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# GC-MS profiling, FTIR, metal analysis, antibacterial and anticancer potential of *Monotheca buxifolia* (Falc.) leaves

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**Abstract**: *Monotheca buxifolia* has traditionally been employed in folk medicines to cure of infectious diseases. Current study was aimed to standardize the *M. buxifolia* leaves extract and evaluate its antibacterial and anticancer activity. Phytochemical analysis was carried through GC, GC/MS, FTIR, and ICP-OES analytical techniques. Antibacterial assay of the crude extract was performed by using tetrazolium micro plates. The extract treated bacteria were observed under (AFM) atomic force microscope and PCR was used for DNA amplification. The anti-proliferative activity of *M. buxifolia* leaves extract was examined through MTT cytotoxicity assay. The bacterial strains employed in this study were *S. epidermidis* ATCC (13518), *S. aureus* ATCC (25923), *P. aeruginosa* ATCC (10145), and *E. coli* ATCC (10536). Minimum inhibitory concentration ($MIC_{50}$) against gram positive bacteria was significantly ($p$<0.01) achieved at 50 and 75µg/mL. $MIC_{50}$ against *E. coli* and *P. aeruginosa* was also significant at 100µg/mL ($p$<0.01). *M. buxifolia* leaves extract damaged the cell walls gram-positive and gram-negative bacteria, while biofilm around gram positive bacteria was significantly damaged. The DNA decantation was also inhibited of *S. aureus* and *S. epidermidis*, however, no any impact was observed on *E. coli* and *P. aeruginosa* DNA decantation. The cytotoxicity findings suggested that the crude extract of *M. buxifolia* leaves at 1000µg/mL gives significant inhibition 73.96±2.0%, 83.76±1.2%, 77.66±1.2% and 72.67±1.6% against MDA-MB-231, MCF-7, HeLa and H460 cell lines respectively at ($p$<0.001). It may be concluded that *M. buxifolia* leaves extract have significant and promising antibacterial and anti-cancer activities which could be helpful to establish new antimicrobial and anticancer agents.

**Keywords**: *Monotheca buxifolia*, GC/MS, FTIR, ICP-OES, AFM, PCR

## INTRODUCTION

Intra-abdominal infections (IAIs) are a diverse group of diseases ranges from appendicitis, pancreatitis, peritonitis to intestinal infections. Such IAIs are the gravest health issues which culminate into different kinds of morbidities and mortalities. A number of studies have suggested that gram-negative bacteria (*E. coli, P. aerugonosa*) and gram-positive bacteria (*B. subtilis, S. aureus*) are the key pathogens of IAIs (Bartlett *et al*., 1978; Lopez *et al*., 2011). Currently, the use of various antibiotics against these causative agents has been limited due to development of bacterial resistance and drug toxicity (Eggleston *et al*., 2010). Thereby, a natural remedy is the best available alternative to deal with the above given nuisance. Since natural products also termed as green medicines and are the richest sources of antimicrobials agents with less no of toxicities. Apart from being cost effective, naturally available antimicrobial agents are easily approachable (Canli *et al*., 2016). Therefore, the development of plant origin medicines that must be safe, effective and economic is a need of the hour. *Monotheca buxifolia* (*M. buxifolia*) belongs to sapotaceae family and is native to the hilly regions of Northern Pakistan and Afghanistan. Its fruits are being sold in the local market, the fruits exhibited anthelmintic, digestive and laxative properties. Apart from this, the fruit extract of *M. buxifolia* was also reported in the treatment of infections of urinary tract (Khan *et al*., 2010). It has, strong *in vitro* free radical scavenging and antioxidant capabilities (Jan *et al*., 2013). Scientific findings also endorsed the use of fruit extract of this plant for anti-nociceptive, anti-inflammatory and anti-pyretic purposes (Ullah *et al*., 2016). It is important to note that *M. buxifolia* fruit extract has also been studied for renal protective effect (Jan and Khan, 2016). The present study was aimed at to standardized leaves extract of *M. buxifolia*, by phytochemical screening and evaluating its antimicrobial and anticancer potential.

## MATERIALS AND METHODS

### *Plant sample collection and its extraction*

*M. buxifolia* was identified with the help of its local name Gurgura and a voucher specimens no. (SB/01/15-MB)

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was submitted in Faculty of Pharmacy, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.

The leaves were collected from Malakand, KPK, Pakistan in the month of July. *M. buxifolia* leaves were subjected to drying process under shade and pulverized into powder. The (5kg) sample was then soaked in commercial grade methanol for two weeks at room temperature, followed by filtration using Whatmann No 1 filter paper. Rotary evaporator B-490, Buchi was used to concentrate the obtained filtrate at 45°C, as reported by (Altuner *et al*., 2014) giving greenish crude methanolic extract (CME) (147.35g, 2.45%).

***Gas chromatography (GC) analysis***

The crude extract of *M. buxifolia* was analyzed on a gas chromatograph (Agilent USB-393752, USA) with the help of HHP-5MS 5% phenylmethylsiloxane capillary column (30m × 0.25mm × 0.25µm) and FID detector. The apparatus was operated as described by (Ayaz *et al*., 2017).

***Gas chromatography–mass spectrometry (GC/MS) analysis***

The crude methanolic extract of *M. buxifolia* leaves was analyzed on gas-chromatography Agilent HP-5973, USA outfitted with an assembly of a selective mass detector. All of the instrumental setup was working in the conditions which have already been specified as the standard conditions for GC as reported by (Ayaz *et al*., 2017). The compounds detected in the crude extract of *M. buxifolia* leaves via GC/MS analysis were authenticated by their retention time comparison with the spectral data stored in Wiley and NIST library (Stein *et al*., 2002).

