IJREB

ISSN 2321-743X

International Journal of Research in

Engineering and Bioscience

Volume 2 (Issue 1)

Journal home page: www.ijreb.org

ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF MEDICINAL PLANT SPECIES SUBJECTED TO ABIOTIC STRESS IN EASTERN GHATS

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ABSTRACT

Abutilon indicum, Achyranthes aspera, Gomphrena celosioides, *Ocimum sanctum* and *Azadirachta indica* are chosen due to their previous history as the plants with various medicinal properties. In order to study the effect of metal stress (abiotic stress), the plants are selected from industrially polluted site. Agar well diffusion method was used to analyze broad spectrum antimicrobial potential of the extracts of the selected plants subjected to abiotic stress by industrial pollution. Of the important human pathogenic microorganisms selected, the gram positive bacteria, *Bacillus cereus* was proved to be highly susceptible to all the extracts followed by *Enterococcus, Bacillus subtilis and Staphylococcus aureus*. Among the gram negative bacteria, *Klebsiella* was resistant to all the extracts, whereas *E.coli* showed average response. The extracts of *Azadirachta indica* subjected to stress exhibited antifungal activity against *Candida albicans*. XTT assay was used to analyze the invitro cytotoxic activities of the extracts of *Ocimum sanctum* and *Azadirachta indica* against human hepatocarcinoma cell line (HepG2). Abiotic stress enhanced the phytochemicals and therapeutic activity in *Ocimum sanctum* only. These observations clearly indicate that *Ocimum sanctum* extracts possess anticancer activity suggesting wide application of the plant species in the above therapeutic preparations.

KEYWORDS: Abiotic stress, antimicrobial, cytotoxic, HepG2 cell lines and Zone of inhibition.

ISSN 2321-743X
International Journal of Research in
Engineering and Bioscience
Volume 2 (Issue 1)
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INTRODUCTION

Higher plant-derived natural products constitute 25% of the total natural products, their derivatives and analogs represent over 50% of all drugs in clinical use (Balandrin *et al*., 1993). The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (Farnsworth & Wilson 1998). Evidence of the importance of natural products is provided by the fact that almost half of the world's 25 best selling pharmaceuticals in 1991 were either natural products or their derivatives (O Neill et *al*., 1993). It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants (Ncube *et al*., 2008).

Current estimates suggest that in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health needs. For historical and cultural reasons herbal medicines have often maintained popularity (Ford 1978). Five medicinal plants used in traditional medicine were collected from Eastern Ghats (unpolluted environment) and polluted environments of HPCL (Visakha Refinery) with respect to metal accumulation and their antimicrobial and antitumor activity.

The acceptance of the traditional medicine as an alternative form of health care and the microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano *et al*., 1996; Hammer *et al*., 1999). Plants containing terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Hostettmann and Nakanishi 1979). Phenolic compounds are known to be major contributors to antimicrobial activity derived from spices and culinary herbs (Kisko & Roller, 2005).

Essential oil and their constituents have been used as flavouring agents in the formulation of different pharmaceutical products. (Cowan 1999). Natural products have been used as anticancer agents (Frie

ISSN 2321-743X
International Journal of Research in
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1982), such as vincristine and vinblastin from *Catharanthes roseus* (Johnson 1963). Even vegetables and fruits may help reduce the risk of cancer in humans (Moon *et al*., 2011; Chen *et al*., 2006).

The extracts that gave promising results in antimicrobial screening have been selected for testing the anticancer potential of these plants against the. Based on the results of antimicrobial activity, *Ocimum sanctum* and *Azadirachta indica* have been selected. Further study investigated the invitro cytotoxicity of these two plants against the human hepatocarcinoma (HepG2) cell line, Compared with the standard drug cis platin.

MATERIALS AND METHODS

In the present study five plant species were selected based on the information collected from literature and through field observation. The plant materials were collected from Visakhapatnam district, Andhra Pradesh, India. Whole plants were screened for their antimicrobial activity. The collected materials were washed thoroughly under running tap water and finally with sterile distilled water and then the materials were air dried on a sterile blotter under shade to constant weight for a period of 45 days. The collected plant specimens were identified with the Herbarium available in the Department of Botany, Andhra University, Visakhapatnam. The collected plant materials are the following

1) *Ocimum sanctum* Linn., Lamiaceae

2) *Gomphrena celosioides* Mart-Beitr., Amaranthaceae

3) *Achyranthes aspera* Linn., Amaranthaceae

4) *Abutilon indicum* Linn., Malvaceae

5) *Azadirachta indica* A. Juss., Meliaceae

In-vitro antimicrobial assay:

The crude extracts of different plant parts were subjected to antimicrobial assay using well plate method. Antimicrobial screening of different species of microorganisms was carried by following the method of Agar-well diffusion assay by Mbata *et al*., 2006 and Valgas *et al*., 2007. This method is highly effective for rapidly growing microorganisms. The inhibition of the growth of the microorganism around the well, known as zone of inhibition was measured. The activity of the test compound was expressed by measuring the diameter of zone of inhibition (mm) around the well. Generally, the more

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susceptible the organism, the larger is the zone of inhibition.

Collection of Microbial Cultures:

Based on common diseases in human beings 8 pathogenic bacteria species were selected to perform the antimicrobial action of test samples. Name of the culture is listed in Table 4. All the cultures were collected from TRIMS, Visakhapatnam, Andhra Pradesh.

Media used for Antimicrobial Assay:

For bioassay studies the media used is Mueller-Hinton agar. The addition of the agar to the medium creates a solid matrix and by avoiding any significant mixing, the culture is good for inoculating microbes on surface of the medium as required for isolation of pure cultures.

