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Benth**

## ***In vitro* anti-oxidant and cytotoxic analysis of *Pogostemon mollis* Benth**

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### **Abstract**

In the present study, the anti-oxidant and cytotoxic effects of the different solvent extracts of *Pogostemon mollis* were analyzed. The phenolic, tannin and flavonoid contents were highest in the ethyl acetate extract and analogous to the antioxidant activity results. The extracts showed activities similar to the standard anti-oxidants. The extent to which the extracts protect free radical damage on DNA was evaluated and showed good genoprotective effects. Bacterial cells and fungal spores and hyphae showed visible damages due to the treatment of ethyl acetate extract. Finally, in the cytotoxic analysis, IC<sub>50</sub> value was calculated based on the absorbance value of different concentrations. It concluded that *P. mollis* is a prospective candidate for the various therapeutic applications especially its ethyl acetate extract.

### **Introduction**

Herbal drugs possess remarkable properties and effects on different biochemical pathways, controlling several organ systems simultaneously. Studies reveal the therapeutic effects of medicinal plants result from the combinations of different secondary metabolites (Beidokhti and Prakash, 2013). They function as anti-oxidants in various mechanisms and their ability in scavenging the radicals can be analyzed employing different antioxidant assays (Kutlu et al., 2014). Presently the screening for anti-oxidant properties of medicinal and edible plants is being extensively carried out (Saravanan and Parimelazhagan, 2014). Plant-based antimicrobials are eco-friendly and safer to use. Taking into account the vast potential of the medicinal plants as antifungal and antibacterial agents, systematic studies should be done to make it further applicable. Utilization of phytochemicals and anti-oxidants against carcinogenesis is a novel consideration (Ghaffari et al., 2014). Experimental analyses validated the anti-cancer abilities of various plant products (Cai et al., 2004; George et al., 2015). Cytotoxicity study using enzyme based methods like

MTT determines cell viability and gives feedback on the response of different cell lines to the extract (Wallet and Provost Lab, 2007).

*Pogostemon* and other Lamiaceae plants are used as age-old herbal remedies. Their pharmacological activities have also been scientifically validated (Caldas et al., 2014; Lu, 2011; Kumar et al., 2007). But the phytochemical properties and therapeutic effects of *Pogostemon mollis*, used by tribals in India are not much worked upon. Here, the *in vitro* anti-oxidant activity, cytotoxicity and HPLC analyses of various solvent extracts of the aerial parts of *P. mollis* are studied.

### **Materials and Methods**

#### ***Collection and identification of plant material***

The aerial parts of the plant *P. mollis* were collected from Kattapettu, Nilgiris, India. The taxonomic identity of the plant was confirmed by the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu. The herbarium specimen was deposited in



Bharathiar University Herbarium (006242). The plant collected was washed under running tap water to remove the surface pollutants and was shade dried. Then it was homogenized into the fine powder using mixer grinder and used for further studies.

#### Chemicals

2,2-diphenyl-1-picryl hydrazyl (DPPH), potassium persulfate, 2,2'-azinobis (3-ethyl-benzothiozoline)-6-sulfonic acid disodium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin and ciocalteau's phenol reagent, sodium nitroprusside, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, dimethyl sulfoxide, Dulbecco's Modified Eagle Medium (DMEM), Minimal Essential Eagle's Medium (MEM), Luria Bertani broth, agarose, etc. were obtained from Himedia Laboratories, Mumbai; Sisco Research Laboratories (SRL), Mumbai and Sigma-Aldrich (USA). All the chemicals and solvents used were of analytical grade.

#### Extraction of plant material

The plant powder was packed in a small thimble and extracted successively using petroleum ether, chloroform, ethyl acetate, acetone and methanol. The residual powder was dried and macerated using hot water. The extracts were concentrated by rotary vacuum evaporator (Equitron, Medica Instruments Mfg. Co.) and then air dried.

#### Quantitative analysis of total phenolics, tannins and flavonoids

The total phenolic and tannin content of the plant extracts were determined according to the method described by Makkar (2003). The absorbance was read at 725 nm against the reagent blank and the results were expressed in Gallic Acid Equivalents (GAE). The total phenolic as well as non-tannin phenolic contents were estimated in Tannic Acid Equivalents (TAE). From these two results, the tannin content of the plant samples was calculated as follows:

Tannins = Total phenolics - Non tannin phenolics

The flavonoid contents of all the extracts were quantified according to the method described by Zhishen et al. (1999). The pink color developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. Rutin was used as the standard for the quantification of flavonoids. All the experiments were done in triplicates and the results were expressed in Rutin Equivalents (RE).

#### DPPH radical scavenging activity

The anti-oxidant activities of the extracts were determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Braca et al. (2001). Radical scavenging activity of the samples was expressed as IC<sub>50</sub> which is

the concentration of the sample required to inhibit 50% of DPPH' concentration.

#### ABTS radical cation scavenging activity

The total anti-oxidant activity of the samples was measured by ABTS radical cation decolorization assay by the method of Re et al. (1999). Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated against the blank (ethanol) absorbance at 734 nm and then was plotted as a function of trolox concentration.

#### Ferric reducing anti-oxidant power (FRAP) activity

The anti-oxidant capacities of different extracts of samples were estimated according to the procedure described by Pulido et al. (2000). Methanolic solutions of known Fe (II) concentration (FeSO<sub>4</sub>.7H<sub>2</sub>O) were used for the preparation of the calibration curve.

#### Phosphomolybdenum activity

The anti-oxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto et al. (1999) with slight modification. The results are reported in mean values expressed as milligrams of ascorbic acid equivalents per gram extract.

