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Study of Antioxidant and Antimicrobial Activity of Medicinal Plants Utilized in Cancer Treatment

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Abstract

In our modern society word Cancer is becoming very common as various types of Cancer are effecting large population worldwide. Need of hour is to find the effective treatment of these cancers through research on different plants which can be a good source of components effective in its treatment. The state of uncontrolled growth of cell is Cancer. Various species of plants have been used in the preparation of drugs utilized for the treatment of malignant diseases. Phytochemicals are the chemical substances producing definite physiological action on human body. They found to have antioxidants which are also called free radical scavangers protecting the cells from different types of Cancer. In our studies we have selected six medicinal plants as Aegle marmelos (Leaves), Vernonia anthelmintica (Seeds), Zingiber officinalis (Rhizome), Tinospora cordifolia (Stem) and Phyllanthus acidus (Leaves) of Gujarat region which are reported to have role in cancer therapy due to the presence of different phytochemicals like phenolics, tannins, flavonoids etc. Aqueous and Organic extracts of different parts of these plants were prepared. Antioxidant activity was measured through DPPH and ABTS radical scavenging assay and antimicrobial activity through Agar Well Diffusion method. Antimicrobial activity was tested on some disease causing microorganisms like Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Salmonella typhii, Aspergillus niger and Penicillium notatum. DPPH assay had revealed that organic extract of Vernonia anthelmintica and aqueous extract of Zingiber officinalis was found to have IC_{50} values 124 and 132 µg/ml respectively. Antimicrobial activity of different extracts had shown that maximum diameter of Zone of Inhibition (24mm at 1g/ml) was obtained with organic extract of Tinospora cordifolia. No antifungal activity was observed with any of the plant species extracts. It can be concluded from the studies that the extracts of all test six plants possessed significant antioxidant and antibacterial activities which conforms their role in cancer studies.

Keywords: Cancer, medicinal plants, antioxidant activity, DPPH, ABTS, antimicrobial activity.

Introduction

Cancer is often dreadful and has effect on large population of the world. It is caused by viruses, environmental factors like exposure to UV, ionizing and non-ionizing radiations causing mutations, modern sedentary life style, food habits, cancer causing genes, defective suppressor genes etc. Various drugs derived from different medicinal plants are used in the treatment of cancer. Whatever the treatments are available for Cancer these days they are associated with a number of undesired side effects. Chemical compounds utilized in the treatment are leading to other kinds of the problems in the patients suffering from Cancer like killing normal cells alongwith the cancerous cells. In our Indian system of treatment various herbal medicines derived from plants are used as folk medicines[1-2]. India is a land of great diversity and treasure of traditional knowledge. Scientific research on those folk medicines can be promoted to determine their potential to be used in the treatment of cancer. Antioxidants are the class of compounds having their role in free radical scavenging. These free radicals are formed as a result of various catabolic and anabolic reactions occurring in our body. They are very reactive species and have deleterious impact on the normal human cells. They cause damage to normal cells leading to abnormal functioning of the cells. Different types of extracts are prepared from different parts of the medicinal plants which are the source of these antioxidants. The antioxidants are generally dissolved in organic or aqueous compounds used while making extracts from different parts of different medicinal plants thus becoming the good source of antioxidants which can be tested on different cancerous cell lines and later identified as proper chemical compounds. Medicinal plants therapeutic value can be evaluated on the basis of presence of chemical substances producing definite physiological action on human body involved in the defense mechanism of plants called phytochemicals. These photochemical are of different categories like Tannins, Cardiac glycosides, Phenolics, Flavanoids, Alkaloids, Anthocyanins, Essential oils, Terpenoids etc which got extracted to organic and aqueous phases according to their polarity while making extracts of different medicinal plants. These aqueous and alcoholic extracts are the source of antioxidant molecule that inhibits the oxidation of other molecules. The chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent is called oxidation and it is this oxidation which leads to formation of free radicals in the body. Antioxidants present in the extract as phytochemicals act on these free radicals and neutralize them protecting the cells against damaging effects of reactive oxygen

species. These antioxidants are classified as natural and synthetic which are further divided in primary and secondary antioxidants. Plants are the source of natural antioxidants like ascorbic acid, carotenoid, tocopherol and synthetic like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroquinone and Gallic acid esters. Reports have shown that synthethic antioxidants have negative health effects so use of them is discouraged and substituted with naturally occurring antioxidants.

