Chapter 7

Phytopharmaceutical Studies of Selected Medicinal Plants Subjected to Abiotic Elicitation (Stress) in Industrial Area

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Additional information is available at the end of the chapter

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Abstract

Plants are a source of large amount of drugs comprising antispasmodics, emetic, Anticancer, anti microbial and anticancer activities etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and today they are extensively used by the people and the metal Components in the plants grown in polluted area seemingly increase the concentration of phytochemicals. Recent times the flora and fauna of any region is directly or indirectly exposed to the all types pollutants which may result into adverse effects rarely the metal pollutants may trigger the production of phytochemicals. The present study deals with Industrial pollution of the area selected for study, metal up take, and their effect on phytochemical, antimicrobial and anticancer activities that explore the research on five medicinal plants namely *Adhatoda vasica*, *Eucalyptus globulus*, *Hyptis suaveolens*, *Ricinus communis* and *Tinospora cordifolia* that thrive well and grow luxuriantly in industrial polluted area and the same five plants from natural area of Visakhapatnam District. The aim of this study is to analyze the effect of Metal elements on phytochemical productivity and antimicrobial and anticancer activity of these medicinal plants. Metal analysis is done ICP-MS (PerkinElmer Sciex Instrument, model ELAN DRC II, USA). Alkaloids, flavanoids, terpenoids and phenols screening is done in solvents Hexane, Chloroform and methanol and checked for antimicrobial activity and anti-cancer activity of *Eucalyptus globulus* and *Tinospora cordifolia* were determined by XTT assay on MCF-7 cell lines. The results are discussed in comparison of Natural with pollutant grown plants. The plants that showed better production of phytochemicals due to the presence of metal elements could be recommended to phytopharmaceutical industries as they comparatively showed better production of phytochemicals further proposing a definite way to eliminate toxic metals from them.

Keywords: Abiotic Elicitation, Industrial Area, Medicinal Plants

1. Introduction

The stupendous glory of India is unparalleled and unmatched. No other country, worldwide, can compare itself to this glory of India. Whether it is the magnificent waterfalls and sacred rivers, lush canopies and verdant valleys, or the varied and rich flora and fauna, India has it all including ecological islands, such as Visakhapatnam in Andhra Pradesh, which harbours endemic plants [1]. There are more than two thousand plant species acknowledged to have medicinal value in the traditional Asian system of medicine. This health-giving store, India is blessed to have experienced every creation of "Mother Nature."

The Eastern Ghats properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [2]. They are grouped as alkaloids, glycosides, corticosteroids, coumarin, flavonoids, and essential oils. Flavonoids are chemical compounds that are active against microorganisms. They have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms [3].

Phytochemicals are naturally occurring, biologically active chemical compounds that protect plants against bacteria, fungi, and viruses and have potential benefits for health. The presence of certain types of phytochemicals in some plants can act as a natural defense system providing protection against such things as attacks from insects and grazing animals. In contrast, other plants produce phytochemicals that provide colour, aroma, and flavor and improve immune function. They stop toxic substances from becoming carcinogens in your body, protect and repair your DNA, reduce inflammation, normalize hormone levels, and slow the growth of cancer cells. Phytochemicals have recently become of great interest owing to their versatile applications.

Medicinal plants are the richest bioresource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [4]. Presently, herbal remedies are preferred due to less or no side effects for many diseases, and due to financial constraints [5]. Despite enormous developments in synthetic medicines, several other diseases have grown due to their harmful side effects after prolonged use [6, 7]. Since the dawn of history, plants have played an important role in the treatment of human ailments. Over 50% of all modern clinical drugs are of natural origin and natural products play an important role in drug development programs in the pharmaceutical industry. Herbal drugs have gained importance in recent years because of their efficacy and cost-effectiveness [8]. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs [9], antimicrobial drugs [10], and antihepatotoxic compounds [11, 12]. According to the World Health Organization (WHO), medicinal plants are the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicines, which have compounds derived from medicinal plants.

However, such plants should be investigated to better understand their properties, safety, and efficiency. Knowledge of the chemical constituents of plants is desirable because such information will be of value for synthesis of complex chemical substances [13, 14, 16].

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These medicinal plants that are rich biosources of several drugs in the treatment and prevention of many diseases are constantly exposed to pollutants directly or indirectly. Especially plants growing in industrial area (surrounded by oil refinery) are subjected to a range of pollutants (external factors) that may adversely affect the growth and development of cultivated plants and crops. These factors may impose an abiotic stress on medicinal plants too, which may alter metabolic profiles and developmental trajectories and thereby induce the production of various secondary metabolites. This in turn assists the plant to adapt varied environmental changes.

In the area selected for my study, the sources of pollutants are mainly industries. Taking into consideration the above mentioned three factors:

1. The promising effects of phytochemicals and antimicrobial activity
2. The negative effect of pollutants on medicinal plants and, in turn, on human health,
3. The interesting and contrasting positive role of metal elements in increasing and eliciting the secondary metabolites and their antimicrobial and therapeutic activity; we have selected two areas of research interest in the Eastern Ghats.

Visakhapatnam in Andhra Pradesh, the area of my study, popularly known as Vizag, is a booming industrial city on the east coast of India. It is located on the eastern shore of India nestled among the Eastern Ghats' Hill Ranges and facing the Bay of Bengal on the East. It is a port city with the biggest ship-building yard in India and is also the headquarters of the Eastern Naval Command. The district presents two distinct geographic divisions. A strip of land along the coast called the plain division, having an altitude not more than 75 m and a hilly area of Eastern Ghats flanking it on the North West called agency division having an altitude of 900 m. Vizag supports a rich diversity of plant wealth due to its annual rainfall.

The first area of my study is a large residential area at the lower altitude of Eastern Ghats Malkapuram in Visakhapatnam, where there are more than fourteen major hazardous industries, among which seven are present in the Malkapuram cluster, namely, HPCL (R), HPCL (T), IOCL (T), LPG bolting plant, CFL, APCL, and BPCL. The soil and medicinal plants that are thriving well and have luxuriant growth in the Malkapuram cluster were chosen as the source in the industrially polluted zone. The area may be polluted with various heavy metals such as cadmium, lead, copper, manganese, cobalt, nickel, aluminum, zinc, iron, chromium, etc. Owing to the low-quality fuel that is processed in refineries, these substances exert abiotic stress on the plant species that inhabits those areas. The plants grown in natural conditions may have less or no abiotic stress [16] (Plate 1).

The second area of my study is the Paderu division of Visakhapatnam district, the higher altitude zone in the hilly tracts of Eastern Ghats of Andhra Pradesh. It has the second highest tribal population in Andhra Pradesh. The tribal people of this division largely depend on herbal medicines, plant products for primary health care, and their daily life [17]. This area is considered to be free from industries and therefore the biosphere in this zone is less polluted.

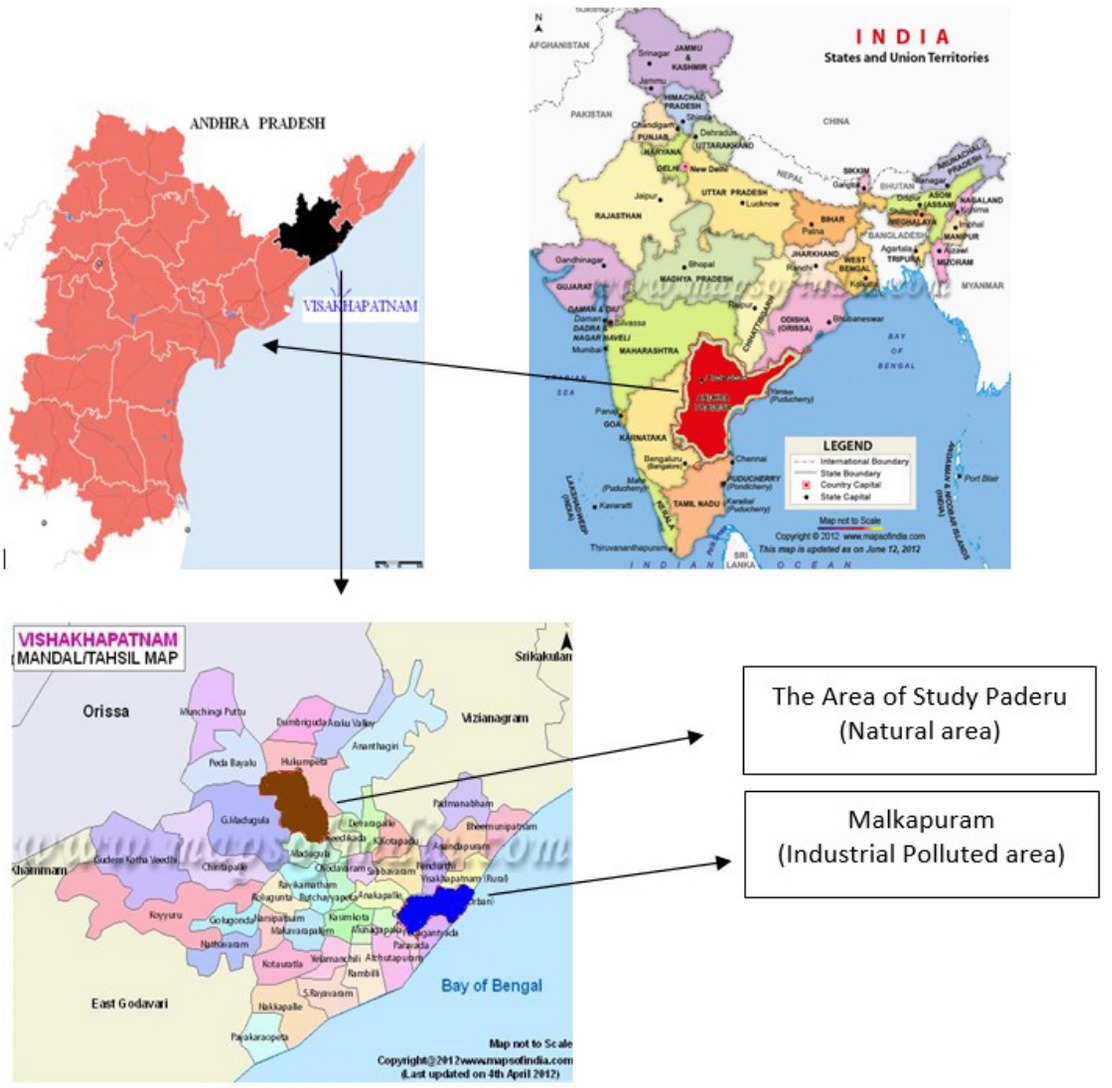

Plate 1. The map of Visakhapatnam in Andhra Pradesh and the areas selected for study.

1.1. Justification

Plants, when exposed to unfavorable environments, such as water deficit, chilling, heat stress, oxygen deficiency, and air pollution, result in some degree of stress and express only a fraction of the plants' genetic potential. Plants adapt to unfavorable conditions through genetically determined stress resistance [18]. Plants grown in a given habitat are exposed to various abiotic stresses that may have significant effects on their growth and productivity. Environmental factors such as light, water, as well as salinity are important variables affecting phytochemical production in plants [19]. Abiotic stresses will affect the pathways involved in the biosynthesis of the 3 principal groups of secondary metabolites: terpenes, phenolics, and nitrogen-containing compounds. These compounds serve as the plants' defenses against herbivores and pathogens, as attractants for pollinators and seed-dispersing animals, and assist with absorb-

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ing harmful ultraviolet radiation or reducing the growth of competing plants among others [20]. Owing to their biological activity, secondary metabolites are used commercially as insecticides, fungicides, pharmaceuticals, fragrances, flavorings, medicinal drugs, and industrial materials.

Oxidative stress induced by heavy metals triggers signaling pathways that affect the production of specific plant metabolites [21]. Similar observations have been noted by several research groups [22]. However, high levels of heavy metal contamination in medicinal or other plants may suppress secondary metabolite production. Alternatively, the presence of heavy metals in medicinal plants may stimulate the production of bioactive compounds in many plant species. In addition, trace element levels have become of prime importance for both the clinical diagnosis and curing of different diseases [23]. Trace elements play a vital role in medicinal plants and are therefore responsible for their medicinal properties [24].