#### Nitric oxide radical scavenging activity

The procedure is based on the method of Sreejayan and Rao (1997), where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. The scavenging activity (%) was calculated as:

Scavenging activity (%) = [(A<sub>0</sub> - A<sub>1</sub>) / A<sub>0</sub>] X 100

Where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the extract/standard

#### HPLC analysis

High performance liquid chromatography was carried out for the ethyl acetate, acetone and methanol extracts. Each sample was prepared at a concentration of 2 mg/mL of methanol. About 20 µL of the sample was injected into the Luna C-18 column (250 × 4.6 mm) by autosampler. The mobile phase used was a binary gradient formed by solvent A (3% acetic acid) and solvent B (acetic acid: acetonitrile: water in the ratio 3:50:47). The detector was adjusted to 320 nm and the running time was 65 min. Rutin, ellagic acid, quercetin, gallic acid, catechin, chlorogenic acid, p-coumaric acid, cinnamic acid, caffeic acid and naringenin were used as reference standards. Retention time for each peak was noted and compared to the peaks formed for the standards. The concentration of each standard was calculated from the standard graphs using the formula,

$C_{(sa)} = [C_{(st)} \times A_{(sa)}] / A_{(st)}$

Where, C<sub>(sa)</sub> = concentration of compound in sample; C<sub>(st)</sub> =

concentration of standard;  $A_{(sa)}$  = area of peak in sample;  $A_{(st)}$  = area of peak in standard

#### Plasmid breakage protection assay

The ability of the extract to protect the prokaryotic DNA against damage is measured in this assay. The method of Phani Kumar *et al.* (2013) was followed here. Plasmid DNA isolated from bacteria was treated with UV and  $H_2O_2$ . This was kept for 6-10 hours incubation in dark and then the extent of damage caused was analyzed using gel electrophoresis.

#### Assessment of antimicrobial activity with scanning electron microscopy (SEM)

The bacterial strain *Escherichia coli* (MTCC-9747) and the fungal strains *Aspergillus flavus* (MTCC-1783), *A. niger* (MTCC-4325) and *Fusarium graminearum* (MTCC-2089) were used for studying antimicrobial activity according to Shi *et al.* (1996) with some modifications.

#### Preparation of bacterial cells for SEM

In a 5 mL nutrient broth *E. coli* cultures were inoculated and kept for overnight growth. This bacterial culture was treated with ethyl acetate extract of *P. mollis* (20  $\mu$ g). The extract was prepared in 9:1 water: DMSO (v/v). After treatment with the extract, the cells were harvested by centrifugation at 5000  $\times$ g for 10 min. The bacterial cells were fixed with 2.5% glutaraldehyde on a glass slide at 4°C overnight. The sample was then dehydrated by passing through an ethanol series of concentrations (10, 25, 50, 70 and 100%). Gold palladium was sputtered on the samples to avoid charging of the microscope. An FEI Quanta 200 ICON 200 (Oregon, USA) microscope was used for the study. The secondary electron images were taken at low electron energies between 2-2.5 keV.

#### Preparation of fungal strains for SEM

The mother cultures of the fungal strains to be studied were grown in Czapek-Dox broth. The spores were then grown on a glass slide (using potato dextrose agar). After the fungus has grown on the slide, 20  $\mu$ g of

the ethyl acetate extract of *P. mollis*, was added and incubated overnight. The difference in the hyphal morphology of control and treated samples were analyzed with scanning electron microscope set in ESEM mode.

#### In vitro cytotoxicity by MTT assay

MTT assay is a frequently used cytotoxic assay to identify the toxicity level of the test sample. The protocol of Mosmann (1983) with slight modifications was followed in this study. RAW 264.7 (mouse leukemic monocyte macrophage cells) maintained in DMEM medium, MCF-7 (human breast cancer cells) and Caco2 (human colonic adenocarcinoma cells) in MEM were used to check the extent of cytotoxicity of the extracts (100 ng - 100  $\mu$ g).  $H_2O_2$  was used as the positive control. The intensity of the violet color was measured using an ELISA reader at 570 nm. The graph for percentage viability was plotted using the absorbance values and the  $IC_{50}$  values were calculated. The images of the cells were viewed using Olympus CKX41 microscope and were photographed by Olympus imaging corp. digital camera (Model E-330).

#### Statistical analyses

All the experiments were done in triplicates and the results were expressed as Mean  $\pm$  Standard Deviation. The data were statistically analyzed using one-way ANOVA followed by Duncan's test. Mean values were considered statistically significant at  $p < 0.05$ .

## Results

The results showed that ethyl acetate extract has the maximum amount of total phenolics with 474.8 mg GAE/g extract (Table I). Methanol and acetone extracts contain 311.1 and 309.8 mg GAE/g extract respectively. The highest amount of tannins were found to be for ethyl acetate extract with 148.2 mg TAE/g extract followed by methanol and acetone extracts with 117.8 and 105.3 mg TAE/g extract. The results of tannin

Table I

### Quantification of total phenolics, flavonoids and tannins

Extract	Total phenolics (mg GAE/g extract)	Tannins (mg TAE/g extract)	Flavonoids (mg RE/g extract)
Petroleum ether	54.2 $\pm$ 7.1	6.8 $\pm$ 5.4 <sup>d</sup>	174.5 $\pm$ 24.9 <sup>c</sup>
Chloroform	244.2 $\pm$ 9.3 <sup>c</sup>	89.9 $\pm$ 3.4 <sup>c</sup>	351.4 $\pm$ 13.7 <sup>b</sup>
Ethyl acetate	474.8 $\pm$ 7.5 <sup>a</sup>	148.2 $\pm$ 4.5 <sup>a</sup>	431.8 $\pm$ 106.7 <sup>a</sup>
Acetone	309.8 $\pm$ 7.5 <sup>b</sup>	105.3 $\pm$ 14.3 <sup>b</sup>	302.8 $\pm$ 11.5 <sup>b</sup>
Methanol	311.1 $\pm$ 11.9 <sup>b</sup>	117.8 $\pm$ 5.3 <sup>b</sup>	302.0 $\pm$ 5.0 <sup>b</sup>
Hot water	244.4 $\pm$ 9.5 <sup>c</sup>	89.1 $\pm$ 6.3 <sup>c</sup>	167.4 $\pm$ 4.7 <sup>c</sup>

Values are mean of triplicate determination  $\pm$  standard deviation; Statistically significant at  $p < 0.05$  where  $a > b > c > d$ ; GAE- Gallic Acid Equivalents; TAE- Tannic Acid Equivalents; RE - Rutin Equivalents

quantification are expressed in tannic acid equivalents. Ethyl acetate extract of *P. mollis* had the highest flavonoid content with 431.8 mg RE/g extract. Chloroform extract also showed a good amount of flavonoids followed by acetone and methanol. Hot water extract had the least flavonoid content.