In our present study, we have selected 6 commonly occurring medicinal plants extracts like Aegle marmelos (Bael), Vernonia anthelmintica (Kalijiri), Zingiber.officinales (Ginger), Tinospora cordifolia, Phyllanthus acidus (Amla) reported to have potential anticancer properties due to the presence of antioxidants like phenolic compounds and antimutagens for example in Ginger presence of phenolic substances such as 6-gingerol and 6-paradol and other constituents shogaols and zingerone has attributed to anticancer properties[3]. It reduces viability of gastric cancer cells and limits the spread of cancer. Geographical location of the plant has effect on the presence or absence of these phytochemicals. Literature studies have revealed that *Tinospora cordifolia* possess chemopreventive ability and inhibits lipid peroxidation phenomenon in the cells[4]. *Phyllanthus acidus (Amla)* also antitumor effects as revealed by studies done on different cell line as well as in animal models[5]. Aqueous and Organic extracts were prepared from different parts of these plants which were used for determining the antioxidant and antimicrobial activities. Antioxidant potential was detected by two protocols DPPH[6] and ABTS[7] free radical scavenging assays with slight modifications and antimicrobial activity was tested on commonly disease causing bacterial species like *Staphylococcus aureus, Pseudomonas aeruginoa, Bacillus subtilis*, *Micrococcus luteus*, *Salmonella typhii* and fungi *Aspergillus niger* and *Penicillium notatum* by Agar Well Diffusion method.

Material and methods

Collection and identification of plant materials: Fresh leaves of *Aegle marmelos* and *Phyllanthus acidus* collected from Chikhli farm and stem of *Tinospora* were collected from Shri Rasiklal Manikchand Dhariwal Cancer Hospital, Waghaldhara, Gujarat; seeds of *Vernonia anthelmintica* and rhizome of *Zingiber.officinales* were collected from local market. The leaves, rhizome and stems were washed, cleaned and chopped into pieces and dried in sunlight until they attained a constant weight. The samples were then crushed into powder using pastle mortar so as to enhance effective contact of solvent with sites on the plant materials. The dried powdered samples were stored in sterile plastic bags for further use.

Preparation of Plant Extracts: Preparation of aqueous extract: 10 gm dried powder of different parts of plant was macerated in 100ml of distilled water taken in 250ml conical flask for 72hr at room temperature. It is then filtered through Whatmann filter paper no.1. The filtrates used as extract were stored in refrigerator at 4°C until they were required for use.

Preparation of Organic extract: Crude plant extract prepared by Soxhlet extraction method.10gm of powdered plant material was uniformly packed into a thimble and extracted with 150ml of methanol. The process of extraction continues till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 50°C till all the solvent got evaporated. High temperature treatment was avoided to minimize the component degradation. Extract was kept in refrigerator at 4°C for their future use.

***In vitro* Antioxidant activity: DPPH radical scavenging activity:** The DPPH radical scavenging method was used to evaluate antioxidant property. The antioxidant activity of extracts was compared with natural antioxidant, Ascorbic acid. The concentration of the plant extracts required to scavenge DPPH showed a dose dependent response. Antioxidant activity was evaluated with IC_{50} values. IC_{50} indicates the concentration of extract at which radical scavenging activity is 50% in DPPH assay. The effect of an antioxidant on DPPH radical scavenging is due to their hydrogen donating ability. DPPH has the advantage of being unaffected by certain side reaction, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibit a deep purple color with an absorption maximum at 517nm.The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

$$(DPPH) + (H\text{-}A) \rightarrow DPPH\text{-}H + (A)$$
Purple Yellow

Antioxidants react with DPPH and reduce it to DPPHH and as consequence the absorbance is decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of antioxidant compounds or extracts of hydrogen donating ability.

DPPH Radical scavenging Assay: The solution of DPPH was prepared by dissolving 9mg of DPPH in 6.90ml methanol. It was protected from light by covering the test tube with aluminum foil.150µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm and it is considered as control .50µl of various concentration such as 40, 80,120,160,200,240,280,320 µg/ml of the plant sample extracts as well as standard compound were taken and volume was made uniformly to 150µl using methanol. Each of samples was then further diluted with methanol up to 3ml and to each tube, 150µl DPPH was added. Absorbance was taken after 20 min incubation in dark at 517nm using methanol as blank on UV-1800 Visible spectrophotometer (Shimadzu). The DPPH free radical scavenging activity was calculated using the following formula:

$$\text{Inhibition } (\%) = [(A_{control} - A_{test})/A_{control}] \times 100$$

Where Acontrol is the absorbance of control (solution without extract) and Atest is the absorbance of samples (extract and ascorbic acid).The antioxidant activity of each sample was

expressed in terms of IC_{50}. The IC_{50} values for each drug compound as well as standard calculated from the graph after plotting inhibition percentage against extract concentration.