A large number of the plants that reportedly possess antibiotic and antioxidant properties in the traditional system are today extensively used by the people, and the metal components in plants grown in polluted areas seemingly increase the concentration of phytochemicals. In recent times, the flora and fauna of any region have been directly or indirectly exposed to all types of pollutants, which may result in adverse effects where rarely the metal pollutants may trigger the production of phytochemicals. Industrial activities have become a threat to medicinal plants as they release metallic compounds to the atmosphere, leading to heavy metal accumulation in crops and medicinal plants. Large residential areas, especially near the industrial belt, are contaminated by heavy metals that mainly originate from industrial activity in the Malkapuram cluster—the area of our study. However, elevated levels of both essential and nonessential heavy metals pose a serious threat for human health and agriculture.

Contrary to many views, the metal components in the medicinal plants grown in polluted areas seemingly increase the concentration of phytochemicals.

Around the world, it is estimated that 9 million new cases of cancer are diagnosed every year and over 4.5 million people die of disease. In India, 7 lakh cases and over 3.5 lakhs of people die of cancer every year, and 2.3 lakhs of new cases are tobacco related. The leading cancer among males is pharyngeal cancer, which accounts for 14.1% of total cancers, whereas the leading cancer among females is cervical cancer, which accounts for 26.7% of cancers, this is followed by breast cancer, which accounts for 16.6% [25, 26]. As per the statistics news of the WHO, the number of people being diagnosed with cancer in the world has leaped to more than 14 million each year.

India is the first of the emerging economics to join the IARC in 2006, and is an active participating state of the global cancer research agency. In India, around 5, 55, 000 people died of cancer in 2010. According to estimates published in *Lancet*, of which 45% were deaths from breast cancer [27]. Breast cancer in urban Indian women is 25–30 and the age is adjusted to 30–35 and the new cases 100, 000–125, 000 breast cancer cases every year in India [28].

A number of undecided side effects that occur during chemotherapy can be reduced by using plant-derived products in cancer treatments. Various active compounds derived from medicinal plants have been assisting for their efficacy and tolerability and treatment of breast

cancer. The present work is an attempt to find out metal analysis, phytochemical, antimicrobial activity, and cytotoxicity of crude extracts of selected medicinal plants particularly *Eucalyptus globulus* and *Tinospora cordifolia* on MCF-7 breast cancer cell lines.

2. Aims and objectives

The present study was aimed to identify and collect the plants that are thriving well in both industrial and natural areas and their comparative analysis of metal, phytochemical, antimicrobial, and anticancer activities.

This research details the uptake of metals by various medicinal plants grown in polluted and natural sources and their effect on phytochemical, antimicrobial, and anticancer activities. The study includes 5 pairs of plants (for a total of 10 samples). *Adhatoda vasica*, Nees, *Hyptis suaveolens*, (L.)Poit, *E. globulus,* Labill., *Ricinus communis* Linn., and *T. cordifolia*, Meirs. These plants that are under metal stress may trigger secondary metabolites of pharmacological importance, which may be a boon to the pharmacological industries to exploit their phytochemical nature and in turn become beneficial to mankind.

Therefore, the present study was undertaken with the following objectives:

- Identification, selection and collection of some medicinal plants that are grown abundantly and luxuriantly in both natural and industrial areas
- Metal analysis of selected medicinal plants and soils from both areas and their comparative study
- Qualitative phytochemical analysis, and estimation of total phenols and antibacterial activity of the medicinal plants that are grown in natural and industrial areas
- The comparative study on the effect of metals on phytochemicals and their antibacterial activity
- Role of phytochemicals on MCF-7 breast cancer cell lines
- Finally, to recommend those plants which elicit phytochemical, antimicrobial, and anticancer activities to the pharmaceuticals for further detailed study and for the usage conformity by the experts.

3. Collection of plant material

In the present study, an initial survey was done in polluted areas that were previously mentioned and found to be dominated by about 20 plant species. Out of these 20 plant species, 10 were screened and selected based on their luxuriant growth and medicinal properties. Furthermore, we restricted our study to 5 plants (Table 1) to make our analysis more concrete. The same species of plant materials were collected from polluted areas and from natural areas

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of Visakhapatnam district, Andhra Pradesh, India. All plants were screened for their metal elements and, among them, five plants from each area were selected for qualitative and quantitative phytochemical analysis and antimicrobial and anticancer studies. The collected materials were washed thoroughly under running tap water and finally with sterile distilled water, then the materials were air-dried on a sterile blotter under shade to constant weight for a period of 45 days.

S No.	Plant name	Family
1	*A. vasica*, Nees	Acanthaceae
2	*E. globulus*, Labill	Myrtaceae
3	*H. suaveolens*, (L.) Poit.	Labiatae
4	*R. communis*, Linn.	Euphorbiaceae
5	*T. cordifolia*, Miers	Menispermaceae

Table 1. List of medicinal plants selected from industrially polluted and natural areas of Visakhapatnam district

The collected plant specimens were identified with the help of *Flora of the Presidency of Madras* [29] and *Flora of Visakhapatnam* by Subba Rao [30] and *Flora of Srikakulam* by Sheshagiri and Sreeramulu [31], and they were confirmed by the taxonamist Mr. Ramesh of Andhra University, Visakhapatnam. The selected plant materials (Plates 2 and 3) are given with short descriptions.

3.1. *A. vasica*, Nees

A. vasica, Nees belongs to the family called Acanthaceae. All plains Districts, perhaps wild in the *N. Circars*, elsewhere cultivated as a hedge plant and runs wild near villages. A dense shrub with a foetid scent, the flowers are white with the throat barred with red or yellow. The leaves are used in native medicine, and an infusion can be used as an insecticide. Vern. Hind. Arusha; Ur. Basung; Tel. Addasaram; Tam. Adatodai [32] (Plate 2a).

3.2. *E. globulus*, Labill.

E. globulus, Labill. belongs to the family called Myrtaceae, largely grown in forests on the Nilgiris and other hills of the W. Ghats and frequently found self-sown. The Blue Gum is a lofty tree with very grey young leaves and narrow, green, linear-lanceolate, curved, vertically hanging old leaves; white, rather large flowers, and ribose capsule, all parts very aromatic with a valuable essential oil which is largely extracted. The bark is grey, with deciduous the outer layers; wood grey with darker streak, moderately hard, used in building but most especially for fuel [33] (Plate 2b).

3.3. *H. suaveolens*, (L.) Poit.

H. suaveolens Poit. belongs to the family of Labiatae. Most plains Districts, on roadsides and waste ground, introduced from Tropical America and runs wild. A tall, sweet-smelling herb

with tetragonal hispid stems, ovate, cordate, denticulate leaves reaching 4.5 in. long and small blue flowers, the fruiting calyx campanulate and ribbed with 5 arista teeth. Vern. Hind. Wilayati tulasi [32] (Plate 2c).

3.4. *R. communis*, Linn.

R. communis, Linn. belongs to the family called Euphorbiaceae. Cultivated and found running wild in the fields and gardens, by roadsides, and on wastelands. The castor-oil plant is often almost a small tree with a thin greyish-brown bark and soft white wood. It is cultivated for the oil which is extracted from the seeds and used for burning, as a lubricant and in medicine. Vern. Hind, Arend; Tam, Sitta-munuk; Tel. Amadam; Kan. Haralu [33] (Plate 3a).

3.5. *T. cordifolia*, Miers

T. cordifolia, Miers belongs to the family called Menispermaceae. A climbing shrub with succulent stems, the bark is papery at first then corky, leaves glabrous, flowers yellow, on nodes on the old wood; drupes red and sessile; endocarp with a few isolated tubercles or smooth. It is grown in forests and among trees in almost all districts. Vern. Hind. Goluncha; Tel. Tippa tiga; Tam. Chintil [32] (Plate 3b).

(a) *Adhatoda vasica, Nees*

(b) *Eucalyptus globulus, Labill*

(c) *Hyptis sua veolens, Poit*

Plate 2. Medicinal plants selected for the present study.

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(a) *Ricinus communis*, Linn

(b) *Tinospora cordifolia*, Miers

Plate 3. Medicinal Plants Selected for the Present Study

3.6. Experimental: Metal analysis

3.6.1. Instrumentation

A Perkin Elmer SCIEX model Elan® DRC II ICP-MS (Ontario, Canada) at CSIR-National Geophysical Research Institute, Hyderabad, was used throughout for trace elemental analysis of plant and soil samples. The sample introduction system consisted of a standard Meinhard® nebulizer with a cyclonic spray chamber.

3.6.2. Materials

A hot plate with digital temperature controller with a maximum temperature of 250°C was used for digestion. Teflon® beakers were thoroughly cleaned, soaked in 1:1 HNO_3 for 6 h, and thoroughly rinsed with Milli-Q water were used for digestion. Thoroughly acid-cleaned 100 and 250 ml standard flasks were used for volume make up. Whattman filter paper no. 42 was used for filtration purposes.

3.6.3. Determination of trace metals in soil

About 0.05 g soil samples were weighed and taken in a clean PTFE Teflon® beaker. Each sample was moistened with a few drops of water. Then, 10 ml of the acid mixture containing a 7:3:1 ratio of HF, HNO_3, and $HClO_4$ was added to each beaker and the sample swirled until completely moistened. The beakers were covered with lids and the samples were left standing

overnight after adding 5 ml of ^{103}Rh (1 μg/ml) as an internal standard. The next day, the beakers were heated on a hot plate at 220°C for about 1 h, after which the lids were removed, and the contents evaporated to near-dryness. The evaporation process was repeated after adding 5 ml of the above acid mixture in each case. Finally, the residue was dissolved by gently heating in 20 ml of 1:1 HNO_3. Clear solutions were obtained for all samples. After cooling to room temperature, the volume was made up to 250 ml, and these final solutions were stored in polyethylene bottles. The concentrations of different metals in these solutions were analyzed by ICP-MS. International geochemical standards SO-1 and SO-2 were used for calibration as well as to check the accuracy and precision.

3.6.4. Determination of trace metals in plants

Open acid digestion method was followed for the determination of metal concentration, wherein representative samples of dried plant tissues (approximately ~0.5 g) were taken in Teflon® beakers and 30 ml conc. HNO_3 were added in each. They were heated on a hot plate (~100°C) for 2 h keeping the lids. At the boiling stage, about 4–5 ml of H_2O_2 was added dropwise and heated further and the volume was reduced to about 10 ml. During this entire process, all organic material gets oxidized and the inorganic contents are extracted into the solution. To this, 5 ml of 1 μg/g Rh solution was added to act as an internal standard and the solution was transferred to a 250 ml volumetric flask and diluted to 250 ml with Milli-Q® water. The solution was analyzed by ICP-MS for trace elements.

3.7. Phytochemical screening

3.7.1. Solvent extraction of plant material

The completely shade-dried plant materials were ground into fine powder using an electric blender. The powdered plant material was subjected to successive solvent extraction taking from nonpolar to polar solvents like hexane, chloroform, and methanol, 70 g of powdered plant material was subjected to soxhlet extraction for 8 h with 300 ml of the various solvents. Each time before employing the solvent of higher polarity marc was dried. The extracts obtained were later kept for evaporation to remove the excessive solvents and brought to complete dryness over a water bath to yield the crude extracts. These extracts were collected, labeled, and stored at 4°C for further study.

3.7.2. Qualitative analysis of phytochemicals (Brindha et al. 1981)

The extract was tested for the presence of bioactive compounds by using the following standard methods given by Brindha et al. [34].

a. **Detection of alkaloids:** Extract (0.5 g) was dissolved in 10 ml of dilute HCl (0.1 N) and filtered. The filtrate was used to test for the presence of alkaloids. Mayer's test/Mayer's reagent: 1.358 g of mercuric chloride in 60 ml of water and 5 g of KI in 10 ml water. Mix

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the 2 solutions and dilute to 100 ml water. Filtrate was treated with Mayer's reagent. The formation of yellow cream precipitate indicates the presence of alkaloids.

b. **Detection of terpenoids**: Five milliliters of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

c. **Detection of saponins**: The ability of saponins to produce frothing in aqueous solutions was used as a screening test for the sample. Dried extract (0.5 g) was shaken with water in a test tube; froth which persist upon warming was taken as evidence for the presence of saponins. Honeycomb froth indicated the presence of saponins.

d. **Detection of tannins**: To the extract, 1% of gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

e. **Detection of phenols:** The extract was treated with 3 to 4 drops of 1% $FeCl_3$ solution. Formation of bluish black colour indicates phenols.

f. **Detection of flavonoids:** Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless upon the addition of dilute acid, indicates the presence of flavonoids.

3.7.3. Quantitative analysis of phytochemicals

a. **Determination of total flavonoid content using Swain and Hills method [35].** Preparation of standard and test solutions: The plant extract (50 mg) was dissolved separately in 50 ml of methanol. These solutions were serially diluted with methanol to get lower dilutions. Phloroglucinol (50 mg) was dissolved in 50 ml of distilled water. It was serially diluted with water to get lower dilutions.