Composition of Mueller-Hinton Agar Medium:

Beef, infusion – 300 gm/lt

Casein – 17.5 gm/lt

Agar – 17.0 gm/lt

Starch – 1.5 gm/lt

Preparation of Culture:

A loop full of clinically tested pre-cultures was reconstituted in sterile peptone water to produce a suspension of microbial cells.

Preparation of Media and Plates for Agar Diffusion Method:

To prepare media, for each organism it requires 20 plates of MTT agar for 500 ml of distilled water 19.5 gm of MH agar was weighed and dissolved in a conical flask. Then it was autoclaved at 15 lbs pressure at 121°C for 20 minutes. After sterilization media was aseptically distributed into petri plates and allowed to solidify. After solidification, using a sterile cotton swab each microbial culture was spread uniformly on to the surface of the agar plates. The most widely used type of identifying antimicrobial activity is the diffusion method which exploits diffusion of antimicrobial compounds through the agar media to demonstrate inhibition of bacteria. The assay was performed by using well-plate method. After inoculation of culture into each petri plate, a well borer of 5 mm diameter was properly sterilized by flame and used to make 4 uniform wells in each petri plate. These wells are labeled with the numbers 1, 2, 3 and 4. To determine the potential of plant extracts they were diluted up to 500 mg/ml, 250mg/ml, 125 mg/ml and 62.5 mg/ml of DMSO solution. And

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from each dilution 20 µl was introduced into wells 1, 2, 3 and 4 respectively and allowed to diffuse for 45 min. The plates were incubated at 37°C for 24 hours. After proper incubation the zone of inhibitions were measured with a ruler. Results were noted and presented.

CYTOTOXIC STUDIES

Cell culture

Human cancer cell lines (HepG2) used in this study were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, antibiotics (BenzylPencillin – 50units/mL, Streptomycin - 50 µg/ml and Amphotericin –B -50 µg/ml), 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

XTT assay

The biochemical procedure is based on the activity of mitochondrial enzymes which are inactivated shortly after cell death. This method was found to be very efficient in assessing the viability of cells. A colorimetric method based on the tetrazolium salt, XTT, was first described by Scudiero (1988). In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 24hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds (12.5,25,50,100,200 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in DMSO. Culture medium and solvent are used as controls. Each well then received 50 µl of fresh XTT (0.9mg/ml in RPMI along with XTT activator reagent) followed by incubation for 2hr at 37°C. At the end of the incubation shacked the 96 micro well plate for 15sec. The Optical Density (OD) of the culture plate was read at a wavelength of 490 nm (reference absorbance at a wavelength of 630 nm) on an ELISA reader, Anthos 2020 spectrophotometer.

- **% cell survival:** 100 – {(At-Ab) / (Ac-Ab)} × 100

Whereas, At = Absorbance of test

Ab = Absorbance of blank

Ac = Absorbance of control

- **% cell inhibition:** 100 - % cell survival

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RESULTS AND DISCUSSION

Antimicrobial activity

Antimicrobial activity is carried on several disease causing gram negative and gram positive pathogens. In the present study(Graph 1) *Abutilon indicum* extracts showed antimicrobial activity only against two gram positive pathogens. Plants in grown in both the soils exhibited more or less same activity. The inhibition zone ranged from 7-15 mm. All the three extracts in hexane, chloroform and methanol solvents displayed varied degree of antimicrobial activity against gram positive *Enterococcus* and *Bacillus cereus*.

Ranjit (2013) carried out the antimicrobial activity of chloroform extract prepared from leaves of *Abutilon indicum* using agar-well diffusion method against both gram positive and gram negative microorganisms. In the present study (Graph 2) extracts of *Achyranthes aspera* showed antimicrobial activity against one gram positive *Bacillus cereus*. Extracts of *Achyranthes aspera* grown in both the sites showed little variation. Inhibition zone ranged from 7-10 mm. Hexane and methane extracts exhibited antimicrobial activity against the fungus strain and gram positive Bacillus cereus. Thus the variation between extracts of both the soils is not effective. Of all the five plant species studied, least activity is shown by *Achyranthes aspera*.

The ethanol, acetone and ethyl acetate root extracts of *Achyranthes aspera* display varying degree of antibacterial activities against the tested bacterial strains. The ethanol root extract is found to be the most effective against *Salmonella typhimurium, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella abony* strains. Acetone extract indicate the activity against *Salmonella typhimurium , Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* strains. Ethyl acetate extract showed activity against *Salmonella typhimurium, Escherichia coli, Bacillus subtilis, Staphylococcus aureus* strains.

The results obtained suggest that the ethanol, acetone and ethyl acetate root extracts of *A. aspera* reveals a significant scope to develop a novel broad spectrum of antibacterial drug formulation. These extracts can be used for development of a new alternative medicine system. The similar results are not obtained

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by the *Achyranthes* extracts in the present study as the whole plant extracts are used and the solvents used are also different.

Methanol extract of dried whole plants of *Achyranthes aspera* was evaluated against bacterial species viz., *Bacillus cereus, Escherchia coli, Acetobacter baumanii Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* and *Salmonella typhi*. All the bacterial species were found susceptible to methanolic extract of whole plants of *Achyranthes aspera* however at high concentrations. *Achyranthes aspera* found to be positive for the presence of carbohydrate, proteins, alkaloids, saponins, terpenoids, tannins and flavonoids. Methanol extract of whole plant of *Achyranthes aspera* was selected to test antibacterial activity against eight different microorganisms using agar well diffusion method. Methanolic extract showed maximum inhibition at 2000µg concentration (Neeta *et al*., 2011). However the whole plant extracts that were used in the present study are in very low concentration (100µmg/ml) therefore the zone of inhibition is very poor.