ABTS Radical scavenging Assay: Another method used for determining antioxidant activity was ABTS radical cation decolorization assay. The ABTS radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) in the dark for 16 hours at room temperature. During this period of incubation ABTS and potassium persulfate react stoichiometrically at a ratio of 1:1. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 hrs had elapsed. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of samples, the ABTS+ solution was diluted with distilled water to an absorbance of 0.70(±0.02) at 734 nm and equilibrated at 30°C. Reagent blank reading was taken (A_0). After addition of 2.0 ml of diluted ABTS+ solution to 2.0ml of varying concentrations (40, 80, 120, 160, 200, 240 and 280 µg/ml) of the extracts and the absorbance readings were recorded at 734 nm. The ABTS+ scavenging capacity of the extract was compared with that of BHT and the percentage inhibition calculated as:

ABTS radical scavenging activity (%) = [(Acontrol – Asample)/Acontrol] ×100

Where Acontrol is the absorbance of ABTS radical + methanol and Asample is the absorbance of ABTS radical + sample extract/standard. The extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting inhibition percentage versus extract concentration.

Antimicrobial activity: **Growth and Maintenance of Test Microorganism:** Bacterial cultures of *Bacillus subtilis*, *Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus luteus, Salmonella typhy A* and fungal cultures of *Aspergillus niger* and *Penicillium notatum* obtained from our department, were used for evaluating antimicrobial activity. The bacteria were maintained on nutrient broth (NB) at 37°C and fungus was maintained on Potato dextrose agar at 28°C.

Preparation of Inoculums: The gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa, Micrococcus luteus, Salmonella typhy A)* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C. The fungal inoculums were prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula.

Media Preparation and Sterilization: For bacterial assay nutrient agar (NA) and for fungus PDA was used for developing surface colony growth. All the media prepared was then sterilized by autoclaving the media at (121°C) for 20 min.

Agar Well Diffusion method: Agar Well Diffusion technique[8] used to determine the antimicrobial activity of the extract. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were poured with 8 hrs old - broth culture of respective bacteria and fungi. Plates incubated at 37°C for 24hr. Wells were made in each of these plates using sterile cork borer. About 100µl of plant aqueous and organic solvent extracts were added into the wells by pipette and allowed to diffuse at room temperature for 24-48 hrs. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

Results and Discussion

Preparation of aqueous and organic extracts: Aqueous and organic extracts of the plants were prepared as the compounds responsible for their antioxidant and antimicrobial activities would get dissolved in these solvents according to their polarity which will be further used as extracts. For each assay the initial concentration of the extract was adjusted to 1 gm/ ml and different concentrations of the extract was taken in assays to study their respective activities.

Determination of *In vitro* antioxidant activity: DPPH radical scavenging activity: DPPH radical scavenging assay is the most common method used in the study of antioxidant activity of plant extracts. It results in the formation of stable free radical which can be detected by common spectrophotometric technique[9]. Decrease in absorbance shows the more efficient antioxidant activity of the extract in terms of hydrogen atom donating capacity. This assay is more indirect type as it measures the inhibition of reactive species (free radicals) generated in the reaction mixture and its results depend on the type of reactive species used[10]. Studies conducted on the free radical scavenging activity of medicinally important plants have shown that the efficiency of each plant species differ depending on the particular assay methodology, reflecting the complexity of the mechanisms involved in total antioxidant capacity. The observed antioxidant of the extracts may be due to the neutralization of free radicals (DPPH), either by transfer of hydrogen atom or by transfer of an electron[11]. Figures 1A and 1B shows the dose response curve of DPPH radical scavenging activity of both aqueous and organic crude extracts of plants respectively and compared with standard Ascorbic acid. Figures 2A and 2B showed the percentage inhibition and comparison of IC_{50} values of different plant extracts obtained from dose dependent curve respectively. IC_{50} value is inversely proportional to antioxidant activity of plant extracts. Among the entire sample analyzed for DPPH radical scavenging assay, organic extract and aqueous extracts of *A. marmelos* with IC_{50} value of 280µg/ml and 236µg/ml respectively had resulted in lowest antioxidant activity and *V. anthelmintica* with IC_{50} value of 124µg/ml, indicate highest antioxidant activity whereas its aqueous extract had not resulted in any activity. Aqueous extract of *Z. officinales* had shown the value comparable to organic extract of *V. anthelmintica* i.e. 132µg/ml. Both aqueous and organic extracts of *P. acidus* had not shown any activity. DPPH assay studies can be concluded both organic and aqueous extracts possessed the