Protocol for flavonoid content estimation: 0.2 ml of extract was taken in a test tube and the final volume was adjusted to 2.0 ml with distilled water. To this, 4.0 ml of vanillin reagent was added rapidly. Exactly after 15 min, absorbance was recorded at 500 nm against a blank. The unknown was read from the standard curves using different concentrations of phloroglucinol.

b. **Protocol for total phenol content estimation**. Total natural phenols of plant extracts were determined using a Folin–Ciocalteu assay with minor modifications [36]. A test tube containing either 500 μl of standard solutions of gallic acid (50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 μg/ml) or crude extracts (diluted 400-fold with distilled deionized water) was prepared, 500 μl of 10% Folin–Ciocalteu's phenol reagent (in DDW) was added into each test tube and mixed. After 20 min, 350 μl of 1 M Na_2CO_3 solution was added into the mixture. After incubation for 20 min at room temperature, the absorbance was determined at 750 nm against the blank prepared in parallel (500 μl of DDW + 500 μl of 10% FC reagent + 350 μl of 1 M Na_2CO_3 solution). The results expressed as gallic acid equivalents from the standard curve.

3.8. Antimicrobial activity

3.8.1. In vitro antimicrobial assay

The antimicrobial activity of the hexane, chloroform and methanol extracts of each sample was evaluated by using well diffusion method or cup plate method modified by Olurinola, which is the most widely used type for identifying antimicrobial activity, and exploited the diffusion of antimicrobial compounds through agar media to demonstrate the inhibition of bacteria and fungi [37, 38].

3.8.2. Collection of microbial cultures

Based on common diseases in human beings, 8 pathogenic species were selected to perform the antimicrobial action of test samples. The names of the cultures are listed in Table 2. All the cultures were collected from TRIMS, Visakhapatnam in Andhra Pradesh.

ID No.	ID Name	Morphology
MTCC 96	*Staphylococcus aureus*	Gram-positive facultative anaerobic, grape-like clusters
MTCC 430	*Bacillus cereus*	Gram-positive large endospore former
MTCC 2274	*Bacillus subtilis*	Gram-positive rod-shaped, motile, peritrichous flagella, aerobic
MTCC 439	*Enterococcus faecalis*	Gram-positive lactic acid bacteria of the phylum Firmicutes
MTCC 7162	*Klebsiella pneumonia*	Gram-negative bacilli, shorter and thicker rods about 1–2 mm encapsulated (thick caps)
MTCC 1457	*Shigella boydii* (serogroup C)	Gram-negative bacilli, short rods measuring from 0.5 to 1.3 m, nonvolatile, non–spore-forming, and noncapsulated
MTCC 443	*Escherichia coli*	Gram-negative, straight rod measuring 1.3 × 0.4 to 0.7 mm, motile capsulated
MTCC 3072	*Candida albicans*	Diploid fungus that grows both as yeast and filamentous cell, Chlamydosporous

Table 2. List of microorganisms used for antimicrobial activity with the diseases they cause

3.8.3. Media used for antimicrobial assay

For bioassay studies, the media used was 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 the surface of the medium as required for isolation of pure cultures.

Composition of Mueller–Hinton agar medium

Beef infusion; 300.0 g/l, Casein, 17.5 gm/l

Agar; 17.0 g/l, Starch, 1.5 g/l

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3.8.4. Preparation of culture

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

3.8.5. Preparation of media and plates for agar diffusion method

This assay was performed using two methods: agar disc diffusion and agar well diffusion. In these two methods, the agar well diffusion assay was used to screen for antimicrobial activity of the hexane chloroform and the methanolic extracts of different plant species. To prepare the media, for each organism, it requires 20 plates of MTT agar for 500 ml of distilled water; 19.5 g of MH agar was weighed and dissolved in a conical flask. Then, it was autoclaved at 15 lbs. pressure at 121°C for 20 min. After sterilization, media was aseptically distributed into sterile 6-in. diameter petri plates and allowed to solidify at room temperature for about 10 min and then kept in a refrigerator for 30 min. After solidification, using a sterile cotton swab, each microbial culture was spread uniformly onto the surface of the agar plates from culture containing approximately 105 CFU/ml of each organism for 24 h slant culture in aseptic conditions. The most widely used type of identifying antimicrobial activity is the diffusion method, which exploits the diffusion of antimicrobial compounds through the agar media to demonstrate inhibition of bacteria. The assay was performed by using a 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 6 uniform wells in each petri plate. These wells were labeled based on the microbes and plant extract used. To determine the potential of plant extracts, they were diluted up to 100 mg/ml of DMSO solution. And from three solvent extract dilutions, 40 μl was introduced into wells, respectively, and allowed to diffuse for 45 min. The plates were incubated at 37°C for 24 h. After proper incubation, the zones of inhibition were measured with a ruler. Results were noted and presented.

3.9. Anticancer activity

3.9.1. Cell culture

Human cancer cell lines used in this study were procured from the National Centre for Cell Science, Pune. All cells were grown in minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, antibiotics (benzyl penicillin, 50 units/m; 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.

3.9.2. 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 [39]. 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 5×10^3 cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 24 h of incubation, the supernatant was discarded and the cells were pretreated with growth medium and subsequently mixed with different concentra-

tions of test compounds (12.5, 25, 50, 100, and 200 μg/ml) in triplicate to achieve a final volume of 100 μl and then cultured for 48 h. The compound was prepared as 1.0 mg/ml concentration stock solutions in DMSO. Culture medium and solvent were used as controls. Each well then received 50 μl of fresh XTT (0.9 mg/ml in RPMI along with XTT activator reagent) followed by incubation for 2 h at 37°C. At the end of the incubation, shacked the 96 micro-well plates for 15 s. 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

Where, At = absorbance of test, Ab = absorbance of blank

Ac = absorbance of control

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

3.10. Statistical Package for the Social Sciences (SPSS)

SPSS is one of the most used programs for statistical analysis in social science. The program is used in many fields. Education researchers, market researchers, health researchers, survey companies, even the government, all use this program.

SPSS has a set of features which simplify programming. The program's datasets contain a table structure with rows and columns. The rows represent cases and the columns represent measurements like age, sex, etc. It has only 2 defined data types (text and numeric). SPSS Statistics 17.0.2 will help users in different fields to work together and obtain the best results for their companies.

These are some important features of SPSS 17.0.2:

- SPSS Missing Values add-on module (a feature that allows you to estimate data in case of missing files)
- Enhanced Syntax Editor: a features that creates, corrects, and test syntaxes fast and with a very small number of errors
- It can generate new interfaces, so that even beginners will consider the program easy to use

Objectives

To understand the analysis of variance models, *F* test for simultaneously comparing factors with respect to their means, the least-significant difference test: the ANOVA table.

ANOVA[b]						
	Model	**Sum of Squares**	***df***	**Mean Square**	**F**	**Sig.**
1	Regression	RSS	K-1	RSS/TSS		
	Residual	RSS	N*K-1	RSS/TSS	MSS/TSS	
	Total	TSS	N-I	SST+SSE		
	ERROR	ESS	N-K			

Table 3. ANOVA table

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4. Results and discussion

4.1. Metal analysis

4.1.1. Metal analysis of soil

The elements that are present in the soil sample of the Malkapuram industrial polluted area and the less-polluted agency area Paderu of Visakha district in Andhra Pradesh were selected for study. There are 24 elements that were detected, namely, As, Ba, Co, Cr, Cu, Mo, Ni, Pb, Rb, Sr, V, Y, Zn, Zr, SiO_2, $A1_2O_3$, Fe_2O_3, MnO, MgO, CaO, Na_2O, K_2O, TiO_2, and P_2O_5 (Table 3). Among which nine are essentials elements (Fe, Co, Mg, Cu, Mo, V, Cr, Mn, and Ni). Mo is the heaviest and is relatively less abundant on the earth's crust. In our analysis of natural and polluted soils, Ni was not detected and Mo is in lower concentrations in natural sources and not detected in polluted soils. Cu is not detected in natural soils and is more abundant in polluted soils. Zn, Co, Cr, and V are more in polluted soils approximately four times. Fe and Mn are in oxide form with no significant increase in polluted soils.

Soil Elements	Natural Source	Polluted Source
As	5.35	6.2
Ba	519.4	857.5
Co	6.05	27.3
Cr	57.8	211
Cu	ND	33.6
Mo	5.2	ND
Ni	ND	ND
Pb	31.8	43
Rb	176.4	44.5
Sr	86.25	104.2
V	43.4	181.4
Y	49.7	25.7
Zn	71.65	338.8
Zr	216.25	177.3
SiO_2	84.4	53.6
Al_2O_3	8.2	6.38
Fe_2O_3	1.7	6.19
MnO	0.06	0.16
MgO	0.59	0.8
CaO	ND	3.26
Na_2O	1.1	1
K_2O	11.9	4.88
TiO_2	0.68	1.86
P_2O_5	0.1	1.79

Table 4. The concentrations of metal elements in the natural and polluted soils Testing of hypothesis for the data (*T* test):

All the representative metals (Na, K, Mg, P, and Ca) are in oxide form in soils and are found at higher concentrations in the soils of both natural and polluted sources. Si is one of the abundant elements on the earth's crust and has been found to be probably essential in one family of plants [40]. Si is present at higher ranges in both soils with not much significant variation. Analysis of five essential nonmetals (C, N, O, S, and Cl) was not included in the analysis. Al, Ti, and Zr are not essential, yet abundant in earth's crust and form extremely insoluble oxides. The presence of Al, Ti, and Zr show no significant variation in natural and polluted soils. As, Y, Sr, Rb, Pb, Ba, V, and Zr appear to be nonnutrient elements. They are neither included in the above essentiality list nor show significant variation in polluted and natural soils.

	Paired Differences					*T*	*df*	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Natural source– - polluted source	6.039	108.491	24.259	86.814	14.736	1.486	19	.154

Table 5. Sample *T* test

The null hypothesis and alternative hypothesis with respect to natural and polluted soils can be stated as follows:

H_0: There is no significant difference between metals in polluted and natural soils

H_1: There is a significant difference between metals in polluted and natural soils

The *t*-statistic value is 1.486 and the significance is 0.154, which is more than 0.05, so the null hypotheses H_0 is not accepted. Hence, it is concluded that polluted soils showed higher concentrations than the natural soils.

4.1.2. (A) Comparison of metal analysis in natural and polluted sources

In a comparative study of these metals grown in polluted and natural sources, the concentration of 15 metals, namely, As Ba, Co, Cr, Cu, Pb, Sr, Zn, Al, Fe, Mn, Mg, Mo, Ti, and P are found to be higher in polluted soils than that of natural soils. This high concentration may be attributed to the release of elements from the surrounding industries of Malkapuram as the same are not found in control soil samples at higher concentrations. Our reasoning and results are in support that the abundant rock-forming elements are O, Al, Si, Fe, Na, K, Mg, and other than this major contribution to metal burden in soils, include discard-manufactured products as in scrap heaps, landfills (As, Cr, Cu, Pb, Mn, Zn), coal ashes (As, Cd, Pb, Mn, Mo, Ni, Se, V, and Zn), and agricultural wastes As, Cu, and Zn [41]. The United States Environmental Protection Agency (U.S. EPA) included 13 metals in their pollutants list: Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Ti, and Zn. Among the most important metals, Cd, Hg, Pb, Cr, and Zn

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are emitted from smelters and refineries. Cd, Se, and Hg are not analyzed in my study. The results are also consistent with the information given by several workers who accounted for the environmentally important metals As, Cd, Cr, Co, Hg, Pb, Mn, Ni, Se, and Zn and the less well-known but environmentally important elements Sb, Ba, Au, Mo, Ag, Th, Sn, Cu, U, and V [42].

In a comparative study of metals in polluted and natural soils, polluted soils showed higher concentrations than the natural soils and all heavy metals fall below the regulatory limits of the heavy metals applied to soils by the U.S. EPA [43] (Table 6).

Heavy Metals	Maximum Concentration in sludge mg/kg (ppm)	Concentration in natural soil	Concentration in polluted soil
As	75	5.35	6.2
Cd	85	ND	ND
Cr	3000	57.8	211
Cu	4300	ND	33.6
Pb	420	31.8	43
Hg	840	ND	ND
Mo	57	5.2	ND
Ni	75	ND	ND
Se	100	—	—
Zn	7500	71.65	338

ND = not detected.

Table 6. The heavy metal concentration of soils with regulatory limits. (Adapted from U.S. EPA 1993).