In the present analysis(Graph 3) *Gomphrena celosioides* didn't show much variation in the extracts of plants under both the soils. Hexane fraction showed against *E.coli.* This is the only antimicrobial activity in gram negative bacteria. Inhibition zone ranged from 7-10mm which is not effective. The activity is comparatively very less on two gram positive and one gram negative bacteria. To compare with, there are no previous reports on the extracts of *Gomphrena celosioides*.

In the present study (Graph 4) *Azadirachta indica* displayed greatest antibacterial effect among all the extracts of the present study. Among the hexane, chloroform and ethanol extracts, chloroform showed highest activity against microorganisms on both fungi and bacteria which includes three gram positive, one gram negative and fungal strain. The plant grown in normal site showed profound antimicrobial activity than the extracts of polluted site grown plants. Inhibition zones ranged from 7-20mm. Only chloroform extract of this plant showed good antimicrobial activity against the fungal strain. All the three extracts showed varying antimicrobial activity against four gram positive bacteria and fungus. However,

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metal stress under which A*zadirachta* is grown suppressed the antimicrobial activity in the extracts.

Azadirachta indica was tested against selected Gram positive and Gram negative bacterial species. Phytochemical leaf extracts of *A. indica* exhibited significant anti-bacterial activity against all the test microorganisms. Among the different extracted used in the study, ethanolic and dichloromethane leaf extracts of *A. indica* were found to be more active towards the bacterial species used in the study. However, petroleum ether and chloroform extracts were not effective against any of the organisms tested, but for *Bacillus cereus* where the chloroform extract was moderately active. The study shows that ethanolic and dichloromethane leaf extracts of *A. indica* can be used as a potential source of antimicrobial agents (Rajasekaran, 2008).

In the present study the antimicrobial efficacy of aqueous extracts of leaf, bark and seeds of *A. indica* were compared against human pathogenic bacteria (*Staphylococcus aureus, Enterococcus feacalis, Proteus mirabilis* and *Pseudomonas aeuroginosa*) and fungi (*Aspergillus fumigatus* and *Candida albicans*). Minimum inhibitory concentration (MIC) of leaf and bark extract was found to be in the range of 500 to 2000µg/ml for all the tested microorganisms (Reddy *et al*., 2013).

The methanol extract of *Azadirachta indica* exhibited pronounced activity against *Bacillus subtilis* (28 mm), high activity against the Gram positive organism *Staphylococcus aureus* (18mm), the Gram-negative bacteria *Proteus vulgaris* (18 mm) and *Salmonella typhi* (20 mm), low activity against *Pseudomonas aeruginosa* (14 mm) and inactive against *Escherichia coli* . These might be due to presence of triterpenoids, phenolic compounds, Carotenoids , steroids , valavinoids, ketones and tetratriterpenoids Azadirachtin. All extracts were inactive against *Aspergillus niger*. The petroleum ether and methanolic extracts of *Azadirachta indica* exhibited high activity against *Candida albicans* (15-18mm); while its aqueous extract was inactive against *Candida albicans* (Grover *et al*., 2011).

The most commonly found pathogenic organisms were *Escherichia* (17 strains) followed by *S. aureus* and *Pseudomonas* (9 strains each). Sharma *et al*., (2009) demonstrated pronounced activity of neem leaf methanolic extract against *S. aureus*. They

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found lower activity of leaf extract against *E. coli* and *Klebsiella*. For *Escherichia* and *Klebsiella* in the present study that leaf extract was more effective.

In the present study (Graph 5) *Ocimum sanctum* extracts grown in polluted soils as shown in the table 5 exhibited second highest activity of all the tested plant species. Inhibition zone ranged from 7-15mm. Antimicrobial activity against the used pathogens is not promising in extracts of *Ocimum sanctum* under unpolluted conditions. However, metal stress enhanced antimicrobial activity against all gram positive bacteria.

Ocimum sanctum leaves possessed antifungal activity against clinically isolated dermatophytes at the concentration of 200 µg/mL. MIC and MFC were high with water fraction (200 µg/mL) against dermatophytic fungi used. *Ocimum sanctum* has antifungal activity and the leaf extracts may be a useful source for dermatophytic infections (Balakumar *et al*., 2011).

Among all extracts, methanolic leaves extract exhibited greatest antibacterial effect showing 20 mm and 18 mm zone of inhibition against *S. enterica* and *E. coli* O26 respectively. All the extracts were relatively less effective against *E. coli* O26. It was also noted that within the zone of inhibition induced by 10 mg concentration/disc, resistant bacterial colonies were present. It was further noted that on prolonged incubation zone of inhibition was narrowed down (Varshney *et al*., 2011).

The order of antimicrobial activity in descending order is as follows *Azadirachta indica* > *Ocimum sanctum* > *Abutilon indicum* > *Gomphrena celosioides* > Achyranthes aspera.

The results obtained in the present study suggest that extracts of *Azadirachta indica* and *Ocimum sanctum* reveals a significant scope to develop novel broad spectrum antimicrobial drug formation. Therefore to study specific therapeutic action, the study proceeded towards cytotoxicity action.