The stable free radical DPPH is commonly employed to analyze the anti-oxidant activity of various compounds. Here the assay was carried out to measure the IC<sub>50</sub> value of *P. mollis* extracts to scavenge DPPH radicals. Ethyl acetate extract with the IC<sub>50</sub> value of 3.1 µg/mL and acetone extract with 3.8 µg/mL were the best. These values were comparable to that of the various standards like gallic acid (1.5 µg/mL) and quercetin (2.9 µg/mL). From the results of BHA it can be concluded that the natural anti-oxidants have a better scavenging capacity than the synthetic ones (Figure. 1). In the ABTS assay, ethyl acetate extract showed the best activity among all the extracts (53737.3 µM TE/mg). Acetone, methanol and chloroform extracts also had good response to this assay. Petroleum ether and hot water extracts showed the least effects to the ABTS radicals (Table II).

The ferric reducing power is a typical characteristic of anti-oxidants and the results are expressed in mM Fe (II)/mg of extract (Table II). The standard anti-oxidants like BHT (155.1 mM Fe (II)/mg), rutin (154.2 mM Fe (II)/mg) and quercetin (154.2 mM Fe (II)/mg) are highly efficient in the reduction of ferric ions. *P. mollis* ethyl acetate extract is significantly similar in its activity as the other anti-oxidant standards. This is clearly seen in the result with 147.9 mM Fe (II)/ mg. Acetone extract with 132.4 mM Fe (II)/mg also showed good activity in this regard. The results are statistically significant with

the highest for the standards followed by ethyl acetate and acetone extracts respectively (Table II).

Ethyl acetate extract has a considerable effect on the formation of the green phosphomolybdenum complex showing a better anti-oxidant activity (362.9 mg AAE/g). This can be compared to the other anti-oxidant assays. Chloroform extract with 343.4 mg AAE/g was also having a good phosphomolybdenum complex formation capability. It can be clearly understood that all the extracts are showing a comparatively good result in this assay. Rutin, quercetin and BHT were used as standard anti-oxidants in this assay.

Reactive nitrogen species are one of the most common free radicals. These are scavenged by various anti-oxidant molecules and thus can be applied as a means to measure the anti-oxidant capacity. Acetone extract (11.7%) showed the highest percentage of nitric oxide inhibition when compared to the other extracts. Ethyl acetate extract with 10.1% inhibition was also having similar activity. It can be noted from the results that the nitric oxide inhibition percentage is somewhat similar in all the extracts of *P. mollis*.

The HPLC analysis of ethyl acetate, acetone and methanol extracts of *P. mollis* were done. The retention time obtained for the samples was compared to illustrate the standard phenolic and flavonoid compounds present in it (Table III). Naringenin was found in all the three extracts and was the only compound found in the ethyl acetate extract (24.0 µg/mg).

The isolated plasmid DNA was treated with free radicals initiated from H<sub>2</sub>O<sub>2</sub> by UV treatment. A separate set with extract alone did not show any streaking of DNA proving that the extract is not causing any

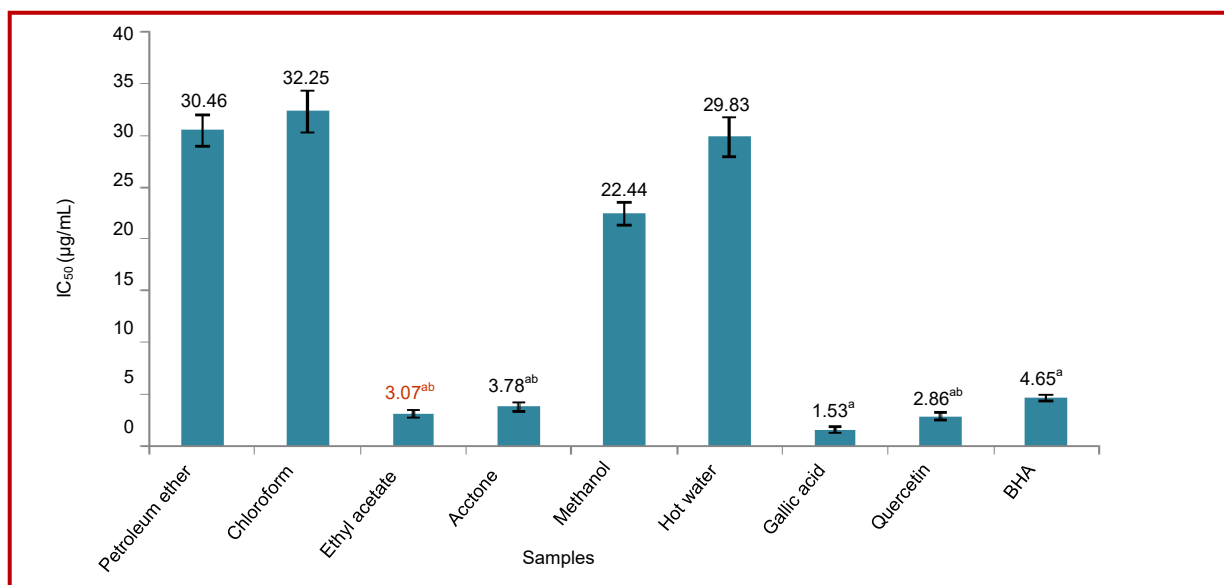


Figure 1: DPPH radical scavenging activity

Values are mean of triplicate determination (n=3) ± standard deviation; Statistically significant at p<0.05 where a > b

damage to the plasmid at the particular dose i.e. 50  $\mu\text{g}$  (Figure 2). The damage to the plasmid DNA can be clearly understood by the absence of any significant DNA band in the third lane. In this experiment, *P. mollis* extracts have prevented the damage caused by free radicals from  $\text{H}_2\text{O}_2$  and this is clearly visible in the treated samples. Even though all the three extracts have a similar extent of protection, ethyl acetate extract has best protected the DNA from damage.