4.1.3. Analysis of metals in plant samples

A total of 26 elements (V, Cr, Mn, Ni, Co, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sb, Ba, Ti, Pb, U, Na, Mg, Al, Si, K, Ca, and Fe) were estimated in the powdered medicinal plant samples of ten, each plant was grown under natural sources and plants thriving under polluted sources (Table 7).

Metal analysis was carried out in five medicinal plants, namely, *A. vasica (A.v), E. globulus (E.g), H. suaveolens (H.s), R. communis (R.c), and T. cordifolia (T.c)* from the Paderu region, which is considered to be a natural and less polluted area, and the same five plants from industrial polluted area of Malkapuram cluster. A total of 26 elements (V, Cr, Mn, Ni, Co, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sb, Ba, Ti, Pb, U, Na, Mg, Al, Si, K, Ca, and Fe) were estimated in the 10 powdered medicinal plant samples, each of the plants were grown under natural sources and thriving under polluted sources. Among these, the role played by 15 elements (Na, Mg, Al, Si, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Mo) as macro- and micronutrients to support the growth and development of the plants is established [44]. As, Se, Rb, Sr, Ag, Cd, Sb, Ba, Ti, Pb, and U are nonessential nutrients.

Ele	*A. vasica* (N)	*A. vasica* (P)	*E. globulus* (N)	*E. globulus* (P)	*H. suaveolens* (N)	*H. suaveolens* (P)	*R. communis* (N)	*R. communis* (P)	*T. cordifolia* (N)	*T. cordifolia* (P)
Fe	0.98796	1.77412	0.68672	1.01536	0.89097	4.2936	0.77276	2.1658	1.38922	0.63875
Co	0.00244	0.00192	0.00948	0.00084	0.00495	0.00474	0.00153	0.0019	0.00237	0.00072
Zn	0.67237	0.57145	0.55601	0.66609	0.38132	0.8085	0.46781	0.6722	0.68254	0.59659
Cu	0.08278	0.08773	0.06837	0.04879	0.09411	0.12817	0.06431	0.0916	0.14323	0.07426
Mo	0.00576	0.02651	0.02532	0.0092	0.00256	0.00383	0.113	0.0353	0.00193	0.00257
V	0.00335	0.00458	0.00299	0.00472	0.00333	0.01382	0.00338	0.0066	0.00693	0.00293
Cr	0.08009	0.09044	0.08066	0.0803	0.08188	0.10096	0.07854	0.0866	0.08381	0.0847
Mn	0.2382	0.20703	4.98842	0.64406	0.60595	0.64673	0.38399	0.4353	0.43473	0.1411
Ni	0.2768	0.03153	0.04767	0.03006	0.02322	0.03344	0.02246	0.0271	0.03843	0.01687
The concentration of essential trace elements (in ppm) in both treated and control plants										
Na	4.095	5.42096	9.95389	25.6549	4.33451	11.0924	4.96097	7.0729	5.77424	10.1774
K	230.648	223.862	67.4462	84.3537	181.501	122.72	234.129	162.33	223.08	247.791
Mg	32.274	64.4634	12.3217	9.15043	27.363	33.8241	23.8091	33.627	15.4185	12.7332
Ca	208.215	261.432	82.4995	52.9004	133.748	144.43	134.994	190.48	85.0588	55.856
The concentration of representative elements (in ppm) in treated and control plants										
Al	0.69115	0.7559	0.56936	0.64118	0.96181	1.87821	0.74413	1.2689	1.46868	0.62012
TI	0.00026	0.00023	0.00013	0.00021	0.00008	0.00026	0.00008	0.0002	0.0001	0.0007
The concentration of nonessential trace elements (in ppm) in both treated and control plants										
Si	2.08043	2.42939	2.23198	2.20235	2.28226	1.9138	2.24237	2.5118	1.86681	2.36288
As	0.0012	0.00205	0.00163	0.00173	0.00122	0.00569	0.00127	0.0031	0.00189	0.0012
Se	0.15301	0.16083	0.17753	0.15543	0.14497	0.16998	0.14665	0.1579	0.14961	0.16122
Rb	0.14468	0.0914	0.15147	0.02595	0.23845	0.09003	0.16182	0.0855	0.1105	0.23519
Sr	0.48	0.77235	0.21404	0.20187	0.58336	0.3376	0.38586	0.4858	0.26037	0.1645
Ag	0.00047	0.00046	0.00061	0.00035	0.00051	0.00059	0.00064	0.0006	0.00046	0.00046
Cd	0.00331	0.00159	0.00134	0.0029	0.00181	0.00259	0.00101	0.002	0.00128	0.0012
Sb	0.00016	0.00025	0.00023	0.00026	0.00021	0.00057	0.00016	0.0004	0.00023	0.00034
Ba	0.30367	0.16086	0.28909	0.2944	1.28744	0.12151	0.18919	0.1079	0.24005	0.06183
Pb	0.02018	0.02472	0.01214	0.03141	0.02817	0.03618	0.0162	0.0259	0.192	0.02445
U	0.00026	0.00049	0.00022	0.00092	0.00025	0.00122	0.00024	0.0007	0.00034	0.00042
The concentration of toxic, heavy metals (in ppm) in treated and control plants										
N = natural P = polled										

Table 7. The concentration of metals (in ppm) in treated and control plants

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The results supported the information given by Craig Dick (2008), who said that there are actually 20 mineral elements necessary or beneficial for plant growth. Carbon (C), hydrogen (H), and oxygen (O) are supplied by air and water.

The six macronutrients, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) are required by plants in large amounts. The rest of the elements are required in trace amounts (micronutrients). Essential trace elements include boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), zinc (Zn), molybdenum (Mo), and nickel (Ni). Beneficial mineral elements include silicon (Si) and cobalt (Co). The beneficial elements have not been deemed essential for all plants but may be essential for some. The distinction between beneficial and essential is often difficult in the case of some trace elements.

The data in Table 7 indicates the uptake of elements (macro, trace, and heavy elements) by the various medicinal plants grown in natural and polluted areas and their comparative analysis is drawn. All the plants grown in polluted areas having more than 12 elements in higher concentration than the natural ones. The descending order in the number of metals found are 21, 18, 15, 13, and 12, respectively, in *R. communis (R.c), H. suaveolens (H.s), A. vasica (A.v), E. globulus (E.g)*, and *T. cordifolia (T.c).* The concentration of metals in natural conditions is less than in polluted conditions. This is because heavy metal pollution can arise from many sources, or more precisely, the activities are carried out in seven industries that are surrounding my area of study. Therefore, the sources of these metals in soils and plants are the industries. There are many correlations observed in the elemental content of both natural and polluted conditions. The metals, even though they are present in soil, the uptake by plants are seemingly less.

	Test value = 0					
	T	*df*	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
A.V (N)	1.585	25	.125	18.5177127	−5.541360	42.576786
A.V(P)	1.659	25	.110	21.62977654	−5.2224022	48.4819552
E.G(N)	1.757	25	.091	7.0129500	−1.208756	15.234656
E.G(P)	1.797	25	.084	6.85068500	−0.9992846	14.7006546
H.S(N)	1.608	25	.120	13.63712846	−3.8335830	31.1078399
H.S(P)	1.736	25	.095	12.4099431	−2.311420	27.131306
R.C(N)	1.525	25	.140	15.52655654	−5.4409577	36.4940708
R.C(P)	1.641	25	.113	15.4493462	−3.942021	34.840713
T.C(N)	1.434	25	.164	12.9387327	−5.645728	31.523193
T.C(P)	1.322	25	.198	12.7596385	−7.119869	32.639146

Table 8. Sample *T* test

Testing of hypothesis for the data (*T*-test)

The *t*-statistic values for different metals are in the above table and significance values are greater than 0.05. So, null hypotheses H_0 is not accepted. Hence, it is concluded that polluted soils showed higher concentrations than the natural soils.

In all the plants that have been studied for metal uptake, six elements, namely, Na, Mg, Al, Si, K, and Ca, are found in high concentrations in both control and polluted plants, ranging from 4 to 354 ppm, whereas Fe shows in *Tinospora*, respectively, ranging between 001 and 5.48 ppm, which is enumerated in Table 7, whereas all other 19 elements fall below 1 ppm in all plants. In addition, this metal concentration is high in plants grown in polluted areas than from the natural areas.

From the study, it was revealed that all the metals accumulated to a greater or lesser extent by all plant species studied. The plants showed a large number of elements and were rich in Na, Mg, Ca, K, Fe, Al, and Si, which are earth's crust elements and the same were found to be abundant in earth's crust also. There is a lot of correlation observed in the elemental content of both soils and plants studied in the natural and polluted conditions.

The higher concentrations of 7 metals (Na, Mg, Al, Si, K, Ca, and Fe) are seen in polluted plants, except for Na, which is highest in the natural condition.

Na ranges between 4.095 and 26.655 ppm, and the highest concentration is seen in *E. globulus* (polluted) having 26.655 ppm. Mg ranges between 9.150 and 64.463 ppm and the highest concentration is seen in *A. vasica* (polluted) having 64.463 ppm. Al ranges between 0.569 and 1.878 ppm and the highest concentration is seen in *H. suaveolens* (polluted) having 1.878 ppm. Si ranges between 1.867 and 2.512 ppm and the highest concentration is seen in *R. communis* (polluted) having 2.513 ppm. K ranges between 67.446 and 247.791 ppm and the highest concentration is seen in *T. cordifolia* (polluted) having 247.791 ppm. Ca ranges between 52.900 and 261.432 ppm and the highest concentration is seen in *A. vasica* (polluted) having 261.432 ppm. Fe ranges between 0.1749 and 4.988 ppm and the highest concentration is seen in *E. globulus* (natural) having 5.484 ppm (Figs. 1–5).

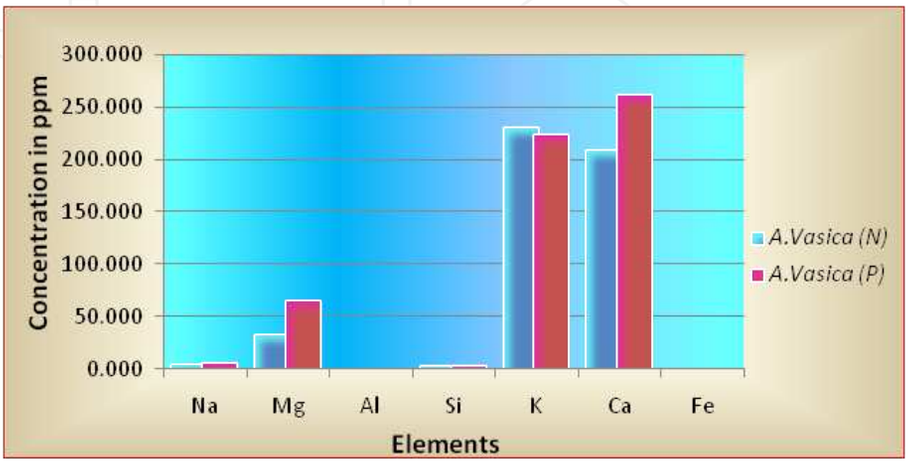

Figure 1. Metals with higher concentration in *A. vasica* (AV) Polluted (P) natural (N).

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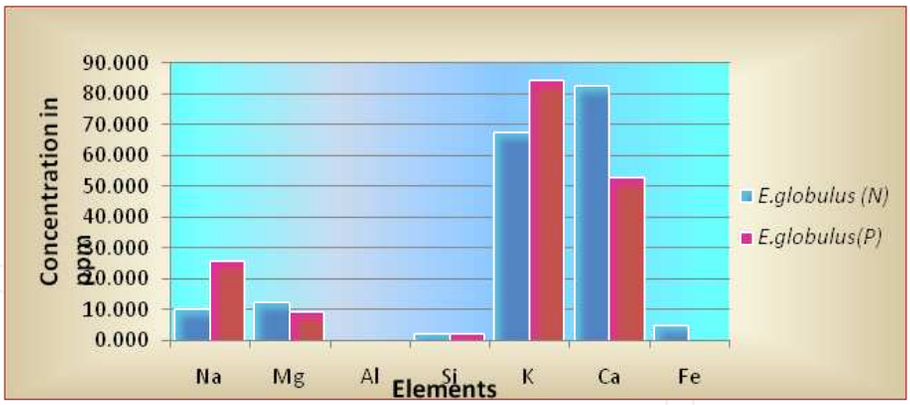

Figure 2. Metals with higher concentration in *E. globulus* (EG) polluted (P) natural (N).