antioxidant activity but at very high concentrations and among all plant extracts organic extracts of *V. anthelmintica* works much better. DPPH antioxidant activity of Organic extract of *emblica officinalis* leaves was performed and obtained IC_{50} value 45.38µg/ml[12]. Free radical scavenging activity of different parts of *Aegle marmelos* was evaluated by using DPPH method. The highest free radical scavenging effect was observed in leaves with IC_{50} is 2.096µg/ml[13]. These findings contradict our findings and this can be explained that in different geographical conditions different plants synthesize different concentrations of antioxidants. High IC_{50} values depicts that the concentration of antioxidants in the extracts is low.

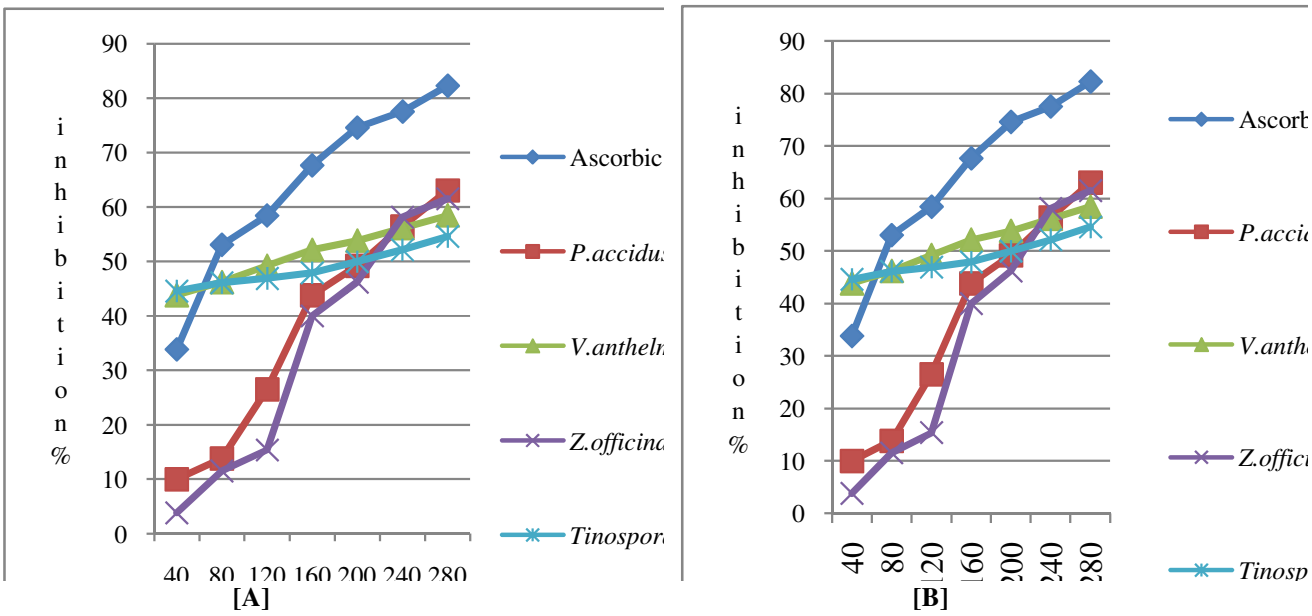
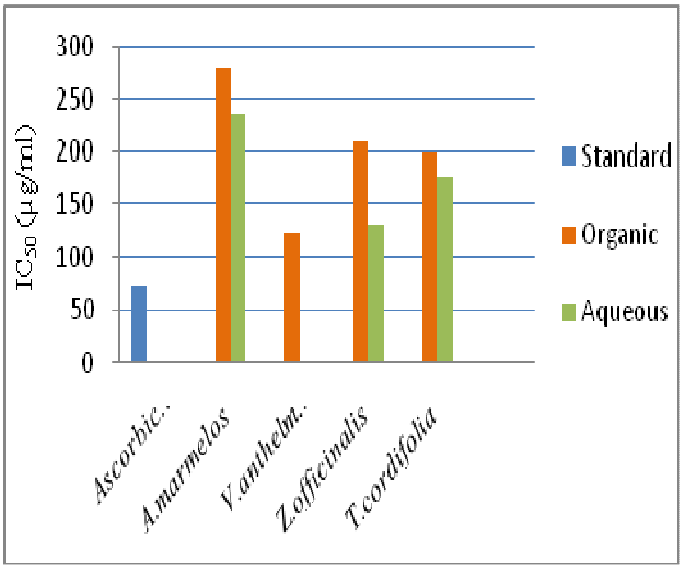