The gram negative bacteria were more resistant to inhibition than gram positive bacteria. This may be due to different nature of gram negative cell envelope that makes access to membrane. More restricted in gram negative bacteria

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due to high content of lipopolysaccharide in the cell wall. (Cox *et al*., 2000 ; Oussalah, 2006)

In the present study among the three gram negative bacteria *Klebsiella* did not show any response to any of the extracts, whereas *E.coli* showed least response. Among 4 gram positive bacteria *B.cereus* showed maximum response by all extracts followed by *Enterococcus, B.subtilis* and *S.aureus*. The observed high susceptibility in unpolluted chloroform and methanolic extracts of *A. indica* c when compared to that of polluted one in case of *O. sanctum* can be attributed to the plants being small shrub. In the case of *Ocimum* (whole plant except roots has been used for extraction of phytochemicals where as *A. indica* being the large tree the dispersion of heavy metals absorbed was comparatively less which can be the possible reason for varied presence of phytochemicals in two plants taken from polluted environment

Cytotoxic effect of the plant crude extracts in Human

Hepatocarcinoma HepG2 cell lines

Upon studying the antibacterial activity, two medicinal plants namely, *Ocimum sanctum* and *Azadirachta indica* that have good response on antibacterial activity have been selected. These two plants are chosen for the study due to their previous history as the plants with various medicinal properties. Invitro cytotoxicity test is mainly performed to screen potentially toxic compounds that effect basic cellular functions. This toxicity is measured with cellular damage using XTT assay. The present study demonstrated the cytotoxicity indices as a measure of percentage cell mortality calculated by XTT assay. Cell viability was determined as previously reported in methodology by using the cleavage of the tetrazolium salt XTT (3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by the mitochondrial enzyme succinate dehydrogenase to give a blue product (formazan). The cell lines included in our study responded to the crude extracts, basically by inhibition of proliferation and induction of cell death (apoptosis and necrosis).

The methanol fraction being most polar fraction is expected to contain active components. Methanol fractions were found to be safer in terms of cytotoxicity and

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abundant in flavonols. Although these plants are famous for their limonoids, it seems flavonols can be in charge for many medicinal effects of leaves of the plants (Jafari *et al*., 2013). In order to understand the characteristic of the cytotoxicity of *Ocimum sanctum* and *Azadirachta indica* extract on cancer cells, the bioactive compounds in extracts of these two plants were tested on Human Hepatocarcinoma (HepG2) cell lines, and in comparison with the standard drug (cis platin). The present study demonstrated the cytotoxicity indices as a measure of percentage cell mortality calculated by XTT assay. The effect of anticancer from *Ocimum sanctum* and *Azadirachta indica* on HepG2 cells was evaluated through microculture tetrazolium assay (MTT). Several concentrations of methanol extracts ranging from 6.25µg/ml to 200µg/ml were used. The percentage of inhibition activity showed steady increase for all the samples, with concentration increment of the extracts. The cytotoxicity increased with the increase in concentration of crude extract of methanol used on the cells. It is found that the crude extracts showed significant cytotoxicity to HepG2 with different IC50 values when compared to the standard synthetic drug, cis platin (Table 3).

Evaluation of morphological changes upon treatment with extracts

Morphological alteration of HepG2 cells upon exposure using extracts of *Ocimum sanctum* and *Azadirachta indica* grown under different abiotic conditions, were observed under phase contrast microscope. The cells indicated the most prominent effects after exposure to the above extracts. The cells are shown in Fig 1-4. The number of dead cells increased with concentration increment of the extracts. The presence of apoptotic bodies could be seen in the treated cells. The cells showed extensive vacuolation in the cytoplasm, indicating autophagy like mechanism of cell death. Autophagy like structures were clearly seen in cells treated with the extracts.

The cytotoxic effect of methanolic extracts of *Ocimum sanctum* on HepG2 cell lines by XTT assay

At the highest concentration of the tested concentration (200µgm/ml), the cytotoxicity of the extracts of *Ocimum*

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sanctum grown in unpolluted and polluted sites was 52% and 71.7% respectively. The effects were dose dependant and based on the dose response curve IC50 values were determined. The methanol extracts of *Ocimum sanctum* grown under two different soil conditions i.e. polluted and unpolluted exhibited antiproliferative effect on HepG2 cell lines with IC50 values 185.71µgm/ml and 103.21µgm/ml respectively. The above results on the cytoxicity of crude extracts of *Ocimum sanctum* reached upto 72% whereas the control cis platin showed upto 96.7% at the same concentration. The data is shown in Table 2.

The cytotoxic effects of methanolic extracts of *Azadirachta indica* on HepG2 cell line by XTT assay

At the highest tested concentration (200µgm/ml) the cytotoxicity of *Azadirachta* extract grown in two soil conditions i.e. natural and polluted sites were 69.6% and 50% respectively. The above plant extract grown in two different conditions i.e. natural and polluted sites showed the antiproliferative effect with IC50 values 102.11 µgm/ml and 185.59 µgm/ml respectively. The cytotoxic effect of neem is reduced in the plant grown at polluted site than that of unpolluted site.

However, the cytotoxic effect of *Ocimum sanctum* and *Azadirachta indica* showed little improvement when used together in comparison with the individual samples. The data is shown in Table 6. The extracts of *Ocimum sanctum* and *Azadirachta indica* grown in unpolluted sites showed 82.8% cell inhibition with IC value of 83. /168 µgm/ml when used together. Whereas the extracts of both the plants grown at polluted sites showed 76.2% cell inhibition with an IC value of 79.75 µgm/ml. Highest cell inhibition activity is shown by both the plant extracts grown at unpolluted site, least activity is shown by Azadirachta indica grown under polluted conditions.. the order of cytotoxicity in descending order is as follows,

Ocimum sanctum(N) + *Azadirachta indica* (N) > *Ocimum sanctum*(P) + *Azadirachta indica* (P) > *Ocimum sanctum*(P) > *Azadirachta indica* (N) > *Ocimum sanctum*(N) > *Azadirachta indica* (P).