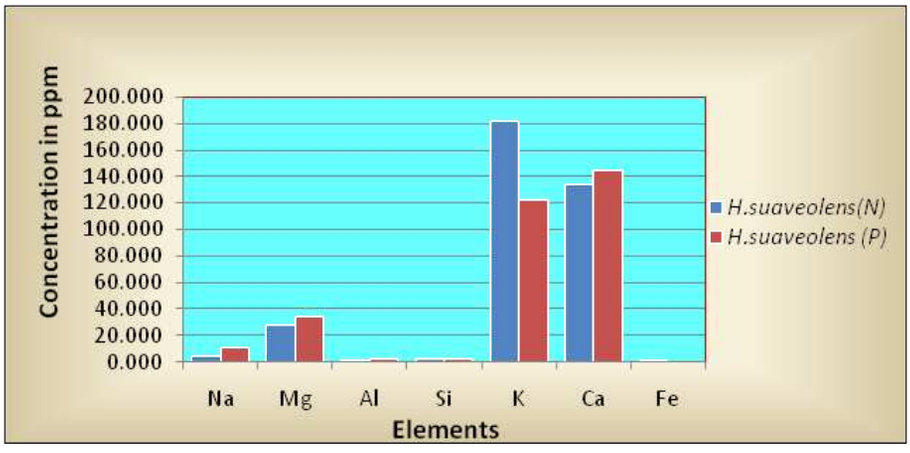

Figure 3. Metals with higher concentration in *H. suaveolens* (HS) polluted (P) natural (N).

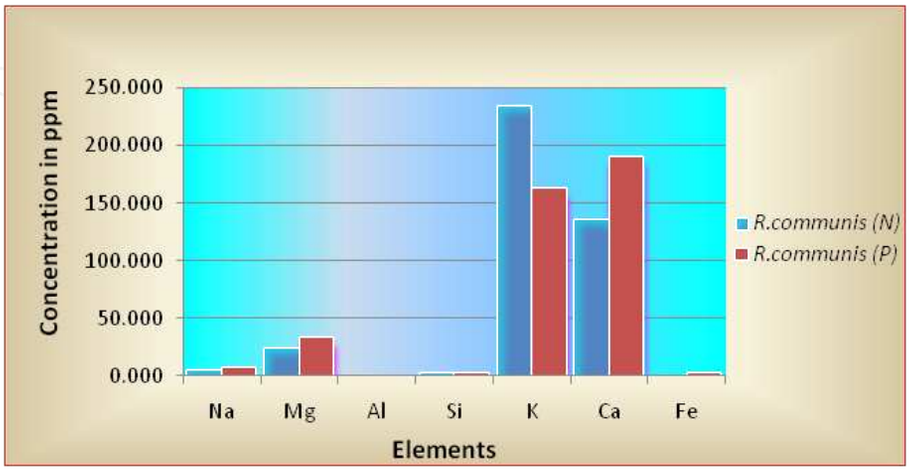

Figure 4. Metals with higher concentration in *R. communis* (RC) polluted (P) natural (N).

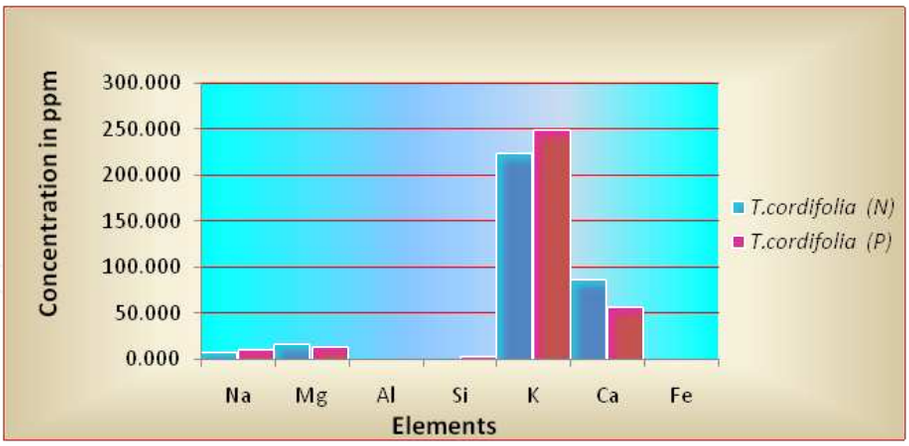

Figure 5. Metals with higher concentration in *T. cordifolia* (TC) polluted (P) natural (N).

The variations in elemental concentration are mainly attributed to the differences in botanical structure, mineral composition of the soil, preferential absorbability, use of fertilizers, irrigation of water, and climatologic conditions [45]. Furthermore, the difference in sampling and presence of pollution sources can also effect the concentration of metals from species to species. The plants that are grown in polluted areas show luxuriant growth irrespective of the higher metal concentration.

Furthermore, differences in sampling and the presence of pollution sources can also affect the concentration of metals from species to species. The plants that are grown in polluted areas showed luxuriant growth irrespective of the higher metal concentration.

Of the elements that are accumulated in higher concentration, Na (in all plants) and Co and Si (in two plants) are beneficial elements. In the above plants showing luxuriant growth in polluted sources, the beneficial elements could be compensating for the toxic effects of other elements or replacing the mineral nutrients in some other less specific function such as maintenance of osmotic pressure. Also, it is surprising to know that the percentage of Na in the soils of both natural and polluted sources was found to be almost the same., and yet, the Na uptake by all plants growing in polluted sources is significantly higher. The reason could be that the plants could have developed some mechanism such as ion flux, which may prevent the plant from absorbing heavy toxic elements even though they are found in higher concentration in the polluted soil sources. Na and K metal concentrations are responsible for maintaining normal hydration and osmotic pressure and K concentration is needed for cell growth and function. Thus, the high content of K could be the reason for the luxuriant growth of medicinal plants in heavily polluted areas.

The high concentrations of Mg, Ca, and K in the plants show that these elements are the most abundant metal constituents in plants [46].

Trace elements play an important role in the production of secondary metabolites, which are responsible for the pharmacological actions of medicinal plants [47]. Furthermore, it is aimed to study the concentration of these metals that are listed at higher level in the selected medicinal plants and later to intensify the study on their biological effects. In general, it is observed in

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my study that even though the concentration of metal elements is high in soil, the uptake of metals by plants is seemingly less.

4.1.4. (a) Heavy metals and medicinal plants

The heavy metal concentration with permissible limits [56] FAO/WHO 1984: In all 5 plants studied out of 11 heavy metals detected, namely, Zn, As, Co, Cu, Cd, Ti, Pb, Ni, Mn, Fe, and Cr. More than 9 metals are in high concentration in plants grown in polluted than in plants grown in natural condition (Table 9). All plants grown in natural and polluted areas have metal concentrations that are below the permissible limits of the FAO/WHO (1984). Zn and Pb concentrations in all plants grown in polluted areas are high when compared to plants grown in natural areas [48].

Elements	FAO/WHO limits	*A. vasica* (N)	*A. vasica* (P)	*E. globulus* (N)	*E. globulus* (P)	*H. suaveolens* (N)	*H. suaveolens* (P)	*R. communis* (N)	*R. communis* (P)	*T. cordifolia* (N)	*T. cordifolia* (P)
Zn	27.4	0.67237	0.55601	0.38132	0.46781	0.68254	0.57145	0.66609	0.8085	0.67223	0.59659
As	1	0.0012	0.00163	0.00122	0.00127	0.00189	0.00205	0.00173	0.00569	0.00306	0.0012
Co	0.48	0.00244	0.00948	0.00495	0.00153	0.00237	0.00192	0.00084	0.00474	0.00185	0.00072
Cu	3	0.08278	0.06837	0.09411	0.06431	0.14323	0.08773	0.04879	0.12817	0.09162	0.07426
Cd	0.21	0.00331	0.00134	0.00181	0.00101	0.00128	0.00159	0.0029	0.00259	0.00203	0.0012
TI		0.00026	0.00013	0.00008	0.00008	0.0001	0.00023	0.00021	0.00026	0.00017	0.0007
Pb	0.43	0.02018	0.01214	0.02817	0.0162	0.192	0.02472	0.03141	0.03618	0.02588	0.02445
Ni	1.63	0.2768	0.04767	0.02322	0.02246	0.03843	0.03153	0.03006	0.03344	0.02708	0.01687
Mn	2.001	0.2382	4.98842	0.60595	0.38399	0.43473	0.20703	0.64406	0.64673	0.43534	0.1411
Fe	20	0.98796	0.68672	0.89097	0.77276	1.38922	1.77412	1.01536	4.2936	2.16584	0.63875
Cr	0.02	0.08009	0.08066	0.08188	0.07854	0.08381	0.09044	0.0803	0.10096	0.08655	0.0847

Table 9. Heavy metal concentration in plants with permissible limits of FAO/WHO 1984 (ppm)

The *t*-statistic value for different metals in Table 10 are greater than 0.05. So, null hypotheses H_0 is not accepted, i.e., in all the 5 plants studied, out of 11 heavy metals detected, namely, Zn, As, Co, Cu, Cd, Ti, Pb, Ni, Mn, Fe, and Cr. More than 9 metals are in high concentration in plants grown in polluted conditions than natural conditions.

Copper (Cu): The copper concentrations in all polluted plants are high, ranging between 0.04879 and 0.12817 than the unpolluted plants, which range between 0.06431 and 0.1472. The permissible limits of FAO/WHO for copper (Cu) is 3 ppm. Copper is an essential enzymatic element for normal plant growth and development but can be toxic at excessive levels. Phytotoxicity can occur if its concentration in plants is higher than 20 mg/kg DW (dry weight).

	Paired Differences					*T*	*df*	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
FAO/WHO - A.V(N)	5.380567	9.435203	2.983673	−1.368971016	1.213010502E1	1.803	9	.105
FAO/WHOE.G(N)	4.971856	9.819888	3.105321	−2.052869174	1.199658117E1	1.601	9	.144
FAO/WHO - H.S(N)	5.405740	9.529765	3.013576	−1.411443352	1.222292335E1	1.794	9	.106
FAO/WHO - R.C(N)	5.436112	9.514774	3.008835	−1.370347592	1.224257159E1	1.807	9	.104
FAO/WHO - T.C(N)	5.320150	9.378580	2.965767	−1.388882144	1.202918214E1	1.794	9	.106
FAO/WHO - A.V(P)	5.337842	9.325711	2.949048	−1.333370270	1.200905427E1	1.810	9	.104
FAO/WHO - E.G(P)	5.364946	9.438893	2.984840	−1.387231321	1.211712332E1	1.797	9	.106
FAO/WHO - H.S(P)	5.011040	8.924020	2.822023	−1.372819819	1.139489982E1	1.776	9	.110
FAO/WHO - R.C(P)	5.265952	9.249872	2.925066	−1.351008190	1.188291219E1	1.800	9	.105
FAO/WHO - T.C(P)	5.459116	9.494573	3.002447	−1.332892374	1.225112437E1	1.818	9	.102

Table 10. Sample *T* test

The critical concentration for copper in plants is 20–100 mg/kg [49]. As with other heavy metals, some species can tolerate very high amounts of copper [50].

Cadmium (Cd): The cadmium concentrations in all polluted plants are high, ranging between 0.001 and 0.0029 compared with that of unpolluted plants, which range between 0.00101 and 0.0033. The permissible limit of FAO/WHO for cadmium (Cd) is 0.21 ppm. Cadmium is a toxic metal having functions neither in human body nor in animals or plants [51].

Tin (Ti): The tin concentration in all polluted plants is high, ranging from 0.0001 to 0.0007 compared with that of unpolluted plants, which range between 0.00008 and 0.0026. The permissible limit of FAO/WHO for tin is (TI) not set.

Lead (Pb): The concentration of lead in all the plants grown in polluted areas is higher than the natural conditions. The concentration of lead in both types falls below the permissible limits of FAO/WHO for lead (Pb), which is 0.43 ppm. The concentration of lead in all plants grown in polluted areas ranges from 0.0244 to 0.03618, and the concentration of lead in all plants grown in natural conditions is 0.0121–0.2821. Lead is regarded as very hazardous for plants and humans [52]. Obviously, the high lead concentration in the aerial parts of plants from polluted areas is due to the lead coming from the emission of vehicles as well as its presence in the soils polluted with wastes from different operations [53].

Nickel (Ni): The nickel concentrations in all polluted plants are high, ranging between 0.0168 and 0.0384 compared with that of unpolluted plants, which range between 0.022 and 0.2744. The permissible limit of FAO/WHO for nickel (Ni) is 1.63 ppm. Nickel is an abundant element;

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it is required in minute quantities for the body as it is mostly present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in a disorder of the liver [54].