***Fourier transform infrared spectrophotometer (FT-IR) analysis***

Functional groups present in the crude extract were ascertained through Fourier transform infra-red (FT-IR) spectrometer Thermo Nicolet Nexus coupled with DTGS (deuterated tri-glycine sulfate) as reported by (Oliveira *et al*., 2016).

***Metal analysis***

The concentration of metals i.e. Cd, Pb, Na, K, Zn, Mn, Fe, Mg, Ca and Si was measured in crude methanolic extract of *M. buxifolia* leaves with the help of an optical plasma emission spectrophotometer that was inductively coupled (ICP-OES), Perkin Elmer, Optima 2000 Japan as reported by (Gomez *et al*., 2007).

***Antibacterial assay***

*Bacterial strains*

Antibacterial bioassay was carried out on four ATCC bacterial species two gram-positive i.e. *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 13518 and two gram-negative bacteria i.e. *E. coli* ATCC 10536 and *P. aeruginosa* ATCC 10145. These species were of different intra-abdominal infections collected from Jinnah Postgraduate Medical Center (JPMC), Pakistan. The bacteria were cultured on soya agar Oxoid, UK at the temperature of 4ºC to grow colonies. From colonies bacterial suspension of final concentration of as $5\times10^5$cfu/mL was prepared.

***Tetrazolium microplate assay***

Tetrazolium microplates were employed in order to assess the minimum inhibitory concentration ($MIC_{50}$) of *M. buxifolia* leaves extract (Piaru *et al*., 2012). The freshly prepared suspension of microbes *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* at the concentration $5\times10^5$ cfu/mL were inoculated into the 96 well plate. Later on, the serial dilutions of the crude methanolic extract (200-10 µg) were accomplished with the help of Muller Hinton Broth, and incubated at 37 ºC ± 0.5 for 18 to 24 hours. After making serial dilutions, from each of the dilution, a volume of 200 µl was drawn and subsequently added to the wells. This was followed by the process of incubating the inoculated plates for the period of 18 to 24 hours at the temperature of 37±0.5ºC. After incubation 50µl of MTT was added to the microtiter plates. The absorbance was measure at 570 and 650 nm after 30 mints of incubation of the plates. The $IC_{50}$ calculated as previously reported (Sarkar *et al*., 2007).

$$IC_{50} = \left[\left(\frac{\text{O.D. in control} - \text{O.D.of test}}{\text{O.D. of control}}\right)\right]\times 100$$

***Atomic force microscopy study***

The morphological changes in bacterial cells were assessed via atomic force microscopy. 10% 1ml gelatin was used for preparation of mica slides. After that, the extract treated cultures of different bacteria i.e. *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* were harvested from microtiter and were then placed on the slides prepared with the polylsyline mica. The same procedure was followed so as to prepare the samples for negative as well as positive control. After the inoculation of bacterial strains on mica slides, they were dried with the help of temperature of ambient nature. The prepared slides were observed on atomic force microscopy for morphological changes in bacterial strains.

***DNA extraction and PCR amplification method***

A single colony from each plate (extract treated and untreated) was harvested and suspended in Trypton soya broth and then incubated for 24 hrs at 37°C. The incubated tubes containing bacteria were centrifuged at 10000 rpm in Eppendorf for 10 mints. The supernatant of the centrifuged tubes was discard and the pellate were further wash with normal saline solution. The pellets were vortexed and centrifuged again, and the process was repeated 2 times for complete washing of pellate. To the pellets 1mL of CTAB and 20µl of protenase k was added and incubated the tubes for 1h at 60°C, followed by addition of 800µl isoamyl alcohol and chloroform (1:24)

and the tubes were mix gently by vertexing several times. The tubes were further centrifuged for 5 min at 14000 rpm. The supernatant was carefully transferred into another tube and 600µl of ice chilled isopropanol was added with gently mixing. The tubes were again centrifuged at 14000 rpm for 5 min to get DNA pellate. The pellets were rinsed with 70% ethanol and dried at room temp. The pellets were further dissolved in TE buffer and stored until use at -20°C (Winnepenninckx, 1993). The purity of DNA (A260/A280) was measured through (Genova Nano micro-volume) spectrophotometer (Jenway Inc).

The PCR products were analyzed on 2% agarose-gel electrophoresis, The PCR products were purified by Ket method (GF-1 Yivantis), Following the manufacturer's instructions before 16S sequencing.

***Cytotoxicity assay***

Cytotoxicity potential of crude extract of *M. buxifolia* was determined against; MCF-7 breast cells, H460 Lung cancer cells, HeLa Human cervical and MDA-MB-231 breast. The cytotoxicity analysis was assessed via MTT bioassay (Scudiero *et al*., 1988). Briefly in DMEM (containing FBS (10%), streptomycin and penicillin (100 units/mL)), the cells were cultured at 37°C in a humidified environment that contained 5% $CO_2$. Cells were seeded into 96-well plates at a density of $8\times10^3$, $1\times10^4$, $6\times10^4$ and $4\times10^4$ cells/well MCF7, MDA-MB-231, HeLa and H460 respectively in culture medium (100µl). The original medium after 24 hours of incubation was substituted by fresh medium (200µl) having concentrations (1000-62.5µg/ml) of test samples and allowed to grow for further 24 hours. To each well 200µl MTT solution of 0.5mg/ml in PBS was added followed by incubation for 24 hours and removal of unreacted dye. The purple formazan crystals were thawed in 100µl DMSO, and the absorbance was recorded on the microplate reader (Spectra Max plus, Molecular Devices, CA, USA) at a wavelength of 570 nm. The inhibitory % was calculated as:

$$\text{Inhibition (\%)} = 100 - \left[ \frac{\text{Mean of O.D. of test compound} - \text{Mean of O.D. of negative control}}{\text{Mean of O.D. of positive control} - \text{Mean of O.D. of negative control}} \times 100 \right]$$

The results were further analyzed by Soft-Max Pro software (Molecular Device, USA) for percent inhibition.