The cell lines included in out study responded to the crude extracts, basically by inhibition of proliferation and induction

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of cell death (apoptosis and necrosis). In the present study IC50 values are above 100 µgm/ml when the extracts of *Ocimum sanctum* and *Azadirachta indica* are used individually. However when the crude extracts are used together on HepG2 cells, the extracts were cytotoxic to HepG2 cells with IC 50 values les than 100 µgm/ml. The results are shown in table 3.

The investigation provides evidence for cytotoxicity, which may be due to existing phytochemicals in the studied extracts as mentioned previously. Abiotic stress to which these plants are exposed enhanced the anticancer activity of only *Ocimum sanctum* and it is not so in *Azadirachta indica*. Further *Ocimum sanctum* grown under polluted soils showed higher anticancer activity. Robust anticancer activity is exhibited by *Ocimum sanctum* and *Azadirachta indica* when used in combination that are grown in unpolluted soil. Further when the extracts of both the plants are added together no significant difference is noted between the plants grown under different conditions. These observations clearly indicate *Ocimum sanctum* extracts possess potential anticancer activity followed by *Azadirachta indica*. As these plants have excellent safety profile, they may be ideal candidate for a prospective trial for different cancer cell lines and cancer patients as well.

The sensitivities of cancer cells to cell death by flavonoids are accordance with this finding from previous reports in literature. In another study, the presence of alkaloids with flavonoids in *Onobis hirta* was reported expressing superior activity against cancer cells (Mahasneh 2011). In the present study also in both the plant species terpenoids, flavonoids, phenols were found. However, alkaloids, tannins and saponins were not detected. This finding suggests that the reduction observed in the viable cells following treatment with the present extracts is due to cell death.

In conclusion, the present observations provide preliminary data exposing the present extracts to have potent cytotoxic activity against HepG2 cells. This calls for further studies on the active components for proper assessment of their chemotherapeutic properties as well as their possible development as promising anticancer drugs.

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The crude extracts of both the plants of our study too consisted various polyphenolic and flavonoid content (Table 1). Therefore the presence of phenolic and flavonoid compounds in the crude extracts was partly attributed to the anticancer activity of the crude extracts. Abiotic stress enhanced phytochemicals in certain plants. Flavonoids are more in polluted grown extracts of Gomphrena *celosioides*. Phenols are more in polluted grown extracts of *Abutilon indicum*, *Gomphrena celosioides* and *Ocimum sanctum*.

The methanolic extracts of two plants namely, *Azadirachta indica* and *Ocimum sanctum*, are assayed against HepG2 cell lines. Methanol extract is the safest extract in terms of cytotoxicity. Melianone possesses cytotoxic properties (Itokawa *et al.*, 1992) and activates hepatic metabolic enzymes in rat *(*Kim *et al.*, 1996) Methyl kulonate is reported to have anticancer properties (Pettit *et al.*, 2002). Neem is known worldwide as commercial natural insecticide, pesticide and agrochemical (Koul & Seema, 2004) and is abundant in cytotoxic limonoids (Carpinella *et al.*, 2006; Jafari *et al.*, 2013).

REFERENCES

1. Balandrin, N F Kinghorn, A D.1993. Farnsworth, Human Medicinal Agents from Plants ACS Symposium Series 534, pp 2-12.
2. Bisignano G, Germano MP, Nostro A, Sanogo R. 1996. Drugs used in Africa as dyes: antimicrobial activities. Phytotherapy research 9: 346-350.
3. Chen T-J, Jeng J-I-E, Liu C-W, Wu C-Y, Chen Y-C. 2006. Quercetin inhibition of ROS dependent and independent apoptosis in rat glioma C6 cells. Toxicology, 1-2: 113-126.
4. Cowan M. M. 1999. Plant products as antimicrobial agents. Clinical microbiology reviews. 12:564-82.
5. Farnsworth N R, Wilson E O, 1998. Biodiversity. National Academy Press, New York.
6. Ford. R.I ed, 1978. The nature and status of Ethnobotany. Mus. Anthrop. University of Michigan, Ann Arbor.
7. Freie. 1982. The national cancer chemotherapy program. Science, 217: 600-606.
8. Hammer KA, Carson CF, Riley TV. 1999. Antimicrobial activity of essential oils and other plants extracts. J. Appl. Microbiol. 86: 985-990.
9. Hostettmann-kaldas, M. & Nakanishi, K. 1979. Mronoic acid, a simple

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International Journal of Research in
Engineering and Bioscience
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triterpenooids keto acid with antimicrobial activity isolated from Ozoroa mucronata. Planta Medica 37(4), 358-360.

10. Kisko G & Roller, S. 2005. Carvacrol and P-cymene inactivate *E.coli* 0157: H7 in apple juice. BMC Microbiology.

11. Moon, J.Y., Mosaddik A, Kim H, Cho M, Choi H-L, Kim, Y. S. & Cho SK. 2011. The chloroform fraction of guava (*Psidium cattleianum* Sabine) leaf extract inhibits human gastric cancer cell proliferation via induction of apoptosis. Food Chem., 125:369-375.

12. Rajasekaran, C., Meignanam, E., Vijayakumar, V., Kalaivani, T., Ramya, S., Premkumar, N., Siva, R. and Jayakumararaj, R. 2008. Investigations on Antibacterial Activity of Leaf Extracts of *Azadirachta indica* A. Juss (Meliaceae) -A traditional medicinal plant of India. *Ethnobotanical Leaflets* 12: 1213 - 1217.