Manganese (Mn): The manganese concentration in all polluted plants ranges between 0.141 and 0.64673 compared with that of unpolluted plants, which range between 0.238 and 4.9884. The permissible limit of FAO/WHO for manganese (Mn) is 2.001 ppm.

Manganese is also an essential element for plant and animal growth. Its uptake is controlled metabolically. Soils derive manganese from the parent material and its contents in rocks is higher than the concentration of other micronutrients apart from iron [55]. The main sources of manganese in the soil are fertilizers, sewage sludge, and ferrous smelters.

Iron (Fe): The iron concentrations in all polluted plants are high, ranging between 0.6387 and 4.2936, whereas that of the unpolluted plants range between 0.6867 and 1.3892. The permissible limit of FAO/WHO for iron (Fe) is 20 ppm.

Iron is very essential for plants and animals. Its deficiency in plants produces chlorosis disease; however, high concentrations also affect plant growth. The plant samples were collected from polluted areas and, in general, the concentrations of iron in polluted areas are higher.

Chromium (Cr): The chromium concentrations in all polluted plants are high, ranging between 0.0803 and 0.10096, whereas that of the unpolluted plants range between 0.07854 and 0.08381. The permissible limit of FAO/WHO for chromium (Cr) is 0.2 ppm.

Chromium is one of known environmental toxic pollutants in the world. The main sources of chromium contamination are tanneries, steel industries, sewage sludge application, and fly ash [56].

Pb, Cd, and Fe also accumulated more in plants growing in polluted sources. Higher concentrations of Pb and Cd in plants grown in polluted sources might reflect the concentration of these commonly encountered metals in polluted soils, which are being continuously released from the surrounding industries such as Coromandel Fertilizers, Hindustan Petroleum Corporation Limited, Hindustan Shipyard, steel plants, etc. Chelate-assisted phytoextraction has been developed because plants do not naturally accumulate important toxic elements, e.g., Pb, Cd, and As, that would be significant in remediation: continuous phytoextraction of metals relies on the properties of plant that lead to accumulation in aerial plant tissues [57].

Positively, the concentration of heavy metals in my study falls below the permissible limits and thus do not interrupt any regular functions of the plant and this could be the reason why plants grown in polluted areas have normal and luxuriant growth.

The heavy metal concentrations in my results were justified by the works of Yadav (2010), where plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage and disturbance of cellular ionic homeostasis. To minimize the detrimental effects of heavy metal exposure and their accumulation, plants have evolved detoxification mechanisms mainly based on chelation and subcellular compartmentalization. These reasons also could support why the concentration of heavy metals did not affect the growth of plants in the polluted areas of my study [58].

4.2. Phytochemical analysis

The five plants that have luxuriant growth have been selected for further study after metal analysis. The phytochemical characteristics of five medicinal plants grown in polluted and natural conditions were tested. The results revealed the presence of medically active compounds in methanolic extracts (Table 11). Almost all plants showed the presence of phytochemicals, namely, terpenoids, flavonoids, phenols, and alkaloids either in polluted or in control extracts of methanolic solvent. Hexane and chlorofoam showed no presence of phytochemicals.

Sample	Terpenoid	Flavonoids	Phenols	Saponins	Alkaloids
A. vasica Nees, (N)	+	+	+	−	+
A. vasica Nees (P)	−	−	−	−	+
E. globulus (Labille) (N)	+	+	+	−	−
E. globulus (Labille) (P)	+	+	+	−	+
H. suaveolens,(L.) Poit. (N)	+	+	+	−	+
H. suaveolens,(L.) Poit. (P))	−	+	+	−	+
R. communis Linn. (N)	+	+	−	−	−
R. communis Linn. (P)	+	+	+	−	−
T. cordifolia, Miers (N)	+	−	+	−	−
T. cordifolia, Miers (P)	+	+	-	-	+

Table 11. Preliminary phytochemical analyses of selected medicinal plants from natural and polluted sources

In addition, my work is supported by the results of Arvind Kumar et al. (2014), where methanolic extracts showed the presence of a large number of phytochemicals [59]. The results showed the concentration of phenols, flavonoids, and alkaloids in methanolic extracts. Flavonoids are not found in hexane and chloroform extracts [60]. The methanolic extract showed greater inhibition against *Pseudomonas* and *B. subtilis*. According to the results of Venkataswamy [61], the methanolic extracts showed the presence of flavonoids and triterpenoids. The presence of tannins and saponins are in contrast to my results. Except in *A. vasica* and *H. squaveolens*, all other three plants, namely, *Eucalyptus, Ricinus*, and *Tinospora* from polluted areas showed more phytochemicals than the plants grown in natural conditions. Flavonoids and phenols are seen in polluted plants of *Eucalyptus, Ricinus*, and *Hyptis* whereas alkaloids are seen in *Adhotoda* and *Hyptis* of both control and polluted plants.

4.2.1. Preliminary phytochemical analysis

The preliminary phytochemical analysis of plants showed that flavonoids, phenols, terpenoids, and alkaloids are seen in plants *A. vasika* and *H. suaveolens* that are grown in natural conditions and in *E. globulus* that is grown in polluted conditions.

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The results obtained in this study thus suggest that the identified phytochemical compounds may be bioactive constituents and that these plants are proving to be an increasing valuable reservoir of bioactive compounds of substantial medicinal merit. The results are at par with the results of Daniel and Daniang (2011), where many phytochemicals found in plants are either the products of plant metabolism or synthesized for defense purposes [62].

A. vasica has all four phytochemicals in natural condition, only alkaloids in *A. vasica* were polluted. In *Eucalyptus*, except for alkaloids, all three flavonoids, phenols, and terpenoids are found in both plants. But alkaloids present only in polluted plants, this is confirmed where the crude extract of medicinal plant studied was found to contain one or more of the following phytochemical compounds flavonoids, phenols, terpenoids, alkaloids, and volatile oils [63]. Other investigators have reported the presence of these components in members of the family Myrtaceae to which the plant used in the present study belongs. The inhibitory effects of this medicinal plant on the microorganisms may therefore be due to the presence of the above phytochemical components [64].

In *Hyptis* flavonoids, phenols, and alkaloids are found in both types of plants. Terpenoids are present only in plants in the natural condition. *Ricinus* (natural) has only terpenoids and flavonoids but *Ricinus* (polluted) has terpenoids, flavonoids, and phenols. *T. cordifolia* (natural) has only terpenoids and phenols and *T. cordifolia* (polluted) has terpenoids, flavonoids, and alkaloids. The presence of phenols in all five plants except in *A. vasica* (polluted), *R. communis* (natural), *T. cordifolia* (polluted) may help these plants to be very effective in their usage as a herbal medicine.

As the previous studies explained that phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [59]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection, and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities [65].

The flavonoid contents of the plants in my study are consistent with the results of Okwu, as flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to have antimicrobial substances against a wide array of microorganisms in vitro [66].

The results also showed the presence of alkaloids in *A. vasica* (natural), *A. vasica* (polluted), *E. globulus* (polluted), *H. suoveolens* (natural), *H. suoveolens* (polluted), and in *T. cordifolia* (polluted). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [67, 68]. Several workers have reported the analgesic, antispasmodic, and antibacterial properties of alkaloids.

The investigations have opened up the possibilities of the use of these plants in drug development for human consumption possibly for the treatment of gastrointestinal, urinary tract, wound infections, and typhoid fever. Preliminary phytochemical analysis revealed the presence of phenols, terpenoids alkaloids, and flavonoids. It is not surprising that there are differences in the antibacterial effects of plant groups due to phytochemicals properties and different among species.

4.2.2. Quantitative analysis of phenols and flavonoids

The amount of total phenols was determined with the Folin–Ciocalteu reagent. Galic acid was used as a standard compound and the total phenols were expressed as μg/mg gallic acid equivalent using the standard curve equation: $y = 0.0061x + 0.0396$, $R^2 = 0.9991$, where y is absorbance at 760 nm and x is total phenolic content in the different extracts expressed in mg/g. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [69].

Except for *A. vasica*, all four plants *E. globulus* (polluted): 279.4, *H. suoveolens* (polluted): 298, *R. communis* (polluted): 80.36, and *T. cordifolia* (polluted): 283.6 showed high concentrations of phenols compared with controls (Fig. 6). Interestingly, *Adhatoda* shows lower concentrations than controls. The highest concentration is seen in *H. suoveolens* (polluted) with 298 μg/mg. The next highest was *E. globulus* (polluted) at 279.4 μg/mg.

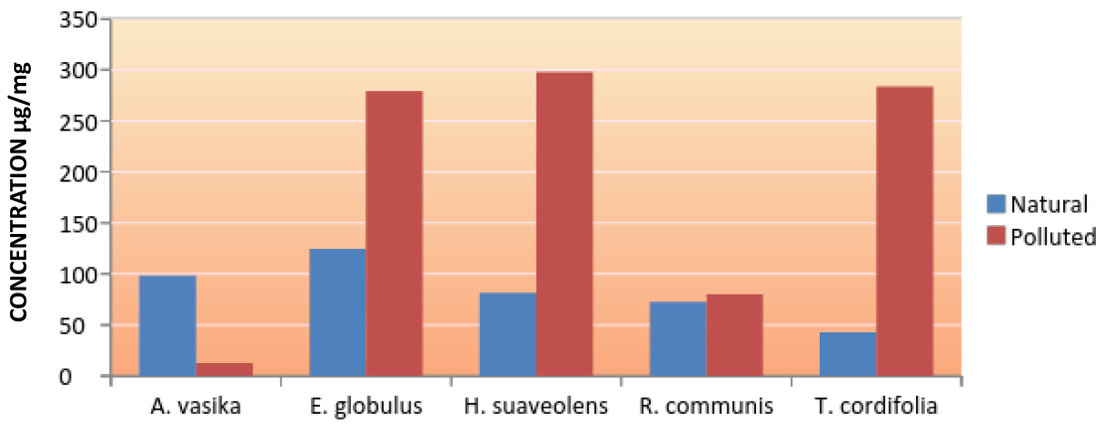

Figure 6. Total phenol content in plants grown in natural and polluted areas.

	Test Value = 0					
	t	*df*	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
GAE (μg/mg dry mass for methanol)	4.038	9	.003	1.373582	60.4171333	214.2988667

Table 12. One-sample test

Testing of hypothesis for the data (*T*-test)

The *t*-statistic value is 4.038, that is, more than 0.05. So, the null hypotheses H_0 is not accepted. Hence, it is concluded that except for *A. vasica*, all other four plants *E. globulus*, *H. suoveolens*, *R. communis*, and *T. cordifolia* showed high concentrations of phenols than controls.

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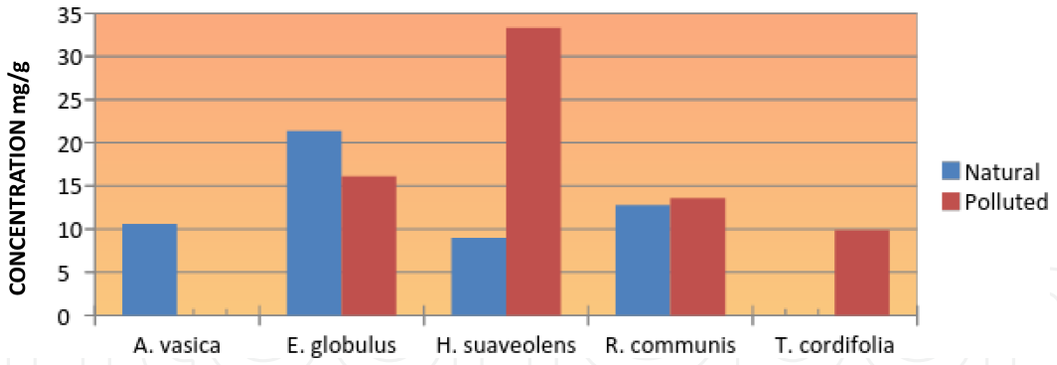

Figure 7. Total flavonoid content in plants grown in natural and polluted areas.

Estimation of flavonoid content using Swain and Hillis method (1959) used by Zachariah et al. (2012) employed in my work to estimate flavonoids [70]. In our results, high concentrations of flavonoids were found in *H. suoveolens* (polluted): 33.1 mg/g, *R. communis* (polluted): 13.6 mg/g, and *T. cordifolia* (polluted) in comparison with their natural varieties that have 19 mg/g, 12.8 mg/g, and 0 concentrations, respectively (Table 16). Three out of five plants from polluted areas showed high flavonoid contents and the remaining two showed low concentrations than the plants grown in natural conditions. The highest concentration is observed in *H. suoveolens* (polluted) is 33.1 mg/g (Figure 7).