Figure-1
Graphs showing percentage inhibition comparison of Aqueous [A] and Organic [B] extracts of plants with standard Ascorbic acid

IC_{50} (µg/ml)			
Plant Extracts	Standard	Organic	Aqueous
Ascorbic acid	72	-	-
A.marmelos	-	280	236
V.anthelmintica	-	124	-
Z.officinalis	-	210	132
T.cordifolia	-	200	176
P.acidus	-	-	-

[A]



[B]

Figure-2
IC_{50} values (calculated from % inhibition graphs) of Ascorbic acid and different plant extracts [A], Comparison of IC_{50} values of different plant extracts by DPPH Radical scavenging assay [B]

ABTS Radical scavenging assay: Other antioxidant assay which we had performed on different plant extracts was ABTS radical scavenging assay. This assay is based on the principle of decolorization as antioxidants present in plant extract scavenge free radical. The observed antioxidant of extracts may be due to the neutralization of radical (ABTS), either by transfer of hydrogen atom or transfer of an electron. The scavenging effect of all extracts increased with increasing concentration. Aqueous plant extracts had not shown any antioxidant activity with this assay and organic extracts had shown very interesting results. The organic extract of leaves of *A. marmelos* has shown the IC_{50} value appreciably low (57.5µg/ml) in comparison with the standard BHT. It means that this plant extract would be effective in even less concentration as antioxidant in comparison to standard pure BHT. Other two plants extracts *Z. officinales* and *T. cordifolia* had also shown values of IC_{50} comparable with standard presenting that the crude extracts of these plants were as effective as pure standard BHT. *V. anthelmintica* and *P. acidus* plants extracts were found to be effective in little more concentrations. So in all this antioxidant assay was found to very suitable for the plants extracts selected by us in our studies. The percentage inhibition and comparison of IC_{50} values of different plant extracts were shown in Figure 3A and 3B respectively.

Antimicrobial activity: In other part of our study we had performed antimicrobial activity of these plant extracts on commonly disease causing bacterial and fungal species by Agar Well Diffusion method. The results obtained for the antimicrobial test performed on different extracts of medicinal plant at the concentration of 1gm/ml were presented in Table 1 and figure-4. Activity was observed as Zone of inhibitions obtained in the presence of different plant extracts. Results obtained in the present study revealed that the tested five medicinal plants extracts posses potential antibacterial activity against *P. aeruginosa, S. aureus* , *B.subtilis*, *S.typhi and M.luteus* and but no antifungal activity. Aqueous extracts had not shown any antimicrobial activity but the organic leaf extract of *A. marmelos* exhibited highest activity against *M.luteus* (16mm), lowest in *S.typhi* (12mm).Seed extract of *V. anthelmintica* showed highest antibacterial activity against *S.typhi* (15mm) and lowest activity against *P.aeruginosa* (14mm), *M.luteus* (14mm). Rhizome of Ginger showed highest activity against *M.luteus* (20mm) and lowest activity against *P.aeruginosa* (14mm). Stem of *Tinospora* showed highest activity against *S.typhi* (24mm) and lowest activity against *P.aeruginosa*, *M.luteus* (20mm). Leaves of *A. marmelos* showed activity against only *M.luteus* (15mm). Antibacterial activity was not observed against *Bacillis subtilis* with any of the plant extracts used in studies. Antimicrobial activity of fresh and dry Ginger oil was done and obtained 9.06 mm diameter of zone for *Pseudomonas aeruginosa*[14]. Antibacterial activity of Organic extract of *Tinospora cordifolia* was studied and at 50 mg/ml, 2.2 cm diameter of zone for *S.typhi* and 2.5cm diameter of zone for *P.aeruginosa* was obtained.