13. Sharma, D., Lavania, A.A. & Sharma, A. 1988. In vitro comparative screening of antibacterial and antifungal activities of some common plants and weeds extracts. Asian J. Exp. Sci. 2009; 23:169-72.

14. O Neill, M. J., Lewis, J. A., Kinghorn, A. D. & Balandrin. 1993. Human Medicinal Agents from Plants, ACS Symposium Series 534, pp 48 – 55.

15. Ncube, N., Afolayan, S.A.J. & Okoh, A.I. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotechnol. 7(12): 1797-1806.

16. Valgas C, Souza, S. M., Elza, F. A., Mania, S. & Mania, A. Jr. 2007. Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology 38:369-380.

17. Ranjit, M. 2013. Antimicrobial and Antihelminthic Activities of Various Extracts of Leaves and Stems of Abutilon indicum (Linn.) International Journal of Pharmaceutical & Biological Archives 4(1):235-239.

18. Sharma Raj Neeta, Bala Jyoti, Singh Anjuvan and Kaur Prabhjot. 2011. Antibacterial activity of Achyranthes aspera Linn procured from Himachal Pradesh, Punjab and Haryana, India. Research journal of Chemical Sciences. Vol. 1(8), 80-82.

19. Reddy, R. R., Y, Krishna Kumari C, Lokanatha O, Mamatha S, Damodar Reddy C. 2013. Antimicrobial activity of Azadirachta Indica (neem) leaf, bark and seed extracts. Int. J. Res. Phytochem. Pharmacol., 3(1), 1-4.

20. Varshney, P., Dash, S. K. & Bhatia. A. K. 2011. *In vitro* and *in vivo* antibacterial Effects of Leaf Extracts of Ocimum

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Engineering and Bioscience
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sanctum and *Argemone mexicana*. 21914546

21. Cox, S. D., Mann, C. M., Markham, J. L., Bell, H.C., Gustafson, J. E., Warmington J. R. & Wyllie, S.G.2000. The mode of antimicrobial action of the essential oil of Melaleuca *alternifolia* (tea tree oil). Journal of Applied Microbiology, 88, 170–175.

22. Oussalah, M., Caillet, S., Saucier, L., Lacroix, M. 2006. Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. Meat Science, 73, 236-244.

23. Jafari, S., Saeidnia, S., Hajimehdipoor, H., Reza, M., Ali,. S. A. M., Hadjiakhoondi, F. A. & Khanavi, M. 2013. Cytotoxic evaluation of *Melia azedarach* in comparison with Azadirachta indica and its phytochemical investigation. Licensee Bio Med Central Ltd.

24. Mahasneh AM. 2011. Ant proliferative activity of plant extracts used against cancer in traditional medicine. Sci Pharm 78: 33-45.

25. Kim, B., Kim, H., Choi, J.W. & Lee, C.K. 1996. The effects of *Meliae toosendan fructus* on liver function. Iii. Effects of melianone and 28 – deacetyl sendanin on drug metabolism and bile juice secretion. Korean J. Pharmacogn. 27: 47

26. Pettit, G.R., Numata, A., Iwamoto, C., Morito, H., Yamada, T., Goswami, A., Clewlow, P.J., Cragg, G.M., & Schmidt, J.M. 2002. Antineoplastic agents 489. isolation and structures of meliastatins 1-5 and related euphane triterpenes from the tree Melia dubia. J. Nat. Prod. 65, 1886-1891.

27. Koul, O., Seema, W., 2004. Neem: Today and in the New Millennium. Kluwer Academic Publishers, New York.

28. Carpinella, M.C., Defag, M.T., Valladares, G. & Palacios, S.M. 2006. Role of *Melia azedarach* L. (Meliaceae) for the control of insects and acari: present status and future prospects. In Advances in Phytomedicine Series: Naturally Occurring Bioactive Compounds. Volume 3. Edited by Rai M, Carpinella MC. Elsevier 81, Amsterdam.

29. Carpinella, M.C., Giorda, L.M., Ferrayoli, C.G. & Palacios, S.M. 2003. Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. J. Agric. Food Chem., 51, 2506-2511.

30. Scudiero, D.A., Shoemaker, R.H., Paull, K.D., Monks, A., Tierney, S., Nofziger T.H., Currens, M.J., Seniff, D. & Boyd, M.R. 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. 48:48274-33

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International Journal of Research in
Engineering and Bioscience
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Table 1: Dose Response of O.S(N), O.S(P), Az.I(N), Az.I(P), O.S(N)+Az.I(N) and O.S(P)+Az.I(P) on Hep-G2 Cell line

Blank = 0.040

Control = 0.527

Conc(µg/ml)	OD of STD at 490 nm	% CS	% CI	OD of O.S(N) at 490nm	% CS	% CI	OD of O.S(P) at 490nm	% CS	% CI	OD of AZ.I(N) at 490nm	% CS	% CI
6.25	0.442	91.2	8.8	0.522	99	1	0.497	93.8	6.2	0.508	96.1	3.9
12.5	0.372	68	32	0.513	97.1	2.9	0.464	87.1	12.9	0.478	90	10
25	0.259	45	55	0.487	91.8	8.2	0.348	63.2	36.8	0.364	66.5	33.5
50	0.190	30.8	69.2	0.424	78.9	21.1	0.302	53.8	46.2	0.288	50.9	49.1
100	0.101	13.8	86.2	0.387	71.3	28.7	0.246	42.3	57.7	0.212	35.3	64.7
200	0.056	3.3	96.7	0.274	48	52	0.178	28.3	71.7	0.188	30.4	69.6