		Paired Differences					*T*	*df*	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Conc. of Flavonoids mg/g of dried extract nol	1.3191	4.212	1.59221	−1.7087	−9.2955	4.285	6	.000

Table 13. Paired samples test

The value of the *t*-statistic calculated is 4.285. The values of the *T*-statistic are greater than 0.05. So the null hypotheses H_0 is not accepted, i.e., that out of three plants from polluted areas showed high flavonoid content and the remaining two showed lower concentrations than plants grown in natural conditions. Therefore, polluted has high concentration.

4.2.3. The relationship between heavy metal concentration and phytochemicals

Table 14 shows the 11 heavy metals detected and 4 out of 5 plants, *E. globulus*, *H. sauveolens*, *R. communis*, and *T. cordifolia* except for *A. vasica*, have heavy metal concentrations that are higher in polluted plants than in controls. Interestingly, the same four plants showed high numbers of phytochemicals and quantity of phenols. But only two plants (*R. communis* and *T.*

cordifolia) showed high quantities of flavonoids. This indicated that there is a positive correlation existing between heavy metal concentration and phytochemical concentrations. The works of Milan and Stankovic, where birches grown in polluted substance showed higher total phenols than birches grown in unpolluted areas, support my present work.

Plants		Heavy Metal Concentration (PPM)											Total Phenolic (μg/mg)	Total Flavonoids (mg/g)
		As	Cd	Cr	Cu	Pb	Co	Ni	Zn	Mn	Fe	Ti		
A. vasica Nees	N	0.0012	0.00331	0.08009	0.08278	0.02018	0.00244	0.2768	0.67237	0.2382	0.98796	0.00026	98.26	10.6
	P	0.00205	0.00159	0.09044	0.08773	0.02472	0.00192	0.03153	0.57145	0.20703	1.77412	0.00023	12.68	ND
E. globulus	N	0.00163	0.00134	0.08066	0.06837	0.01214	0.00948	0.04767	0.55601	4.98842	0.68672	0.00013	124.42	21.4
	P	0.00173	0.0029	0.0803	0.04879	0.03141	0.00084	0.03006	0.66609	0.64406	1.01536	0.00021	279.4	16.1
H. suaveolens	N	0.00122	0.00181	0.08188	0.09411	0.02817	0.00495	0.02322	0.38132	0.60595	0.89097	0.00008	81.5	9.00
	P	0.00569	0.00259	0.10096	0.12817	0.03618	0.00474	0.03344	0.8085	0.64673	4.2936	0.00026	298.4	33.3
R. communis	N	0.00127	0.00101	0.07854	0.06431	0.0162	0.00153	0.02246	0.46781	0.38399	0.77276	0.00008	72.5	12.8
	P	0.00306	0.00203	0.08655	0.09162	0.02588	0.00185	0.02708	0.67223	0.43534	2.16584	0.00017	80.34	13.6
T. cordifolia	N	0.00189	0.00128	0.08381	0.14323	0.192	0.14323	0.03843	0.68254	0.43473	1.38922	0.0001	42.86	ND
	P	0.0012	0.0012	0.0847	0.07426	0.02445	0.07426	0.01687	0.59659	0.1411	0.63875	0.0007	283.6	9.9

Table 14. The effect of metal concentration on the total phenols and flavonoids content

4.3. Antimicrobial activity

The hexane, chloroform, and methanol extracts of five plants, namely, *A. vasica,* Nees, *H. suaveolens*, (L.) Poit., *E. globulus,* Labill. *R. communis* Linn., and *T. cordifolia*, Meirs grown in both industrial polluted and natural areas were subjected to antimicrobial activity by using the well diffusion method. Table 2 shows the pathogens used, morphology, and the diseases they cause. All five plants in the study were treated against 8 pathogens (Table 2): *S. aureus, C. albicans, B. subtilis, Enterococcus, Shigella, Klebsiella, B. cereus*, and *E. coli.* Among the Gram-negative bacteria are *K. pneumonia, Shigella*, and *E. coli*, whereas the Gram-positive bacteria are *S. aureus, B. subtilis, B. cereus*, and *E. faecalis. C. albicans* is a fungus.

The overall results showed that all the plant extracts have antimicrobial activity against all Gram-positive bacteria, namely, *S. aureus, B. subtilis, B. cereus*, and *Enterococcus*, and the zone of inhibition between 5 and 20 mm is observed. These plant extracts have not shown any activity or insignificant activity against Gram-negative bacteria *Klebsiella, Shigella*, and *E. coli*. The zone of inhibition (ZOI) was analyzed in all the plants and among the three solvent extracts, the methanolic extract showed zones of inhibition better than the hexane and

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chloroform solvents, where they showed minimum or no zone of inhibition. The methanol extracts of all plants showed ZOI against all pathogens except *Shigella*, *Klebsiella*, and *E. coli* (Plates 4-6).

Methanolic extracts of *A. vasica* (natural) showed antimicrobial activity against all pathogens except *Shigella*. *A. vasica* (polluted) showed antimicrobial activity against *S. aureus, B. subtilis, B. cereus, C. albicans*, and *E. coli* (10 mm) only in methanol extracts. Methanolic extracts of *A. vasica* showed ZOI to all the pathogens varying between 7 and 12 mm except for *Enterococcus* and *Klebsiella*. The plants grown in natural areas has more ZOI (13 mm) against *C. albicans* when compared with the plants grown in industrial polluted areas. The least was 7 mm.

Among all five plant extracts, the extract of *E. globulus* (natural) and *E. globulus* (polluted) showed antimicrobial activity against all pathogens except *Klebsiella* and *E. coli*. The highest zone of inhibition was 20 mm against *B. cereus* in *E. globulus* (natural) and 18 mm in *E. globulus* (polluted). All the pathogens except *Klebsiella* and *E. coli* were susceptible to methanolic extracts of *E. globulus* showing high ZOI when compared with all other four plants.

All the pathogens except *Klebsiella* were susceptible to methanolic extracts of *H. sauveolens.* The maximum ZOI was 14 mm against *E. faecalis*. In the plant extracts from industrially polluted *Hiptis*, all the pathogens except *C. albicans* and *K. pneumonia* were susceptible to its extracts like the natural one where it shows 16 mm ZOI against *Enterococcus pneumonia* more than the natural one.

R. communis showed less antimicrobial activity. Only *B. cereus* and *E. pneumonia* are mainly susceptible. All others resisted or activity is insignificant. *R. communis* (natural) showed antimicrobial activity against one pathogen (*B. cereus*). The zone of inhibition was 11 mm. *R. communis* (polluted) showed antimicrobial activity against four pathogens (*S. aureus, B. subtilis, E. faecalis*, and *B. cereus*). The zone of inhibition was found between 7 and 10 mm. Hexane extracts of polluted plants showed 19 mm ZOI against *E. faecalis*.

T. cordifolia showed antimicrobial activity against all except *Shigella, Klebsiella*, and *E. coli.* The maximum ZOI showed against *B. cereus* 11 mm in chloroform of natural extracts and 17 mm of industrially polluted extracts. The most susceptible microorganisms in my study for plant extract in descending order were *B. cereus, S. aureus*, and *E. faecalis*.

The highest degree of antimicrobial activity was shown by *E. globulus* and had the highest ZOI at 20 mm among all the plants and *R. communis* having 5 mm had the minimum ZOI. The antimicrobial activity of both plants grown in polluted and natural conditions showed more similar activity where *A. vasica* and *E. globulus* grown in natural conditions showed better activity than plants grown in industrial polluted and *H. suaveolens, R. communis*, and *T. cordifolia* showed higher activity than plants grown in natural conditions.

From the MIC assays of 10 plant extracts, four plant extracts showed antimicrobial activities at minute quantities, 10 to 20 mg/ml concentration, as shown whereas a few plant extracts did not show any inhibitory effect in one or another bacterial and fungal species. The largest zone of inhibition belongs to *A. vasica* in both polluted and naturally grown extracts against *C.*

albicans, *E. globulus*, *R. communis*, and *T. cordifolia* against *B. cereus* and *H. sauveolens* against *E. faecalis* in all microbial studies.

The present study was designed to obtain information on the antimicrobial effect of 10 plant extracts on certain plant pathogenic microorganisms. The well diffusion/cup plate method was used in this study since it was found to be better than the disc diffusion method. All the medicinal plant extracts showed antimicrobial activity against the selected pathogens.

Hexane extract and chloroform extracts showed much lower or no antimicrobial activity compared with methanolic extracts. This may be due to little diffusion properties of these extracts in the agar or because fresh plants contain active substances which may be affected or removed by the steps of extraction methods. The methanolic extracts of all the medicinal plants screened exhibited greater antimicrobial activity. The antimicrobial action of methanolic extracts is due to compounds such as thiocyanate, nitrate, chloride, and sulphates beside other high-polarity soluble compounds which are naturally occurring in most plant materials [71].

This study showed that plant extracts were effective against the three Gram-positive strains (*S. aureus*, *B. subtilis*, and *B. cereus*) but no activity was observed against Gram-negative bacteria (*K. pneumonia*, *E. coli*, and *S. boydii*). This is consistent with previous studies reporting that Gram-negative bacteria are more resistant to antimicrobials than Gram-positive microorganisms due to their outer lipopolysaccharide membrane [72].

The presence of one or more of the secondary metabolites in my study indicated that the antibacterial activity was due to these active compounds present in different parts of the tested plants. The Gram-positive bacteria were slightly more susceptible to the extracts and showed greater inhibition zone than the Gram-negative bacteria, which in recent years have widely been reported in the literature [73].

Available reports tend to show that the secondary metabolites such as alkaloids, flavonoids, tannins, and other compounds of phenolic nature are responsible for the antimicrobial activities in higher plants [74]. The most studied chemical component in *A. vasica* is bitter quinazoline. The alkaloid vascine due to which the antimicrobial activity is seen [75]. The results provided evidence that *Hyptis* is indeed a potential source of natural antioxidant and antimicrobial agents. The antimicrobial activity could be due to the active chemical components savenine, alfa triterpines, 1–8 cinotene, cineole, and bita caryophyllene [76]. The results from *E. globulus* are promising and much higher than the earlier works [77]. The *Eucalyptus* antimicrobial activity is due to the active compound terpines.

The therapeutic activity of *R. communis* could be due to the recin chemical compound which is present and it also used experimentally in anticancer medicine [78]. The results indicated that the extracts of *R. communis* showed antibacterial activity, mainly against the Gram-negative bacteria (*E. coli*). The phytochemical components of *R. communis* have been established in previous studies and these include tannins, saponins, alkaloids, carbohydrates, phenols, flavonoids, sterols, and resins [79]. Several studies have linked the presence of these bioactive compounds in plant materials to antibacterial activity.

The antimicrobial activity of *T. cordifolia* is due to clerodane-derived diterpinoids, which comprise a large class of natural products with a wide range of biological activities [80]. The

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plants *E. globulus* and *T. cordifolia* showed highest antimicrobial and high concentration of phenols and flavonoids are selected to work upon MCF-7 breast cancer cell lines for its anticancer activity. The difference in ZOI can be due to the environmental differences. The differences between minimum and maximum ZOI could also be attributed to the effects of Gram-positive and Gram-negative bacteria.

Among polluted and naturally grown plants, the plants grown in polluted areas showed more zones of inhibition than that of plants grown in natural conditions. Except for *A. vasica*, all four plants (*E. globulus*, *H. suaveolens*, *R. communis*, and *T. cordifolia*) grown in polluted areas showed zones of inhibition in more than four pathogens compared with plants grown in natural areas. Almost all five plants showed antimicrobial activity against *B. cereus* and zones of inhibition varied between 7 and 20 mm.

The phytochemically quantitative data revealed that the flavonoid and phenolic concentrations are higher in pollutant plants when compared to the control plants. Because phytochemicals could either have antioxidant or hormone-like actions for treating health condition including cancer, heart disease, diabetes, high blood pressure, and for preventing the formation of carcinogens on their target tissues. Flavonoids have been found to be antimicrobial substances against a wide array of microorganisms in vitro [81]. The plants studied here can be seen as a potential source of useful drugs. Further studies are to be investigated in order to isolate, identify, characterize, and elucidate the structure of bioactive compounds.