The SEM analysis was carried out to study the varia-

tions in the *E. coli* treatment with the ethyl acetate extract of *P. mollis* showed critical shrinkage and alterations in the normal morphology when compared to the control. Shrinkage and fragmentation of cells finally led to the death of the organism (Figure 3).

The normal morphology of *A. flavus* was damaged by the addition of the ethyl acetate extract of *P. mollis*. The hyphal and spore morphology showed great variations like deformation in the spores, and was found to be in non-uniform clusters. The conspicuous degradation of

Table II

## Results of ABTS, FRAP, phosphomolybdenum and nitric oxide scavenging assays

Extract	ABTS <sup>•+</sup> assay ( $\mu\text{M TE}/\text{mg extract}$ )	FRAP assay ( $\text{mM Fe (II)E}/\text{mg extract}$ )	Phosphomolybdenum assay ( $\text{mg AAE}/\text{g extract}$ )	Nitric oxide scavenging assay (% inhibition)
Petroleum ether	2297.1 $\pm$ 106.1	17.4 $\pm$ 0.1	254.4 $\pm$ 4.1	5.1 $\pm$ 0.5
Chloroform	14777.5 $\pm$ 1591.9 <sup>d</sup>	42.5 $\pm$ 6.2	343.4 $\pm$ 3.2 <sup>e</sup>	8.6 $\pm$ 0.5 <sup>c</sup>
Ethyl acetate	53737.3 $\pm$ 530.5 <sup>b</sup>	147.9 $\pm$ 2.0 <sup>b</sup>	362.9 $\pm$ 2.2 <sup>d</sup>	10.1 $\pm$ 0.4 <sup>c</sup>
Acetone	27594.1 $\pm$ 2773.5 <sup>c</sup>	132.4 $\pm$ 3.2 <sup>c</sup>	258.9 $\pm$ 2.1	11.7 $\pm$ 0.3 <sup>b</sup>
Methanol	14574.5 $\pm$ 565.2 <sup>d</sup>	123.4 $\pm$ 1.3 <sup>d</sup>	191.4 $\pm$ 3.0	4.4 $\pm$ 0.2
Hot water	9358.8 $\pm$ 459.4	86.2 $\pm$ 1.0	112.0 $\pm$ 2.4	8.9 $\pm$ 0.5 <sup>c</sup>
BHT	185617.7 $\pm$ 509.4 <sup>a</sup>	155.1 $\pm$ 2.0 <sup>a</sup>	453.1 $\pm$ 5.2 <sup>c</sup>	-
Rutin	156402.0 $\pm$ 7307.7 <sup>a</sup>	154.2 $\pm$ 1.5 <sup>a</sup>	631.7 $\pm$ 5.3 <sup>b</sup>	34.2 $\pm$ 0.8 <sup>a</sup>
Quercetin	-	154.2 $\pm$ 0.9 <sup>a</sup>	966.3 $\pm$ 7.4 <sup>a</sup>	-

TE - Trolox Equivalents; AAE - Ascorbic Acid Equivalents; Fe(II)E - Ferrous Equivalents; Values are mean of triplicate determination (n=3)  $\pm$  standard deviation; Statistically significant at  $p < 0.05$  where a > b > c > d > e

Table III

## Concentration of antioxidant standards by HPLC analysis

Standard	Retention time (Min)	Concentration ( $\mu\text{g}/\text{mg extract}$ )		
		Ethyl acetate	Acetone	Methanol
Gallic acid	6.778	-	-	-
Chlorogenic acid	21.298	-	0.2	0.9
Caffeic acid	23.452	-	0.3	0.2
p-Coumaric acid	31.206	-	-	-
Ferulic acid	36.586	-	-	-
Trans-cinnamic acid	59.242	-	1.7	-
Catechin	19.032	-	3.1	18.9
Rutin	42.617	-	-	27.3
Quercetin	51.09	-	0.9	-
Naringenin	56.757	24.0	5.4	1.3