Conc (µg/ml)	OD of Az.I(P) STD at 490 nm	% CS	% CI	OD of O.S(N)+Az.I(N at 490nm	% CS	% CI	OD of O.S(P)+Az.I(P) at 490nm	% CS	% CI
6.25	0.522	99	1	0.485	91.4	8.6	0.475	89.3	10.6
12.5	0.514	97.3	2.7	0.476	89.5	10.5	0.422	78.4	21.6
25	0.478	90	10	0.324	58.3	41.7	0.312	55.9	44.1
50	0.426	79.3	20.7	0.262	45.6	54.4	0.246	42.3	57.7
100	0.359	65.5	34.5	0.212	35.3	64.7	0.210	34.9	65.1
200	0.238	50	50	0.124	17.2	82.8	0.156	23.8	76.2

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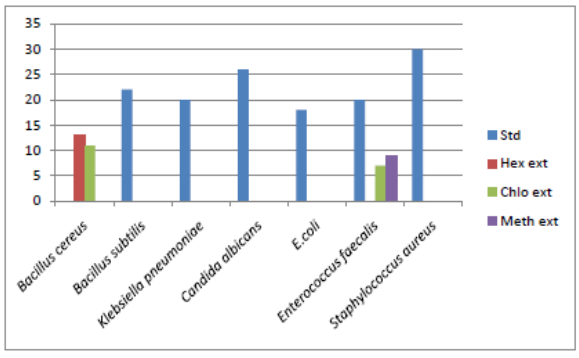
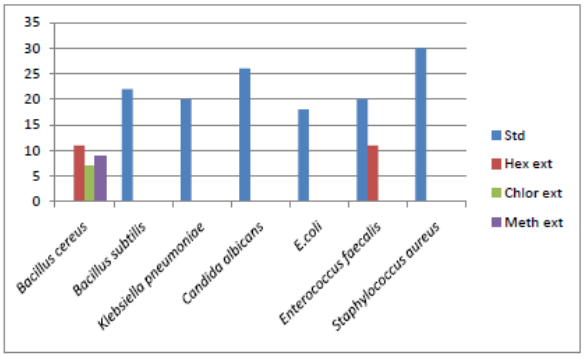
Table 2: IC 50 values of plant extracts used against Hep- G2 cell lines

IC50 of Standard : 13.08µg/mL					
O.S (N)	O.S (P)	Az.I (N)	Az.I (P)	O.S (N)+ Az.I (N)	O.S (P)+ Az.I (P)
IC50 - 185.71µg/mL	103.21µg/mL	102.11µg/mL	184.59µg/mL	83.168 µg/mL	79.75 µg/mL
Slope	-0.25828571	0.30377257	0.31606539	0.25371429	0.35435394	0.2913774
Correlation coefficient	-0.98556761	0.88582356	0.84991127	0.97238896	0.88331379	0.84522807
Intercept	-2.03333333	18.6482587	17.7248756	3.16666667	20.5288557	26.7616915

Graph 1. Zones of inhibition obtained for selected organisms treated with various of extracts of *Abutilon indicum*



Unpolluted environment



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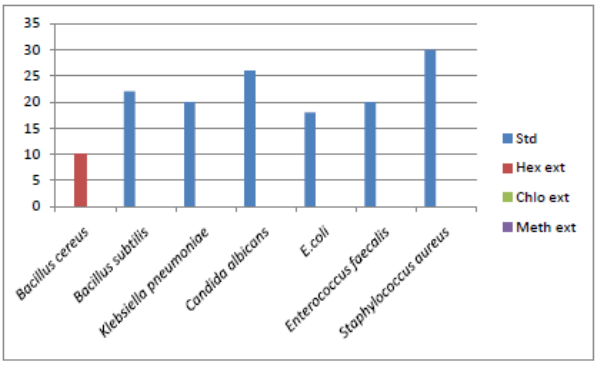
Graph 2. Zones of inhibition obtained for selected organisms treated with various of extracts of *Achyranthes aspera*



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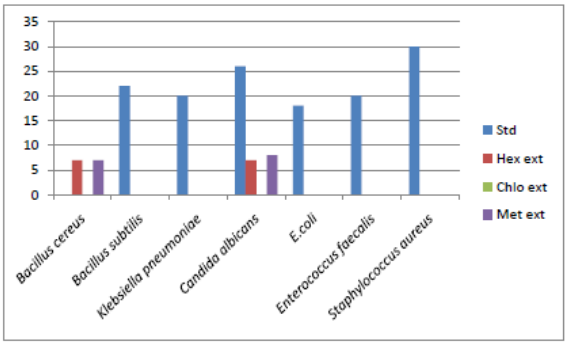

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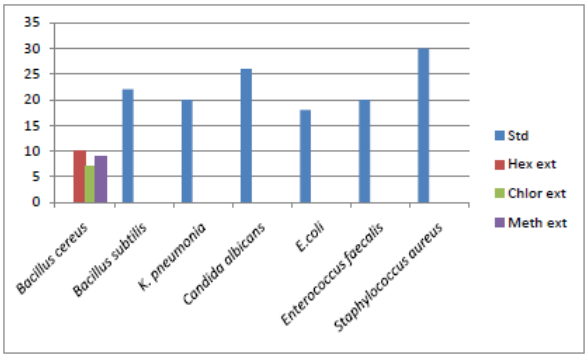
Graph 3. Zones of inhibition obtained for selected organisms treated with various of extracts of *Gomphrena celesoides*