## STATISTICAL ANALYSIS

The antibacterial and anti-proliferative bioassay were performed in triplicate. Analysis of result was carried out using the variance one-way analysis and $*p<0.05$, $**p<0.01$ and $***p<0.001$ were contemplated as statistically important. Graph Pad prism for graphical analysis having specifications; version 5.01, (La Jolla) Software Inc., was used.

## RESULTS

***Phytochemical evaluation***

The GC and GC-MS chromatogram of *M. buxifolia* leaves extract were represented in fig. 1 (a and b). The analysis of chromatogram of CME of *M. buxifolia* depicts that the leaves were enriched with prominent components of immense pharmacological value.

It is notable that the crude extract of the leaves of this plant contained almost more than 160 compounds. However, the major bioactive compounds presented in table 1.

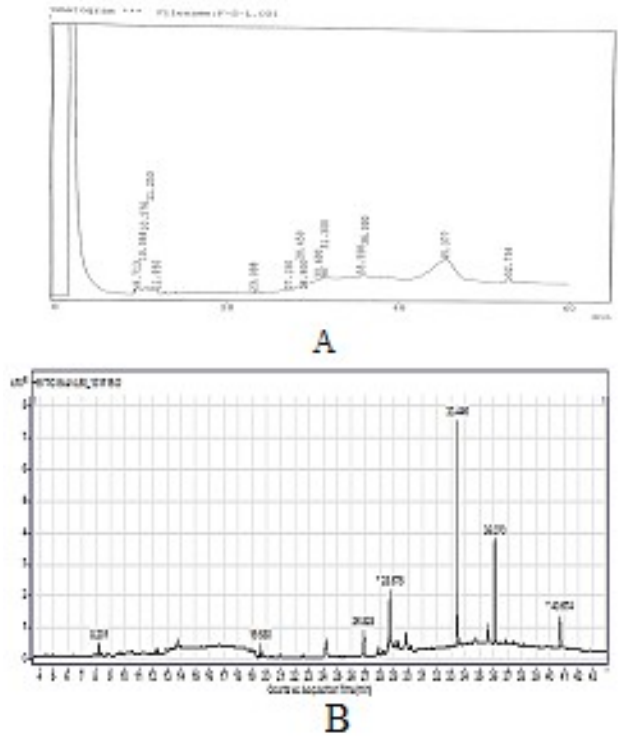



**Fig. 1**: Chromatogram of CME of *M. buxifolia* leaves (a) GC (b) GC/MS

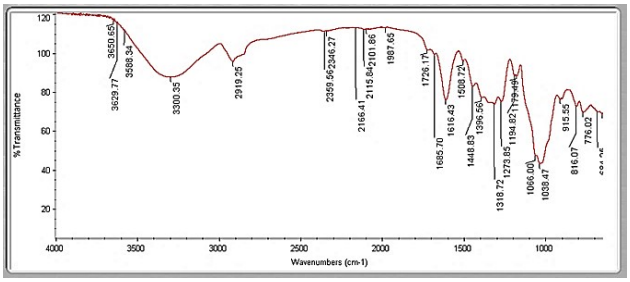



**Fig. 2**: FT-IR spectra of crude *M. buxifolia* leaves extract

It is notable that the crude extract of the leaves of this plant contained almost more than 160 compounds. However, the major bioactive compounds presented in table 1.

**Table 1**: Major components of *M. buxifolia* leaves extract by GC-MS analysis

| No | Compound name | Molecular mass | Activity |
|---|---|---|---|
| 1 | Betulin | 442 | anti-inflammatory, antiviral, antibacterial, antifungal and antitumor |
| 2 | α-Amyrenyl acetate | 468 | Anticancer agent |
| 3 | Lupenol | 426 | Anti-inflammatory and anti-cancer |
| 4 | 3-Deoxyestradiol | 256 | Anti-Inflammatory and antimicrobial |
| 5 | Trilinolein | 878 | anti-inflammatory |
| 6 | α-d-Xylopyranoside, methyl-2,3,4-tris-O-[9-borabicyclo[3.3.1]non-9-yl]- | 524 | - |
| 6 | Ascorbyl Palmitate | 414 | Antioxidant |
| 7 | Ascorbyl 6-stearate | 442 | inhibits human ovarian cell carcinoma |
| 8 | l-(+)-Ascorbic acid 2,6-dihexadecanoate | 652 | antimicrobial and antioxidant activity |
| 9 | cis-13,14-Epoxydocosanoic acid | 354 | anti-inflammatory |
| 10 | Indole | 117.1 | Indole nucleus is anticancer, antimicrobial, analgesic, antioxidant, antidiabetic and antidepressant |
| 11 | 5-Hydroxytryptamine | 176.2 | Antidepressant |
| 12 | alpha.-Tocopherol | 430.7 | Antioxidant, anticancer |
| 13 | 3,4,4-Trimethyl-5-pyrazolone | 126 | Antimicrobial |
| 14 | -Hydroxy-2-methylpyrrolidine-2-carboxylic acid | 145 | - |