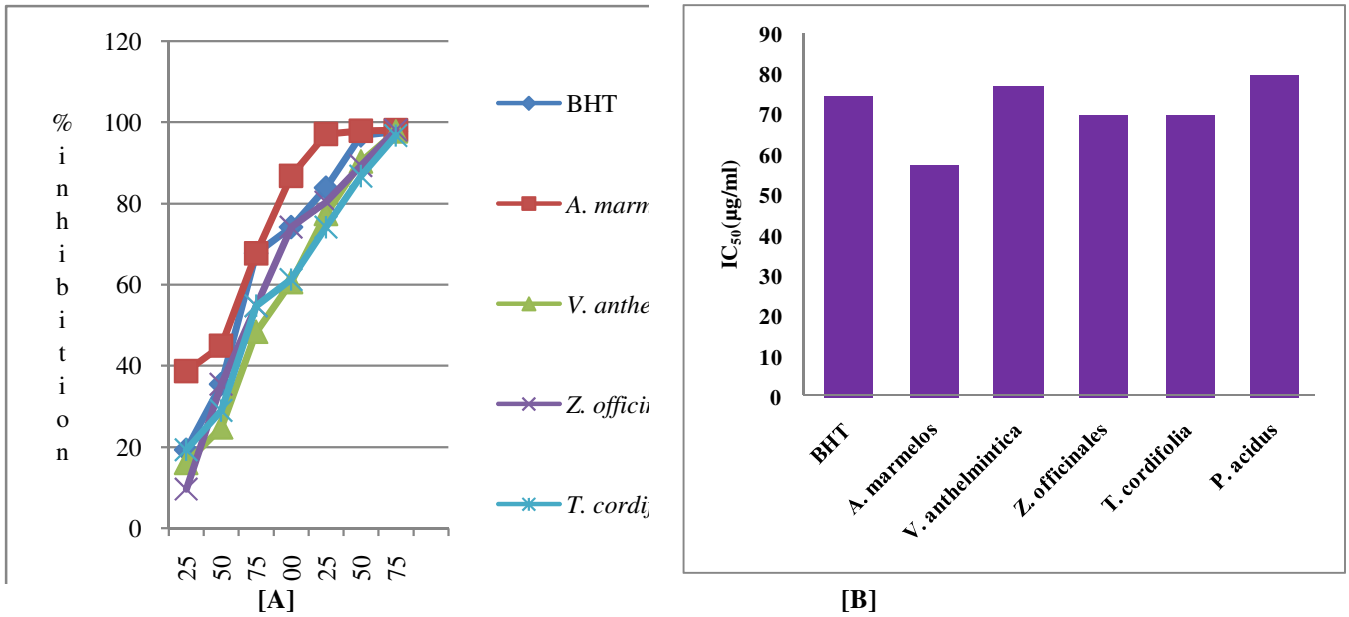
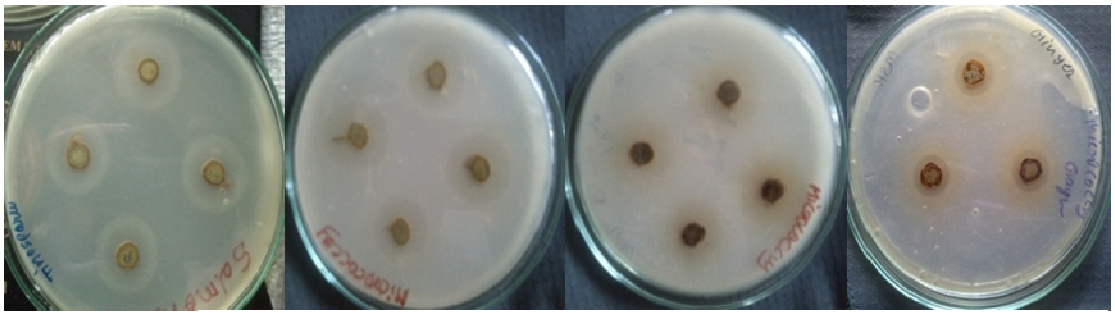


Figure-3
Percentage inhibition by different plant extracts [A], IC_{50} values comparison with standard BHT of different plant extracts [B]



[A] [B] [C] [D]