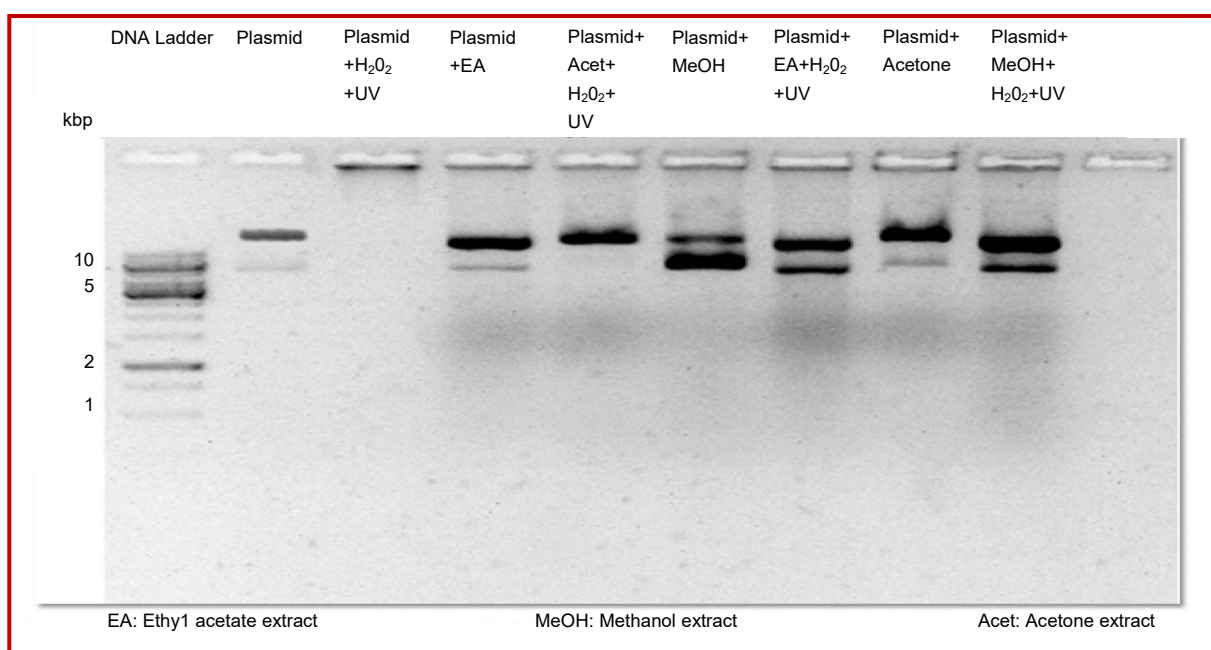
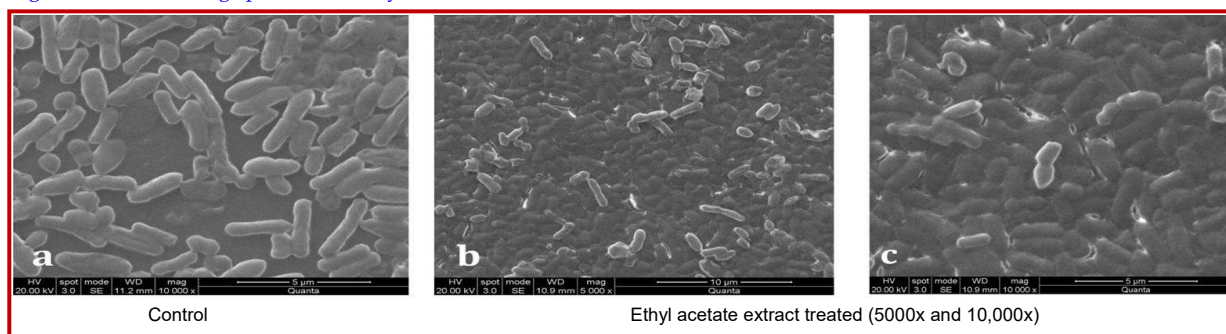


Figure 2: DNA breakage protection assay

Figure 3: SEM images of *E. coli*

hyphae and spores mark the extent of damage. In *A. niger*, the overall growth retardation shows the damaging effect of the extract. While the control spores showed healthy hyphae and well-organized spore arrangements, the extract treated sample had occasional constrictions and bulging. The spherical spores turned into shrunken and almost cubical spores (Figure 4).

The morphology of *F. graminearum* was also analyzed using SEM (Figure 5). The spores which were found neatly arranged in the control sample were found to be disorganized in extract treated sample. The hyphae lost its turgidity and appeared as a flattened loose thread.

The MTT assay employed to estimate the cytotoxicity level of the extracts was carried out on RAW 264.7, MCF-7 and Caco-2 cell lines. The cells were tolerant to a comparatively higher dose of the extracts. The  $IC_{50}$  values calculated using the absorbance is presented in Table IV with the lowest value for ethyl acetate extract. In addition, the observation of the morphology of the cells showed the effects of various concentrations of different extracts on the cells. This has helped to

analyze the extent of damage occurred which has ultimately led to cell death. Figure 6 shows the images of the cells with clear damages in the structural characteristics. The variation of the structural morphology of the cells itself reveal the damage caused to the cells by the extracts. The cell viability percentage of different extracts on the three cell lines is presented in Figure 7. The extracts showed a decrease in the cell viability in a concentration dependent manner.

## Discussion

The present study focuses on *P. mollis*. The quantification of polyphenols is very important in the phytochemical analysis of medicinal plants due to their highly commendable anti-oxidant activities (Zhu et al., 2004). Polyphenols found in most of the plant products have various physiological and biochemical functions in the body and possesses antiproliferative, neuroprotective, cardioprotective effects and regulation of cell functions (Sahelian, 2014). Recently, the role of tannin in

