteins, and finally form a complex with polysaccharides (Kannan et al., 2009). The root of *Vetiveria zizanioides* showed the presence of tannins and is highly responsible for antibacterial activity. Tannin from plant origin showed various antibacterial potentials. Earlier, the antimicrobial property of *Mollugo cerviana* plant extract was studied against various Gram-positive and Gram-negative bacterial strains was studied. The zone of inhibition was registered between 12 and 32 mm and methanol extract was found to be good. Also, n-butanol and ethylacetate solvent extract showed zone of inhibition between 12 and 20 mm (Valarmathi et al., 2012).

### 3.4. Minimum inhibitory concentration of polyherbal extract

MIC values of polyherbal extract ranged from 29 ± 2.5 μg/ml to 34 ± 2.5 μg/ml. MIC value was found to be less against *P. mirabilis* and was high against *S. aureus*. MIC value of polyherbal extract against selected bacteria was described in Fig. 2. Previously, Rios and Recio (2005) reported potent antibacterial properties for organic solvent extract with MIC value less than 100 μg/ml and in our study the evaluated extract showed promising antimicrobial activity with less MIC values. In a study, Barros et al. (2009) analyzed the antimicrobial property of oil from *V. zizanioides* and reported varying MIC values. In their study, the reported MIC value was 150 μg/ml, however, polyherbal extract of our study showed less MIC value.

### 3.5. Antioxidant activity of polyherbal extract

Antioxidant activity of the polyherbal extract was described in Fig. 3. Antioxidant property varied between 49 ± 3% and 95.3 ± 2%. At 20 μg/ml antioxidant activity was reported as 49 ± 3% and it was increased at higher concentrations of polyherbal extract. Antioxidant activity increased in dose dependent manner (Fig. 3). Sadique et al. (1987) studied anti-inflammatory activity of *M. cerviana* in *invivo* using mouse model and reported 26% activity at 100 mg/g body weight. The phytochemicals inhibit the levels of acid phosphatase, lipid peroxides and gamma-glutamyl transpeptidase activity. In recent years, an attention has been paid to find antioxidant molecules from natural sources for various health benefits. Plants produce various secondary metabolites with antioxidant properties. Polyphenols are the one of the important antioxidant compounds and plant tissues are rich of phenolic compounds such as, tannins, phenolic acids and flavonoids. These compounds have various biological activities, including antioxidant activity (Kumaran and Karunakaran, 2006; Chen and Ho, 1997; Rubio et al., 2013). The phytochemicals of *V. zizanioides*, *T. cucumerina* and *M. cerviana* showed the presence of tannin and flavonoids. The combined effect of these plant extracts showed about 95.3 ± 2% antioxidant activity.

### 3.6. Cytotoxic activity of polyherbal extract against HeLa and MCF cells

In our study, two cell lines (HeLa and MCF cell lines) were selected to analyze cytotoxic activity of polyherbal extract. The LC50 value was 467 ± 2.9 μg/ml against HeLa cell line and >800 μg/ml against MCF-7 cell lines. In the present study, the methanol extract of polyherbal plant showed cytotoxicity against tested cell lines. Earlier, crude extract has also been used to identify the lead molecules for the preparation of drugs (Svejda et al., 2010). In recent year's number of medicines have been derived from various medicinal plants and this process continues. The cytotoxic property of medicinal plants plays a potent role in