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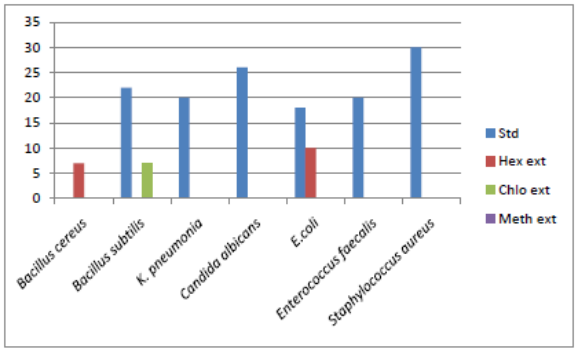

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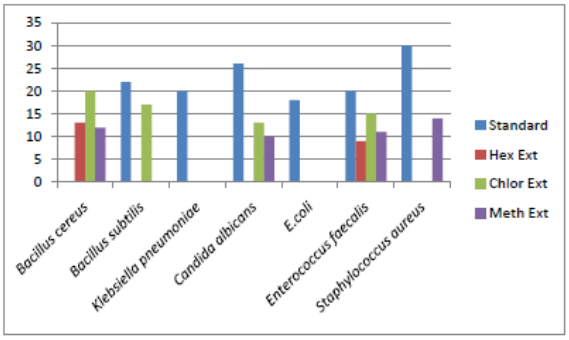
Graph 4. Zones of inhibition obtained for selected organisms treated with various of extracts of *Azadirachta indica*



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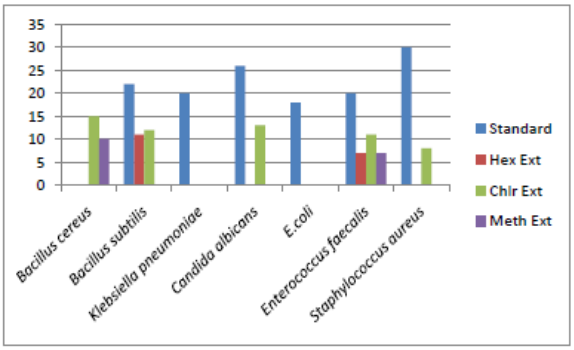

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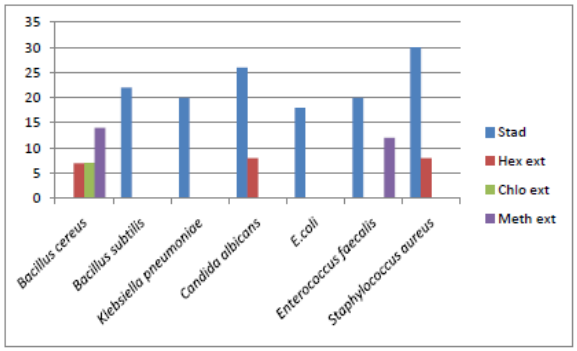
Graph 5. Zones of inhibition obtained for selected organisms treated with various extracts of *Ocimum sanctum*



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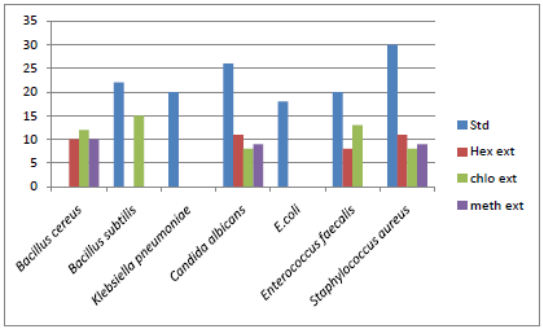

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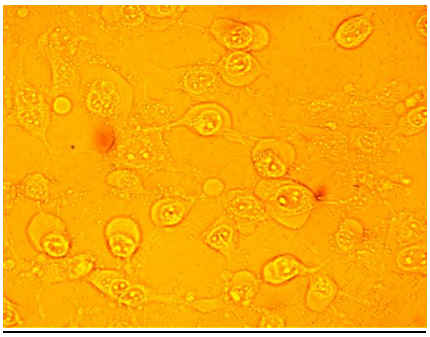

Fig 1 : HepG-2 untreated

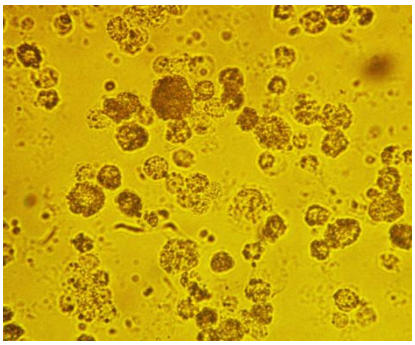

Fig 2 : HepG-2 (STD) treated

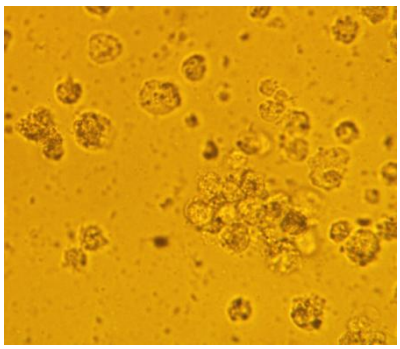

Fig 3: OS.(N) + Az.I (N) on HepG-2 (IC 50 concentration)

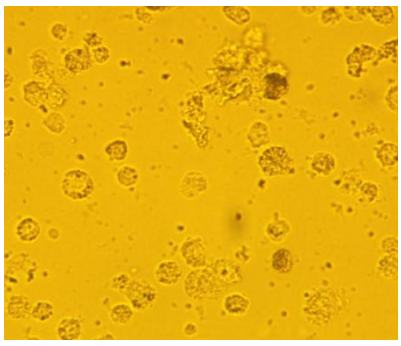

Fig 4: O.S (P) + Az.I (P) on HepG-2 (IC-50 concentration)