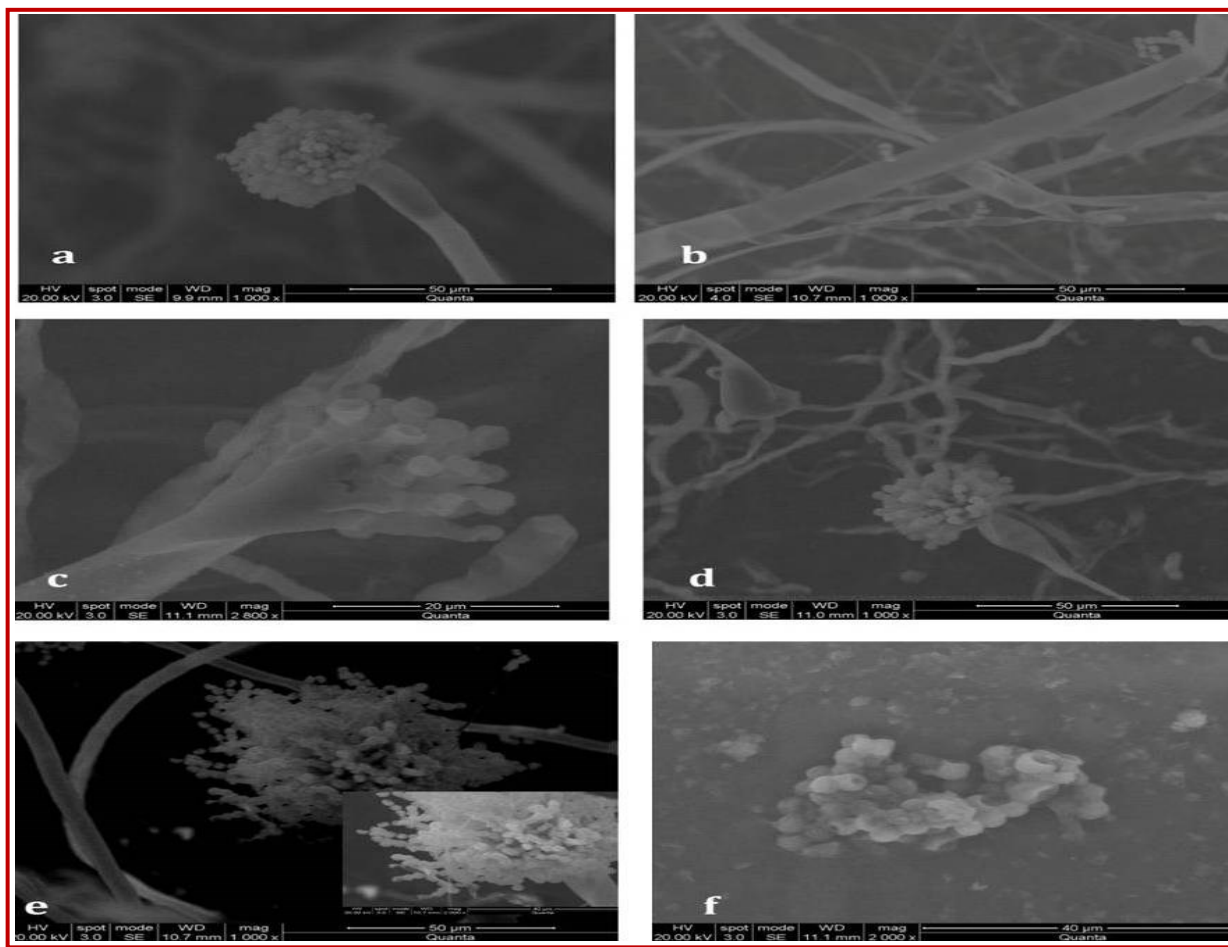


Figure 4: SEM images of *Aspergillus niger* and *A. flavus*  
 a - *A. niger* control; b,c,d - *A. niger* ethyl acetate extract treated; e - *A. flavus* control; f - *A. flavus* ethyl acetate extract treated

Table IV

Cytotoxicity level of the extracts using MTT assay			
Extract	RAW 264.7	Caco-2	MCF-7
Ethyl acetate	28.11	15.07	47.06
Acetone	54.88	31.04	233.50
Methanol	52.29	53.72	201.63

metabolic sensors integrating lipid, drug and liver metabolism, inflammation and glucose homeostasis, anti-oxidant capacities etc. were revealed (Eloranta and Kullak-Ublick, 2005; Beaven and Totonoz, 2006). The biological effects of flavonoids includes free radical scavenging, anti-inflammatory, hepatoprotective, anti-allergic, antiviral, antiulcer properties, etc. (Agarwal, 2011). From the present study we can understand that the ethyl acetate, methanol and acetone are the best solvents for extracting the phenolic compounds as well as the tannins and flavonoids. The low polar solvents like chloroform and petroleum ether has lesser amounts. As *P. mollis* possesses such enormous

amounts of secondary metabolites, it is likely that it can be considered as a potential candidate for the therapeutic effects which needs to be further analyzed properly.

The various anti-oxidant assays performed are concerned in different aspects of free radical scavenging either differing in their mechanism or in the ionic components taking part in the reaction or the scavenging mechanism. In the electron transfer mechanism based DPPH assay the ethyl acetate extract of *P. mollis* showed to contain substantial amounts of the reductants even at low concentrations which react with DPPH radicals to make them stable compared to other extracts (Huangre et al., 2005). The ethyl acetate extract is also showing good ABTS<sup>•+</sup> scavenging ability which is in support to the other Lamiaceae members (Kowalczyk et al., 2012; Ertas et al., 2014). The result of the FRAP assay signifies the efficiency of *P. mollis* extracts to reduce Fe<sup>3+</sup> ions efficiently like the standard compounds. The bioactive compounds present in the ethyl acetate and chloroform extracts of *P. mollis* are able to reduce more amount of Mo (VI) into Mo (V) as revealed by the phosphomolybdenum assay. The scavenging of free radicals is