**Table 2**: Functional groups present in crude extract of *M. buxifolia* leaves

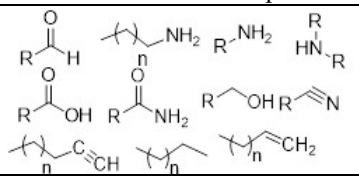
| Functional Group | Common name |
|---|---|
|  | Primary and secondary amines and amides, alkanes, aldehydes, nitriles, carboxylic acid Alkynes terminal, nitriles, Alcohols, Phenols, acids. |

**Table 3**: Essential metals detected in CME of *M. buxifolia* leaves extract

| Metal Name | Percentage % yield | Metal Name | Percentage % yield |
|---|---|---|---|
| Na | 0.08 | Fe | 0.088 |
| K | 0.691 | Mg | 0.041 |
| Zn | 0.161 | Ca | 0.126 |
| Mn | 0.002 | Si | 0.102 |

**Table 4**: Minimum inhibitory concentration ($MIC_{50}$) of crude methanolic extract of *M. buxifolia* against strains of gram-positive and negative bacteria

| Bacteria | $MIC_{50}$ (µg/ml) | *p*-value |
|---|---|---|
| *S. aureus* (ATCC 25923) | 75 | $p<0.01$ |
| *S. epidermidis* (ATCC 13518) | 50 | $p<0.01$ |
| *E. coli* (ATCC 10536) | 100 | $p<0.05$ |
| *P. aeruginosa* (ATCC10145) | 100 | $p<0.05$ |

The FT-IR spectra of crude extract of *M. buxifolia* leaves is represented in fig (2). Results suggested that the crude extract possessed various important functional groups as shown in table 2. The major IR peak at ($cm^{-1}$): 3650.65, 3629.77 (OH, alcohol/phenol, Free), (3500-3000) 3300.35 (OH/NH, hydrogen bonded), 2919.25 (saturated CH stretching), 2166.41 (C-C triple bond), 1616.43, 1508.72, 1448.83 (C-C double bonds /aromatic), 1726.17 (CO ketone carbonyl), 1686.70 (CO ester/amide carbonyl), 1179.49 (C-O, single bond) etc.

The crude extract of *M. buxifolia* leaves also contained important essential metals as presented in table 3. The essential metals found in crude extract were Na; 0.08%, K; 0.691%, Zn; 0.016%, Mn; 0.002%, Fe; 0.088%, Mg; 0.041%, Ca; 0.126% and Si; 0.102%.

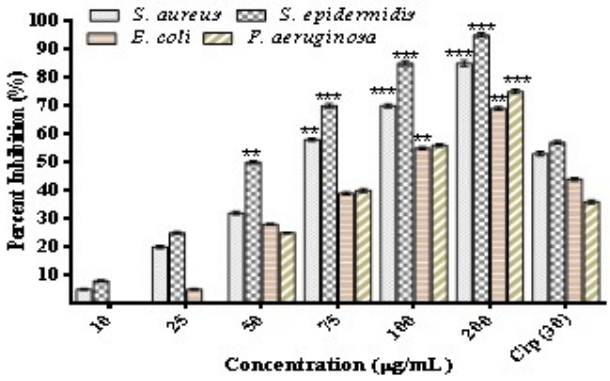



**Fig. 3**: Antibacterial activity of *M. buxifolia* leaves extract against resistant gram negative and gram positive bacteria strains. Each value is represented as mean± SD values are represented as. (n=3). (*$p$<0.05; **$p$<0.01; ***$p$<0.001). Cip= ciprofloxacin

***Antibacterial activity of M. buxifolia leaves***

*M. buxifolia* leaves extract exhibited significantly ($p$<0.001) inhibited (*S. aureus*, *S. epidermidis* and *P. aeruginosa*) and *E. coli* ($p$<0.01) as depicted in fig. (3). *M. buxifolia* at maximum dose of 200µg/mL inhibited, 85 ±1.1% *S. aureus*, 95±0.8% *S. epidermidis*, 75±0.7% *P. aeruginosa* and 69±0.6% *E. coli* as illustrated in fig. (3). The $MIC_{50}$ of *M. buxifolia* calculated for. *S. aureus* and *S. epidermidis* were 75 and 50µmg/mL respectively. The $MIC_{50}$, against *E. coli* and *P. aeruginosa* were found at 100 µg/mL table 4. The results of antibacterial activity of *M. buxifolia* against selected bacteria were significant and comparable with standard drug ciprofloxacin 30 µg/mL.

***Atomic force microscope study***

AFM imaging was being performed in tapping mode at 20 × 20 µm scanning. Extract untreated control cells (fig. 4A-4D) were in normal shape with smooth surface and without any sign of damage. Morphological changes in extract treated bacterial cell were given in (fig. 4a-4d).

In fig. (5A-5D) and fig. (5a-5d) the bacterial cells were magnified and captured in rainbow mode to observe the shape, surface and biofilm around bacterial cells. The biofilm around gram positive and gram negative bacteria were normal biofilm and undamaged as shown in fig. (5A-5D). Whereas, biofilm around extract treated gram positive bacteria were highly rough and damaged. The gram negative bacteria (*E. coli* and *P. aeruginosa*) biofilm were partially damaged fig. (5c and 5d).

***PCR amplification***

The extract treated bacterial DNA decantation in gram positive bacteria was inhibited however no any detrimental impact was seen on the decantation of gram negative bacteria (*E. coli* and *P. aeruginosa*) fig. (6). Besides, this their DNA appeared to be normal and supercoiled.

***Anticancer activity***

The anticancer results of *M. buxifolia* crude leaves extract against different cell lines were given in fig. (7). Docetaxel 50µg/mL was used as standard. The crude extract of *M. buxifolia* leaves inhibited MCF-7 (82.76±2.30%), MDA-MB-231 (73.96±2.01%), HeLa (71.66±1.20%) and H460 (70.67±1.56 %) at the dose of 1000 µg/mL. The anticancer activity results of *M. buxifolia* were comparable with standard drug docetaxel.