Figure-4

The following figure shows the pictures of zone of ihibition of of microorganisms by plants extracts used in studies: [A] Effect of *Tinospora* on *Salmonella typhi* [B] Effect of *Tinospora* on *Micrococcus luteus*[C] Effect of Kalijiri on *Micrococcus luteus* [D] Effect of Ginger on *Micrococcus luteus*

Table-1

Antimicrobial activity of different plant extracts in the form of Zone of Inhibition against common bacterial species of different plant extracts

Bacterial	**Diameter of Zone of inhibition(mm)**				
Species	A. marmelos	V. anthelmintica	Z. officinales	T. cordifolia	P. acidus
P.aeruginosa	-	14	14	20	-
S.typhi	12	15	18	24	-
S.aureus	-	-	-	-	-
M.luteus	16	14	20	20	15
B.subtilis	-	-	-	-	-

Conclusion

The DPPH and ABTS antioxidant assays of different plant extracts had revealed that organic extract of *A.marmelos*, *Vernonia anthelmintica* and aqueous extract of *Zingiber.officinales* had exhibited highest antioxidant activity therefore would be a good source of natural antioxidants. Studies may be further conducted on the identification of specific phytochemical contributing to antioxidant ability of the different plant extracts and purified for effective treatment. Owing to the increase in resistance of pathogens against different antibiotics, research for new chemicals which can inhibit the growth of the pathogenic strains is very much needed. In our case of antimicrobial studies *Zingiber.officinales* and *Tinospora cordifolia* organic extracts had exhibited highest antimicrobial activity against various pathogens suggesting to the presence of important compound which can restrict the growth of these microbes and be the source of some important drug.

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References

1. Ali N., Juelich W., Kusnick C. and Lindequist U., Screening of Yemni medicinal plants for antibacterial and cytotoxic activities, *J Ethnopharmacol*., **74,** 173-179 **(2001)**

2. Nair R., Kalariya T. and Chanda S., Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology*; **29**, 41-47 **(2005)**

3. Nordqvist Christian, Ginger Kills Ovarian Cancer Cells, Medical News Today. Medi Lexicon International limited **(2006)**

4. Dhanasekaran M., Baskar A.A., Ignacimuthu S., Agastian P. and Duraipandiyan V., Chemopreventive potential of Epoxy clerodane diterpene from *Tinospora cordifolia* against diethylnitrosamine induced hepatocellular carcinoma., *Invest new drugs*., **27(4),** 347-55 **(2009)**

5. Lee C., Peng Y., Cheng W.H, ChengH., Lai FNMT. and Chiu T.H., Hepatoprotective Effect of *Phyllanthus* in Taiwan on Acute Liver Damage Induced by Carbon Tetrachloride, *American Journal of Chinese Medicine*; **30(3),** 471- 482 **(2006)**

6. Brand-Williams W., Cuvelier M.E. and Berset C., Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaftund Technologie,* **28,** 25–30 **(1995)**

7. Arnao M.B., Cano A. and Acosta M., The hydrophilic and lipophilic contribution to total antioxidant activity, *Food Chemistry,* **73,** 239–244 **(2011)**

8. Cheesbrough, M., District Laboratory practical in tropical countries, Part 2., *Cambridge university Press, Cambridge, UK*., 137- 150 **(2006)**

9. Blois M., Antioxidant determination by the use of stable free radicals. *Nature*., **26**, 1199-1200**(1958)**

10. Cao G., Sofic E. and Prior R., Antioxidant capacity of tea and common vegetables. *J Agricult Food Chem*., **4**, 3426-3431**(1996)**

11. Matkowski A., Tasarz P. and Szypuła E., Antioxidant activity of herb extracts from five medicinal plants from Lamiaceae, subfamily Lamioideae, *J. Med. Plants Res,*. **2,** 321-330 **(2008)**

12. Naik G., Priyadarsini K., Satav J., Banavalikar M., Sohoni P. and Biyani M., Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry,* **63,** 97-104 **(2003)**

13. Siddique N.A., Mujeeb M., Ansari S.H., Ahmad S., Ali B. and Junaid A., Development of quality standards of *Aegle marmelos* L. leaves. *Journal of Phytology,* **2(2),** 36-43 **(2010)**

14. Sasidharan I. and Nirmala M.A., Comparative chemical composition and antimicrobial activity of Fresh and dry Ginger oils (*Zingiber officinales* Roscoe) *Int. J. Curr. Pharm. Res.,* **2(4)**, 40-43 **(2010)**