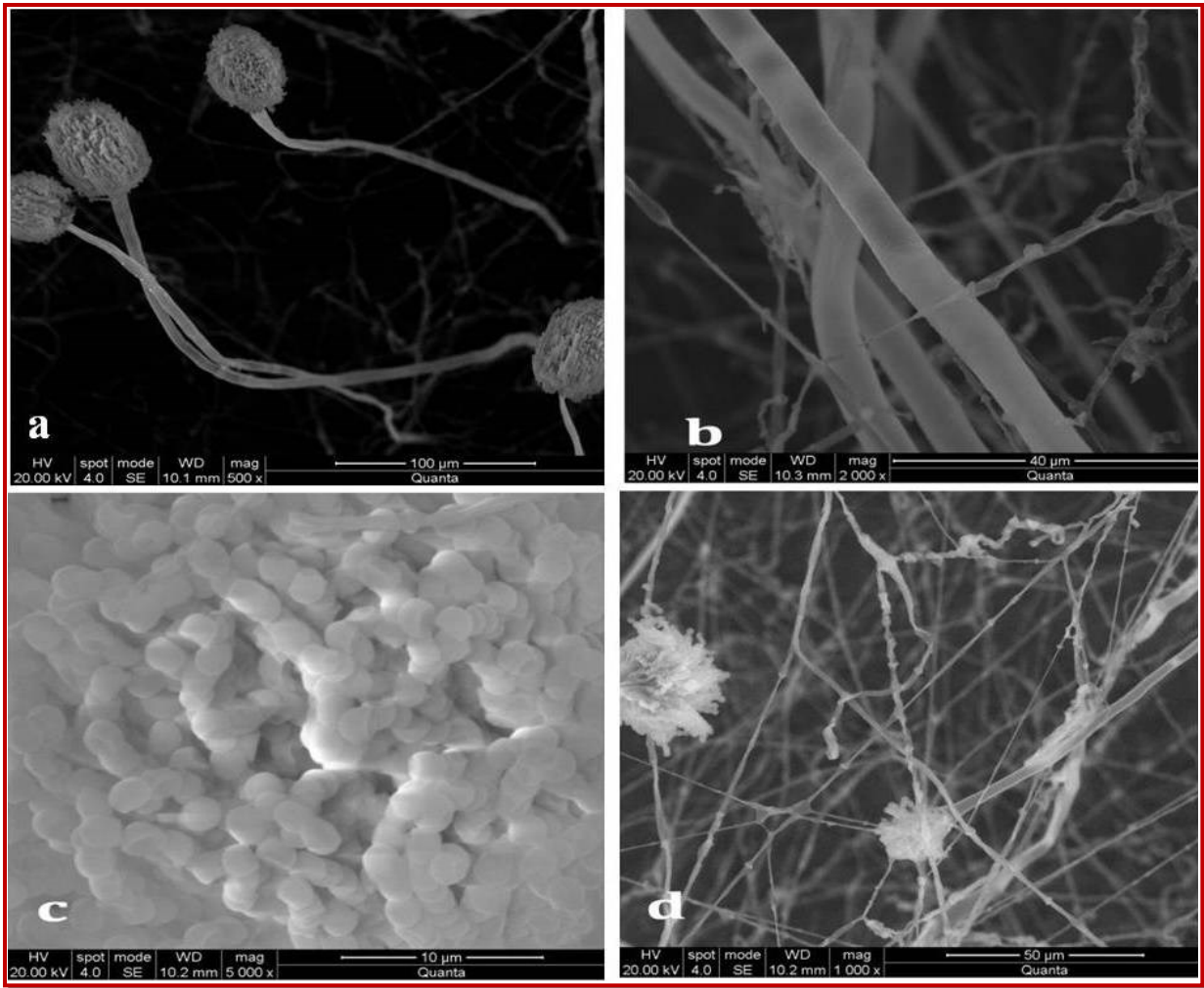


Figure 5: SEM images of *Fusarium graminearum*

a,b - *F. graminearum* control; c, d - *F. graminearum* ethyl acetate extract treated