## DISCUSSION

Natural products are considered to be the richest sources of biologically active compounds (Rawat *et al*., 2018). Additionally, ethno-pharmacologists have already delivered the latest scientific data on therapies from natural resources (Cooper, 2008). The GC, GC-MS and FTIR of *M. buxifolia* leaves extract revealed presence of active principals. Most probably upon library analysis the major components in the crude extract of *M. buxifolia* leaves may contained betulin, lupeol, estradiol, α-tocopherol, urs-12-en-3-ol acetate, (3β)-, ascorbyl palmitate, oleic acid, oxiranedodecanoic acid, 3-octyl-, cis- triarachine, ascorbyl stearate, l-(+)-ascorbic acid 2,6-dihexadecanoate, 4H-pyran-4-one, (Z)-13-docosenamide, 1,2-benzenedicarboxylic acid, 3-octyl-, cis-, 2,3-dihydro-3,5-dihydroxy-6-methyl- 1-ethyl-2-hydroxymethylimi-dazole and 5-hydrxoymethylfurfural.

The metals found in *M. buxifolia* crude extract were (Na, K, Zn, Mn, Fe, Mg, Ca and Si) in a physiologically acceptable quantity. The presence of such heavy metals aided in the therapeutic worth of the leaves extract. It has been proved in scientific findings that zinc carries tremendous role to synthesize, store and release insulin from pancreas (Burki *et al*., 2017). Additionally, zinc serves as a cofactor for more than 300 enzymes, rendering the work of signal transduction, transcription, cell replication, DNA metabolism and DNA repairing (Coleman, 1992). Beside this Mg is supposed to contain anti-bacterial as well as anti-proliferative capabilities (Robinson *et al*., 2010; Bronzetti *et al*., 1996). Similarly,

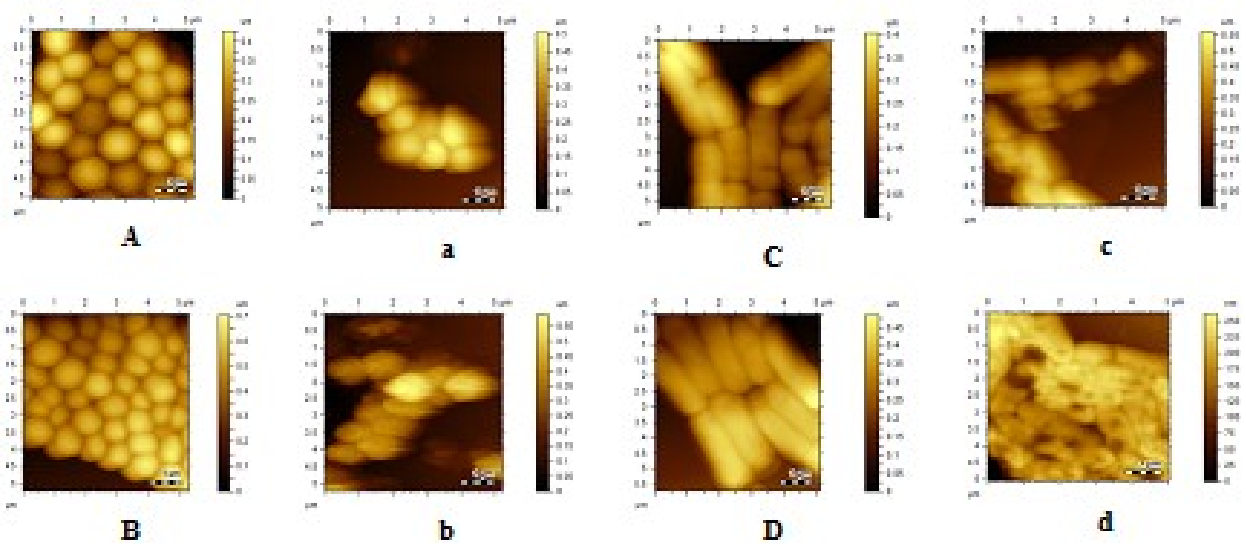



**Fig. 4**: Atomic force microscopic images *of M. buxifolia* crude extract untreated (control) shown as (A) *S. aureus* (B) *S. epidermidis* (C) *P. aeruginosa* (D) *E. coli*, while extract treated as (a) *S. aureus* (b) *S. epidermidis* (c) *P. aeruginosa* (d) *E. coli*