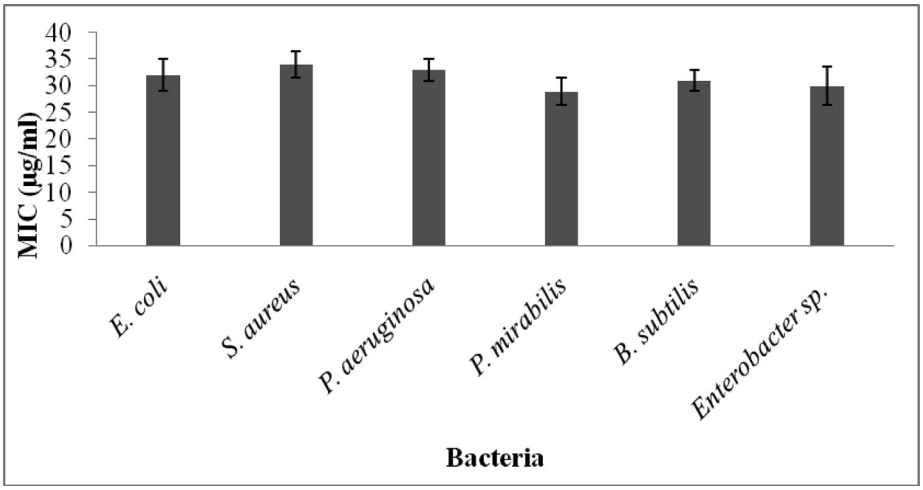


**Fig. 2.** MIC value of polyherbal extract against bacteria.

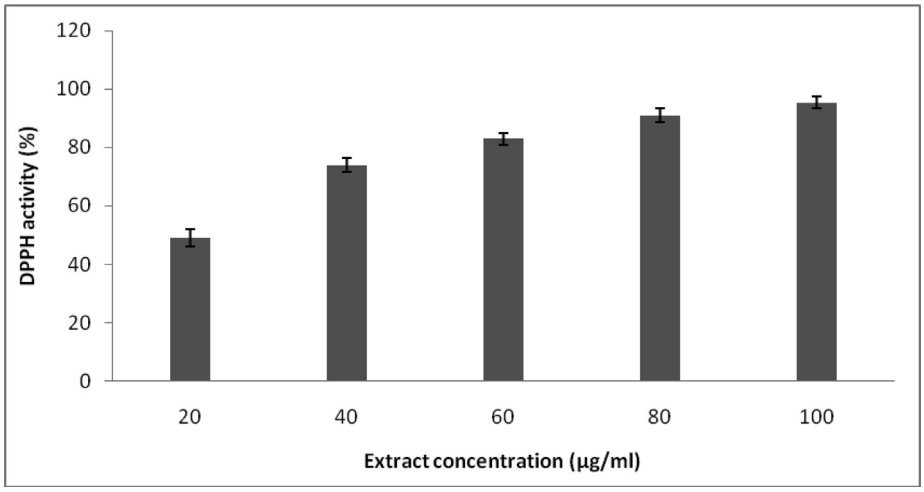


**Fig. 3.** Antioxidant activity of polyhedral extract.

treating various cancers (Svejda et al., 2010). The present findings revealed promising anticancer activity against HeLa and MCF cell lines. Phytochemcials such as, polyphenols and flavonoids have potent anticancer activities by involving regulation of various signal transduction pathways of cancer cell growth and suppression and proliferation of oncogenes and formation of tumor, modulation of enzyme activity, induction of apoptosis, reduction, oxidation, regulation of hormone metabolism and stimulation of the immune system and DNA repair (Aron nad Kennedy, 2008). Also, polyphenols have a significant protective role in inflammation, carcinogenesis, thrombosis, atherosclerosis, and have antioxidant property (Tapiero et al., 2002). Chitra et al. (2014) reported anticancer property of *Vetiveria Zizanioides* against cancer cell lines. Cell growth inhibition was found to be maximum between 31 µg/ml to 37 µg/ml. The phytochemicals from *Trichosanthes cucumerina* showed potential inhibitor activity which delayed the proliferation of hormone-dependent prostrate cancer. Human adrenocortical H295R cells growth was inhibited by the extract of *T. cumerina* (Mollik, 2013).

## 4. Conclusion

Polyherbal extracts showed lot of potential than individual extracts. The selected three medicinal plants (*Vetiveria zizanioides*, *Trichosanthes cucumerina* and *Mollugo cerviana*) showed synergistic activity. The polyherbal extract showed enhanced antibacterial activity due to synergistic effect. Also, polyherbal extract showed promising antioxidant and anticancer activities against HeLa and MEF cell lines. Potential anticancer activity of polyherbal extract was mainly due to the combined activity of *V. zizanioides*, *T. cucumerina* and *M. cerviana*. Also, further investigation is required to evaluate their effect to develop as chemotherapeutic agent.

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