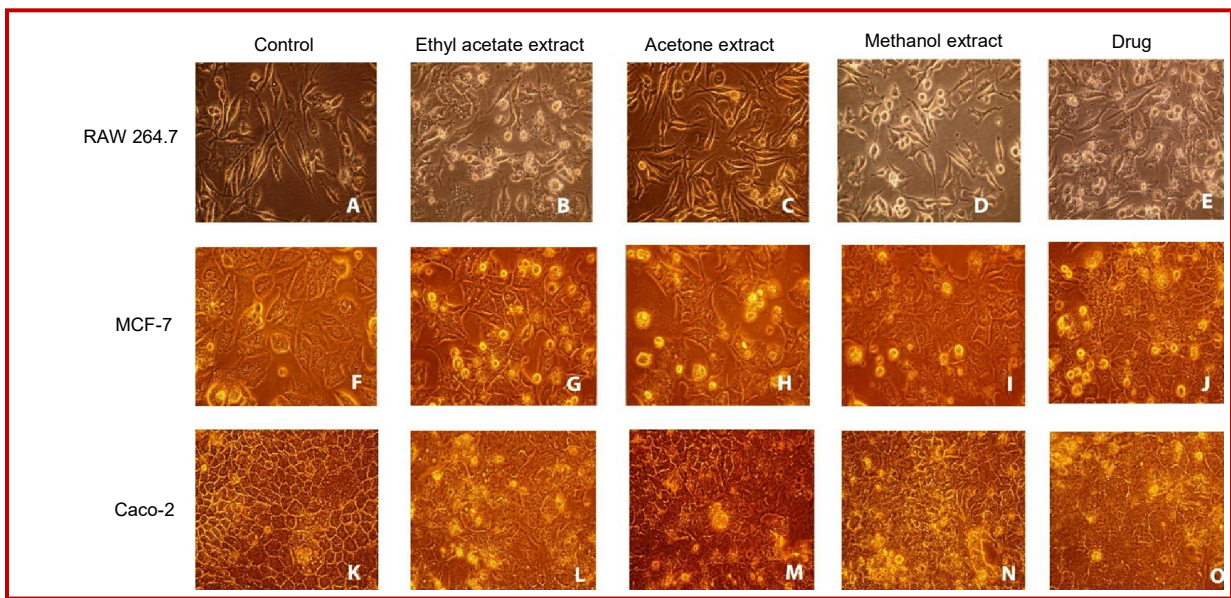


Figure 6: Images of cells in MTT assay by the effect of *P. mollis* extracts

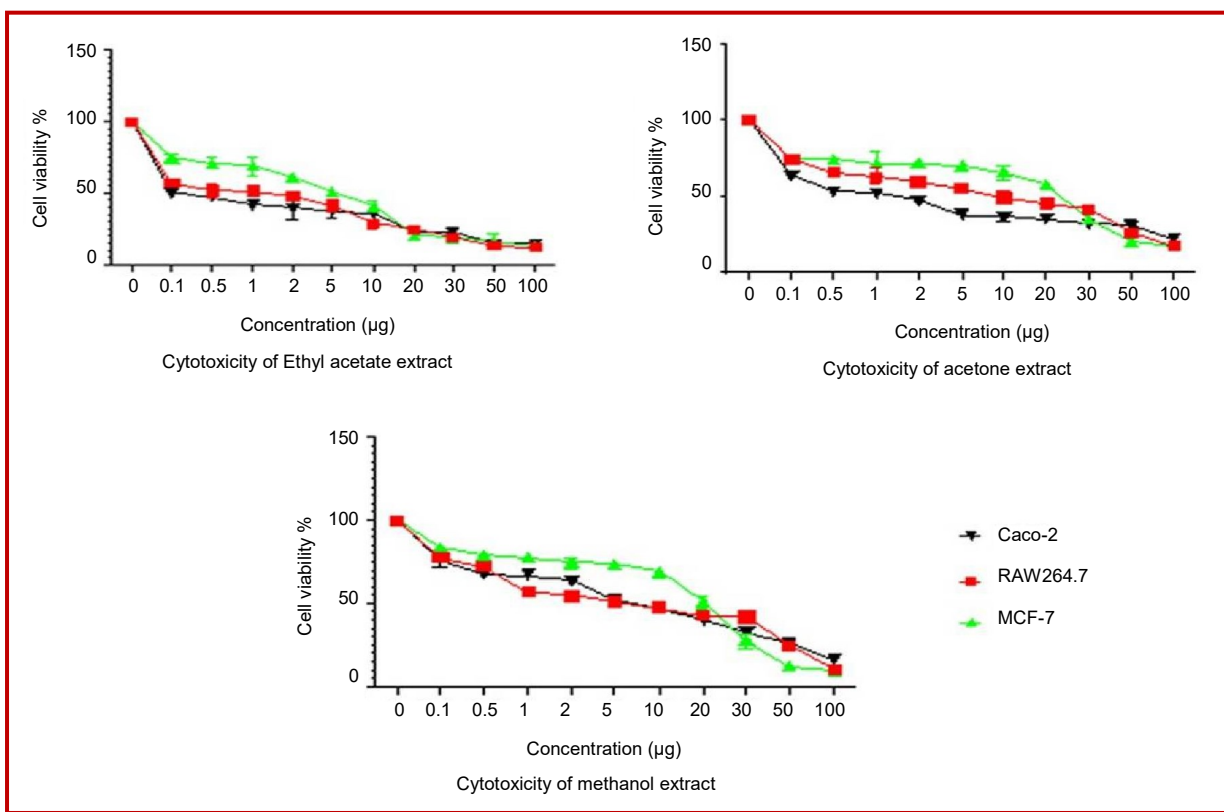


Figure 7: Percentage viability of different cell lines due to *P. mollis* extracts in MTT assay

the chief therapeutic effect of these biologically important entities. And their presence in different extracts comes to the decisive role by this reason. The quantification of the secondary metabolites when correlates with the various anti-oxidant assays affirms the role of these biologically important chemicals in the anti-oxidant capacity. Phenolics, flavonoids, etc. can be considered as the underlying cause for the miraculous healing potential of medicinal plants. It seems that the flavonoids and phenolics in *P. mollis* also attribute to its anti-oxidant activities because ethyl acetate and methanol extracts topped in both.

The HPLC analysis of ethyl acetate, methanol and acetone extracts have given the presence of many specific phenolic and flavonoid compounds. Earlier studies have revealed many of the active principles in *Pogostemon* like luteolin, quercetin, ermanine, kaempferol, vanillic acid, benzyl alcohol, kumatakenin, pachypodol, flavons, pogostone, phenylethanoids (acetoside, isoacetoside, crenatoside), ombuine licochalcone and 5,7-dihydroxy-3'-4'-dimethoxyflavanone, etc. (Li, 2011; Chakrapani *et al.*, 2013). Correspondingly the present study showed the phenolic and flavonoid contents present in *P. mollis*. The presence of naringenin in the ethyl acetate extract is significant as it is this extract which showed the best activity for almost all the assays carried out. It might be this flavonoid which could be responsible for the efficiency

of this extract. The acetone extract with chlorogenic acid, caffeic acid, trans-cinnamic acid, catechin, quercetin and naringenin also showed a good activity in many assays.

Free radicals cause damage to DNA, proteins and cell components like the cell and organelle membranes (Martnett, 1999). The experiment performed in this study has engaged  $H_2O_2$  and UV for causing the damage. The UV irradiation of DNA in the presence of  $H_2O_2$  resulted in the cleavage of the DNA strand which is visible in the lane with  $H_2O_2$  and plasmid. This indicates that the OH radical generated from UV photolysis of  $H_2O_2$ , produced DNA strand scission. The results clearly mark the damage caused by the  $H_2O_2$  and UV. Moreover, the clear protection of the DNA by *P. mollis* extracts is evident from the images as the bands of DNA invisible in the negative control was clearly seen in the test treated samples. Consequently, the identification of natural products which can provide protection against UV radiation-related responses and the generation of oxidative stress may have important human health implications (Kutlu *et al.*, 2014). Thus, the present findings of *P. mollis* extracts showing a good range of protection against the  $H_2O_2$ -induced DNA damage will make it a promising candidate for the same. At this juncture, it should be taken into consideration that the DNA protecting ability is in line with the anti-oxidant capacities of the samples. Thus

the higher level of anti-oxidants in these samples may also function to stabilize the DNA damage as these compounds can neutralize or destroy the free radicals.

The extracts and essential oil of *Satureja hortensis* in a previous study exhibited antimicrobial properties like shrinkage, leakage of cell contents, damage to cell membrane proteins and depression of cell walls (Benli et al., 2007; Burt 2004; Burt and Reinders, 2003). The effect of acetone extract of *Arctotis arctoides* on the growth and ultrastructure of fungi, revealed alteration in fungal morphology by the extract. The conidiophores showed shrinkage, partial distortion and reduced size. There were remarkable morphological variations like deformation of mycelia, flattening and distortion of pseudohyphae and also the whole conidia showed disintegration. Similar to the present findings, many the cells changed from smooth and turgid to distended, rough and flaccid upon treatment (Otang et al., 2011). The SEM images clearly points out that the present investigation reveals the antimicrobial properties of *P. mollis* extract.

MTT assay is an enzyme-based assay which relies on the reduction of coloring reagent in a viable cell to determine the cell viability using a colorimetric method. Among other enzyme based assays, MTT is best known for determining mitochondrial dehydrogenase activities in the living cells. This method of cell determination is useful for the measurement of cell growth, response to mitogens, growth factors, membrane stability, cytotoxicity and to derive growth curves (Akhir et al., 2011). The cytotoxicity studies provide a preliminary knowledge about the nature of the activity of the herbal products on the cancer cells. The ethyl acetate extract with a lower IC<sub>50</sub> can be considered better among the others even though they are all somewhat similar in activity. This can be due to the presence of naringenin which was found to have antiproliferative effect against various cancer cell lines (Erlund, 2004). The images of the cells also prove the damage due to the extracts. The extracts showed a cytotoxic effect in a concentration-dependent manner.

## Conclusion

*P. mollis* is an assorted plant with many innovative implementations and can be a better alternative source of highly potent phytotherapeutics.

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## Conflict of Interest

Authors declare no conflict of interest to reveal

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