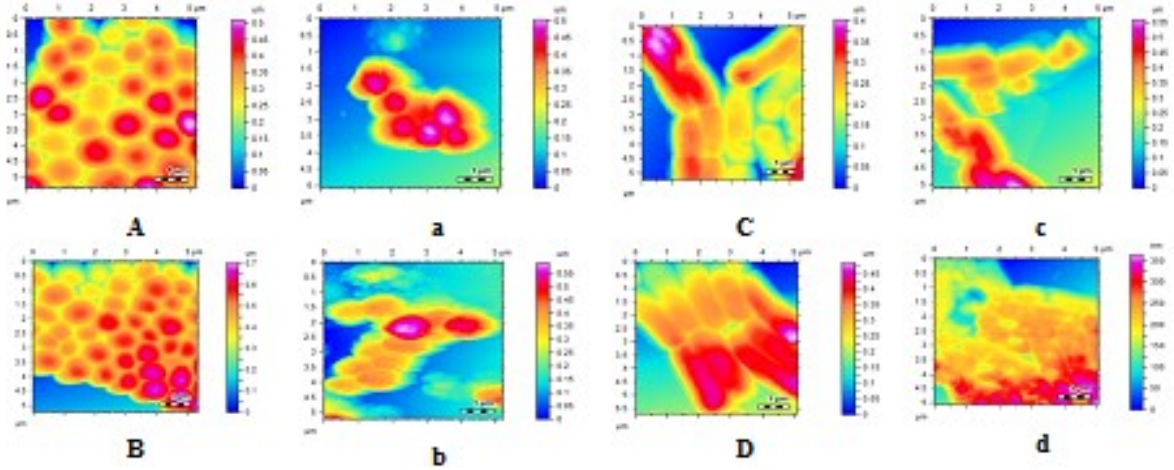



**Fig. 5**: Atomic force microscopic (dye colored biofilm) images *of M. buxifolia* crude extract untreated (control) shown as (A) *S. aureus* (B) *S. epidermidis* (C) *P. aeruginosa* (D) *E. coli*, while extract treated as (a) *S. aureus* (b) *S. epidermidis* (c) *P. aeruginosa* (d) *E. coli.*

Calcium have a key role in the contractions of the smooth muscle. Presence of iron, in the leaves extract of *M. buxifolia* is a core element of hemoglobin to transport oxygen across body (Karaki *et al*., 1997). The antibacterial potential of *M. buxifolia* leaves crude extract has been carried out against gram-positive strains i.e. *S. aureus* and *S. epidermidis* and gram-negative *E. coli* and *P. aeruginosa*. The CME of *M. buxifolia* leaves possessed substantial antimicrobial activity against the above mentioned bacterial pathogens. This potential antibacterial activity of *M. buxifolia* leaves crude extract may be due to important antimicrobial compounds. The compounds identified in the leaves crude extract of *M. buxifolia* include oxiranedodecanoic acid, 3-octyl- 3-deoxy estradiol, indole, cis-,4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 1-Ethyl-2-hydroxymethylimidazole and 5-hydrxoymethylfurfuralin. These compounds have already been reported to contain remarkable bacteria killing potential (Muñoz-Bonilla and Fernández-García, 2012; Medina-Estrada *et al*., 2016; Alizadeh *et al*., 2017). Phenol, 2, 4-bis(1,1-dimethylethyl) is also an important compound frequently reported as antibacterial, anti-tubercular and cytotoxic agent (Kuppuswamy *et al*., 2013). The bactericidal effect of *M. buxifolia* leaves extract was further authenticated by photographs of AFM. In the figures only damaged part of bacteria were magnified, which further authenticate that the leaves extract of *M. buxiflia* have significant bactericidal effect.

The dye colored images further conformed that *M. buxifolia* crude extract also have biofilm inhibitory effect particularly in gram positive bacteria. The antibacterial activity of *M. buxifolia* leaves crude extract was also confirmed by inhibition of DNA replication. The DNA of

extract treated bacteria was subjected to PCR amplification, followed by running on agarose gel. The gram positive (*S. aureus*, and *S. epidermidis*) bacterial DNA was not replicated. On the contrary, no any effect was seen on the DNA decantation of gram negative (*E. coli* and *P. aeruginosa*) bacterial strains. The DNA of both the gram negative bacteria appeared normal and supercoiled. The exact mechanism is not clear, but probably DNA replication inhibition in gram positive bacteria may be due to the fact that the bacterial cell wall was damaged and the compounds in the extract entered the bacterial cell and inhibited bacterial DNA replication. The findings obtained from these assays endorse and validate the local use of this plant to ameliorate the urinary tract infections (Hazrat *et al*., 2013). The cytotoxic bioassay showed the presence of anti-proliferative potential of the *M. buxifolia* leaves extract. The anticancer potential of *M. buxifolia* leaves crude extract may be due to the presence of betulin, lupeol, α-tocopherol, Urs-12-en-3-ol, acetate, (3β)-, ascorbyl palmitate, oleic acid, oxiranedodecanoic acid, 3-octyl-, cis- triarachine, l-(+)-ascorbic acid 2,6-dihexadecanoate, methyl linolenate, methyl palmitate and mysteric acid. As these compounds have already been declared as valuable anti-cancer agents in the literature (Constantinou *et al*., 2008; Król *et al*., 2015; Saleem, 2009; Fang *et al*., 2006; Shimpo *et al*., 1996; Yu *et al*., 2009; Kato *et al*., 1971). In addition to this, the presence of phytol and phytol-trimethylsilyl in the crude extract of *M. buxifolia* enhances its medicinal uses for the cure of hepatic cancer (Kumar *et al*., 2010; Kim *et al*., 2015).

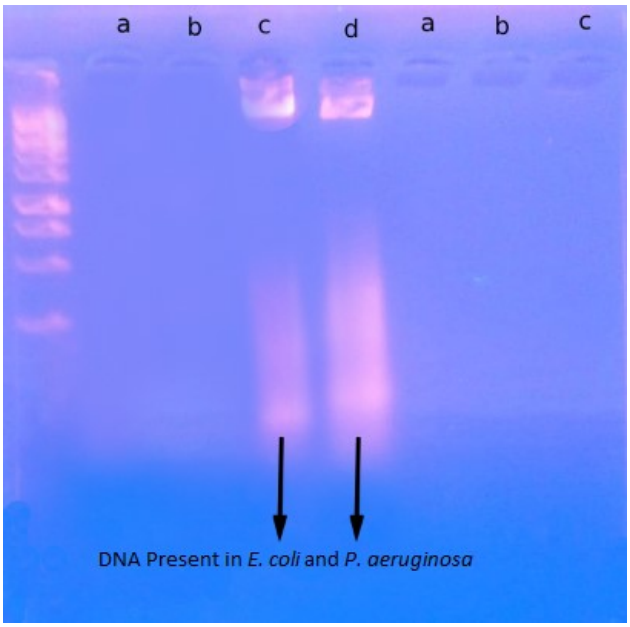



**Fig. 6**: Agarose gel electrophoresis showing *E. coli* and *P. aeruginosa* gene of PCR primer pair (DNA ladder- 1300 bp; DNA negative for (a) *S. aureus* (b) *S. epidermidis*, positive for (c) *E. coli* and (d) *P. aeruginosa*

## CONCLUSION

These results showed that the leaves extract of *M. buxifolia* has strong and distinguished antibacterial and anticancer activity. Further research needs to be carried out to isolate the constituents responsible for antibacterial and anticancer activity and to elucidate the mechanism of action.

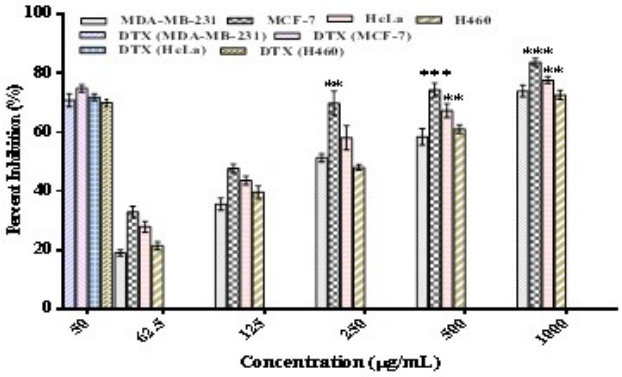



**Fig. 7**: Percent cell cytotoxicity of crude methanolic extract of *M. buxifolia* leaves against MDA-MB-231, MCF-7, HeLa, H460 cell lines at various concentrations, DTX (Docetaxel). Results are represented as mean± SD, (n=3). *$p$<0.05, **$p$<0.01 and ***$p$<0.001

## REFERENCES

Alizadeh M, Khodaei H, Mesgari AM and Saleh Ghadimi S (2017). Assessing the effect of 5-hydroxy-methylfurfural on selected components of immune responses in mice immunised with ovalbumin. *J. Sci. Food Agric.*, **97**(2): 3979-3984.

Altuner EM, Canli K and Akata I (2014). Antimicrobial screening of *Calliergonella cuspidata*, *Dicranum polysetum* and *Hypnum cupressiforme*. *J. Pure. Appl. Microbiol.*, **8**(1): 539-545.

Ayaz M, Junaid M, Ullah F, Sadiq A, Shahid M, Ahmad W, Ullah I, Ahmad A and Syed NIH (2017). GC-MS analysis and gastroprotective evaluations of crude extracts, isolated saponins, and essential oil from *Polygonum hydropiper* L. *Front. Chem.*, **5**: 58.

Bartlett JG, Onderdonk AB, Louie T, Kasper DL and Gorbach SL (1978). A review: Lessons from an animal model of intra-abdominal sepsis. *Arch. Surg.*, **113**(7): 853-857.

BronzettI G, Della CC, Aretini P and Fiori OR (1996). Antimutagenesis and anticancer effects. *J. Environ. Pathol. Toxicol. Oncol*: official organ of the International Society for Environmental Toxicology and Cancer, **15**(2): 59-64.

Burki ZG, Hassan M, Burki S, Farooqui WA, Zeb A and Ahmad S (2017). Effect of zinc supplementation on serum fasting blood sugar and HbA1c in adult diabetics on oral hypoglycemic agents. *Gomal. J. Med. Sci.*, **15**(1): 8-11.

Canli K, Altuner EM, Akata I, Turkmen Y and Uzek U (2016). *In vitro* antimicrobial screening of *Lycoperdon lividum* and determination of the ethanol extract composition by gas chromatography/mass spectrometry. *Bangladesh. J. Pharmacol.*, **11**(2): 389-394.

Coleman JE (1992). Zinc proteins: Enzymes, storage proteins, transcription factors, and replication proteins. *Annu. Rev. Biochem.*, **6**: 897-946.

Constantinou C, Papas A and Constantinou AI (2008). Vitamin E and cancer: An insight into the anticancer activities of vitamin E isomers and analogs. *Int. J. Cancer.*, **123**(4): 739-752.

Cooper EL (2008). eCAM: An emerging linkage with ethnopharmacology? *Evid. Based. Complement. Alternat. Med.*, **5**(4): 365-366.

Eggleston K, Lu M, Li C, Wang J, Yang Z, Zhang J and Quan H (2010). Comparing public and private hospitals in China: Evidence from Guangdong. *BMC. Health. Serv. Res.*, **10**: 76.

Fang Q, Naidu KA, Naidu KA, Zhao H, Sun M, Dan HC, Nasir A, Kaiser HE, Cheng JQ and Nicosia SV (2006). Ascorbyl stearate inhibits cell proliferation and tumor growth in human ovarian carcinoma cells by targeting the PI3K/AKT pathway. *Anticancer. Res.*, **26**(1A): 203-209.

Gomez MR, Cerutti S, Sombra LL, Silva MF and Martínez LD (2007). Determination of heavy metals for the quality control in argentinian herbal medicines by ETAAS and ICP-OES. *Food. Chem. Toxicol.*, **45**(6): 1060-1064.

Hazrat A, Nisar M and Zaman S (2013). Antibacterial activities of sixteen species of medicinal plants reported from Dir Kohistan Valley KPK, Pakistan. *Pak. J. Bot.*, **45**(4): 1369-1374.

Jan S and Khan MR (2016). Protective effects of *Monotheca buxifolia* fruit on renal toxicity induced by $CCl_4$ in rats. *BMC Complement. Altern. Med.*, **16**(1): 289.

Jan S, Khan MR, Rashid U and Bokhari J (2013). Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. *Osong. Public. Health. Res.*, **4**(5): 246-254.

Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano KI, Harada KI, Miyamoto S, Nakazawa H, Won KJ and Sato K (1997). Calcium movements, distribution and functions in smooth muscle. *Pharmacol. Rev.*, **49**(2): 157-230.

Kato A, Ando K, Tamura G and Arima K (1971). Effects of some fatty acid esters on the viability and transplant ability of Ehrlich ascites tumor cells. *Cancer Res.*, **31**(5): 501-504.

Khan N, Ahmed M, Wahab M, Ajaib M and Hussain SS (2010). Studies along an altitudinal gradient in *Monotheca buxifolia* (falc.) ad, forest, District Lower Dir, Pakistan. *Pak. J. Bot.*, **42**(5): 3029-3038.

Kim CW, Lee HJ, Jung JH, Kim YH, Jung DB, Sohn EJ, Lee JH, Woo HJ, Baek NI and Kim YC (2015). Activation of caspase -9/3 and inhibition of epithelial mesenchymal transition are critically involved in antitumor effect of phytol in hepatocellular carcinoma cells. *Phytother. Res.*, **29**(7): 1026-1031.

Król SK, Kiełbus M, Rivero-Müller A and Stepulak A (2015). Comprehensive review on betulin as a potent anticancer agent. *Biomed. Res. Int.*, **2015**.

Kumar PP, Kumaravel S and Lalitha C (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *Afr. J. Biochem. Res.*, **4**(7): 191-195.

Kuppuswamy K, Jonnalagadda B and Arockiasamy S (2013). GC-MS analysis of chloroform extract of *Croton bonplandianum*. *Int. J. Pharm. Bio. Sci.*, **4**(4): 613-617.

Lopez N, Kobayashi L and Coimbra R (2011). A comprehensive review of abdominal infections. *World. J. Emerg. Surg.*, **6**(7): 1-10.

Medina-Estrada I, Lopez-Meza JE and Ochoa-Zarzosa A (2016). Anti-inflammatory and antimicrobial effects of estradiol in bovine mammary epithelial cells during *Staphylococcus aureus* internalization. *Mediators. Inflamm.*, pp.1-16.

Muñoz-Bonilla A and Fernández-García M (2012). Polymeric materials with antimicrobial activity. *Prog. Polym. Sci.*, **37**(2): 281-339.

Oliveira RN, Mancini MC, Oliveira FCSD, Passos TM, Quilty B, Thiré RMDSM and Mcguinness GB (2016). FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. *Materia (Rio de Janeiro)*, **21**(3): 767-779.

Piaru SP, Mahmud R and Perumal S (2012). Determination of antibacterial activity of essential oil of *Myristica fragrans* Houtt. using tetrazolium microplate assay and its cytotoxic activity against Vero cell line. *Int. J. Pharmacol.*, **8**(6): e6.

Rawat P, Kumar A, Singh TD and Pal M (2018). Chemical composition and cytotoxic activity of methanol extract and its fractions of *Streblus asper* leaves on human cancer cell lines. *Pharmacogn. Mag.*, **14**(54): 141-144.

Robinson DA, Griffith RW, Shechtman D, Evans RB and Conzemius MG (2010). *In vitro* antibacterial properties of magnesium metal against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Acta. Biomater.*, **6**(5): 1869-1877.

Saleem M (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer. Lett.*, **285**(2): 109-115.

Sarkar A, Parikh N, Hearn SA, Fuller MT, Tazuke SI and Schulz C (2007). Antagonistic roles of Rac and Rho in organizing the germ cell microenvironment. *Curr. Biol.*, **17**(14): 1253-1258.

Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D and Boyd MR (1988). Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res.*, **48**(17): 4827-4833.

Shimpo K, Takahashi H, Tsuda H, Hibino T, Kawai K, Kimura C, Nagatsu T and Fujita K (1996). Inhibition of hepatocellular carcinoma development and erythrocyte polyamine levels in ODS rats fed on 3'-methyl-4-dimethylaminoazobenzene by hemicalcium ascorbate, 2-O-octadecylascorbic acid, and ascorbyl palmitate. *Cancer. Detect. Prev.*, **20**(2): 137-145.

Stein S, Mirokhin D, Tchekhovskoi D, Mallard G, Mikaia A, Zaikin V and Clifton C (2002). The NIST mass spectral search program for the NIST/EPA/NIH mass spectra library. Gaithersburg, MD: Standard Reference Data Program of the National Institute of Standards and Technology.

Ullah I, Khan JA, Shahid M, Khan A, Adhikari A, Hannan PA, Javed I, Shakeel F and Farooq U (2016). Pharmacological screening of *Monotheca buxifolia* (Falc.) A. DC. for antinociceptive, anti-inflammatory and antipyretic activities. *BMC. Complement. Altern. Med.*, **16**: 273-280.

Winnepenninckx B (1993). Extraction of high molecular weight DNA from molluscs. *Trends. Genet.*, **9**(12): 407.

Yu W, Jia L, Park SK, li J, Gopalan A, Simmons Menchaca M, Sanders BG and Kline K (2009). Anticancer actions of natural and synthetic vitamin E forms: RRR-α-tocopherol blocks the anticancer actions of γ-tocopherol. *Mol. Nutr. Food. Res.*, **53**(12): 1573-1581.