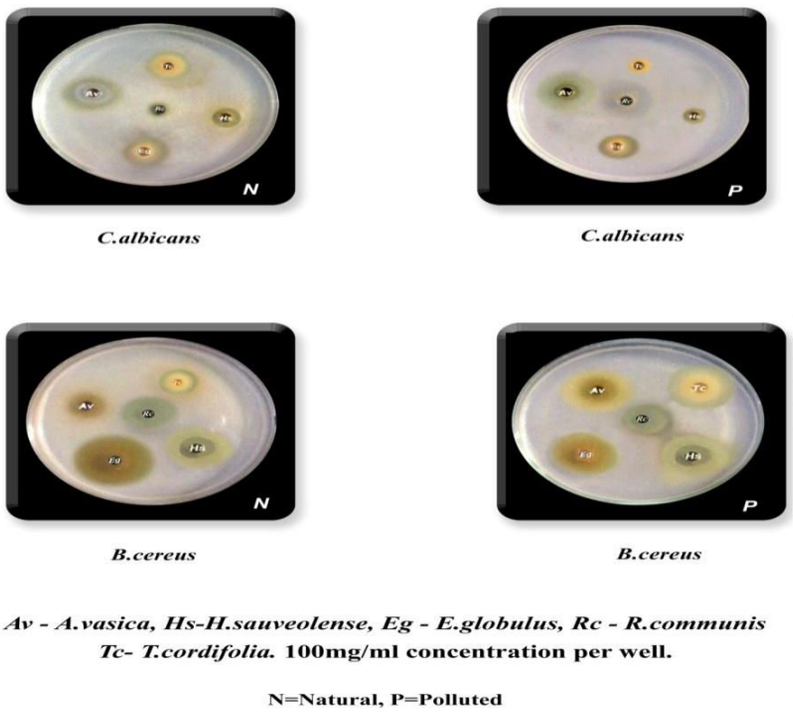

Plate 4. Antimicrobal Activity of Different Methanolic Plant Extracts against *C. albicans & B. cereus*

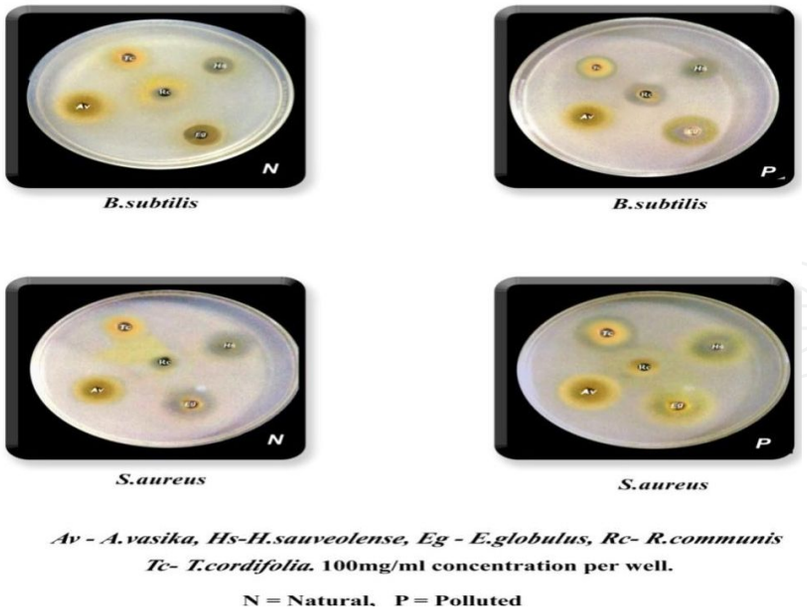

Plate 5. Antimicrobal Activity of Different Methanolic Plant Extracts against *B. subtilis & S. aureus*

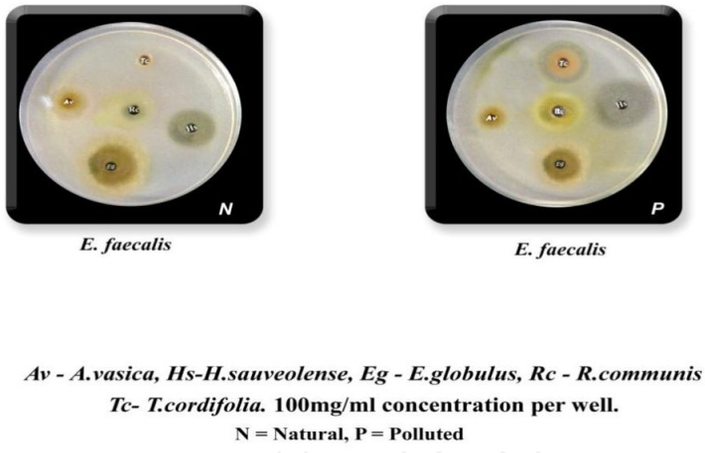

Plate 6. Antimicrobal Activity of Different Methanolic Plant Extracts against *E-faecalis*

4.3.1. The comparison/correlation between the result of phytochemical analysis and antimicrobial activity

The results showed that the phytochemical analysis is inconsistent with the antimicrobial activity that is seen in the same plants, namely, *H. suaveolens*, *Eucalyptus, Ricinus*, and *Tinospora* of the polluted areas which in turn showed increased phenolic and flavonoid content. The overall results and observations indicated that the plants that contained high metal concentrations tend to produce high amounts and high concentrations of phytochemicals, and this in turn resulted in the antimicrobial activity of the plants in coordination with the phytochemical analysis.

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In support of these observations is the antimicrobial activity of the same four plants, *E. globulus, H. suoveolens, T. cordifolia*, and *R. communis*, which showed a zone of inhibition. The order of high total phenolic content was found to be 298.4, 283.6, 279.4, and 80 μg/mg in *H. suoveolens* (polluted), *T. cordifolia* (polluted), *E. globulus* (polluted), and *R. communis* (polluted), respectively. Similarly, the flavonoids order of high content was 33.3, 16.1, 13.6, and 9.9 mg/g in *H. suoveolens* (polluted), *E. globulus* (polluted), *R. communis* (polluted), and *T. cordifolia* (polluted), respectively. But in *E. globulus* (natural), the high concentrations of flavonoids are seen at 21.4 mg/g than polluted.

4.4. Anticancer activity

The cytotoxic effects of the methanolic crude extracts of *E. globulus* and *T. cordifolia* were evaluated on MCF-7 breast cancer cell lines by microculture XTT assay. The multiple concentrations of methanolic extracts from *E. globulus* and *T. cordifolia* were used and effective doses were calculated from the dose–response curve. The results of cytotoxicity evaluated against MCF-7 using extracts at different concentrations (6.25, 1.5, 25, 50, 100, and 200 μg/ml) (Table 15). The methanol extracts of *E. globulus* and *T. cordifolia* exhibited cytotoxic effects on MCF-7 cell lines were confirmed by XTT assay.

Conc (μg/ml)	OD of STD (Tamoxifen) at 490 nm	% CS	% CI	OD of E.g (N) At 490 nm	% CS	% CI	OD of E.g (P) at 490 nm	% CS	% CI	OD of T.c (N) at 490 nm	% CS	% CI
6.25	0.423	83.8	16.2	0.492	98.9	1.1	0.488	98	2	0.485	97.4	2.6
12.5	0.322	61.7	38.3	0.473	94.7	5.3	0.462	92.3	7.7	0.468	93.7	6.3
25	0.246	45.1	54.9	0.386	75.7	24.3	0.352	68.3	31.7	0.361	70.2	29.8
50	0.179	30.4	69.6	0.365	71.1	28.9	0.315	60.2	39.8	0.331	63.7	36.3
100	0.095	12	87	0.272	50.8	49.2	0.285	53.6	46.4	0.288	54.3	45.7
200	0.058	3.9	96.1	0.234	42.5	57.5	0.222	39.8	60.2	0.257	47.5	52.5

Conc (μg/ml)	OD of T.c(P) STD at 490mm	% CS	% CI	OD of E.g + T.c (N) at 490 nm	% CS	% CI	OD of E.g (P) +T.c (P) at 490 nm	% CS	% CI
6.25	0.495	99.6	0.4	0.485	97.4	2.6	0.474	95	5
12.5	0.486	97.6	2.4	0.444	88.4	11.6	0.462	92.3	7.6
25	0.426	84.5	15.5	0.356	69.1	30.9	0.382	74.8	25.2
50	0.372	72.6	27.4	0.257	47.5	52.5	0.276	51.6	48.4
100	0.269	50.1	49.9	0.142	22.3	77.7	0.176	29.8	70.2
200	0.215	38.3	61.7	0.113	16	84	0.126	18.8	81.2

Blank = 0.040, Control = 0.497

E.g = *E. globulus*, T.c = *T. cordifolia*. N = natural, P = polluted.

Table 15. Dose–response of *E. globulus and T. cordifolia on* MCF-7 cell line

At the final tested concentration (200 μg/ml), the cytotoxicity of *E. globulus* grown in natural and polluted areas was 57.5% and 60.2%, respectively. The effects were dose-dependent and based on a dose–response curve, IC_{50} values were determined (Table 16). The methanol extracts of *E. globulus* exhibited antiproliferative effects on both cell lines with IC_{50} values of 145.85 and 136.53 μg/ml, respectively. From these results, it is evident that the effect of *E. globulus* extract from natural areas showed less cytotoxicity than the *E. globulus* from polluted areas. The cytotoxicity of the crude extracts from both are halfway ranging between 50% and 60% to reach the results of control tamoxifen, where it was 96.1%.

Plant	E.g(N)	E.g(P)	T.c(N)	T.c(P)	E.g(N)+T.c(N)	E.g(P)+T.c(P)
IC_{50}	145.85 μg/ml	136.53 μg/ml	156.95 μg/ml	139.91 μg/ml	82.400 μg/ml	91.980 μg/ml
Slope	0.27773134	0.26371002	0.23139161	0.32012509	0.40436674	0.39459844
Correlation coefficient	0.907716280	0.86551911	0.8376033	0.9425528	0.88480409	0.90949691
Intercept	9.49054726	13.9940299	13.681592	5.20845771	16.6800995	13.7044776

E.g = E. globulus, T.c = T. cordifolia. N = natural P = polluted.

Table 16. The IC_{50} values of plant extracts used against MCF-7 cell lines

4.4.1. The cytotoxic effect of methanolic extracts of T. cordifolia on MCF-7 breast cancer cell line by XTT assay

At the final concentration of 200 μg/ml the cytotoxicity of *T. cordifolia* grown in natural and polluted areas were 52.5% and 61.7% respectively based on the dose–response curve IC_{50} values were determined (Table 16). The plant extracts exhibited high antiproliferative effect on MCF-7 cell lines with IC_{50} values of 156.95 and 139.91 μg/ml, respectively. And like in the case of *E. globulus*, the cytotoxicity is less in *T. cordifolia* natural areas than that of polluted areas.

4.4.2. The cytotoxic effect of combined extracts of E. globulus and T. cordifolia from natural and polluted sources on MCF-7 cell lines

The cytotoxic effect of combined samples proved better results in comparison with the individual samples. The combined samples of *E. globulus* and *T. cordifolia* natural areas showed final concentrations of 200 μg/ml, 84% of cell inhibition having IC_{50} value 81.400 μg/ml and the *E. globulus* and *T. cordifolia* polluted areas showed 81.2% of cell inhibition IC_{50} value 91.980 μg/ml (Tables 15 and 16).

Here, contrary to the individual samples, the cytotoxicity exhibited by the combined extract of plants grown in polluted areas were less than that of natural extracts. The cytotoxicity results were also promising as the cytotoxicity of the combined extracts almost neared substituting the control tamoxifen results of 96.1% at the final concentration.

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4.4.3. Evaluation of morphological changes of MCF-7 cell lines upon treatment with extracts

The morphological changes of MCF-7 cell lines treated with extracts from plants grown in both polluted and natural resources were observed under phase contract microscope. The cells indicated most prominent effect after exposure to the extracts of *E. globulus* and *T. cordifolia* from polluted areas and their combined extracts compared with that of individual extracts of *E. globulus* and *T. cordifolia* from natural areas. In the combined sample extracts, 40% to 50% of the cells showed membrane blebbing (demonstrated with small protrusions of the membrane) and ballooning were apparent in the cells.

Cells also showed extensive vacuolation in the cytoplasm, indicating autophagy-like mechanism of cell death. Autophagosome-like structures were clearly seen in the cells treated with extract. At the highest concentration (200 μg/ml) the cells became rounder, shrunken, and showed signs of detachment from the surface of the wells denoting cell death.

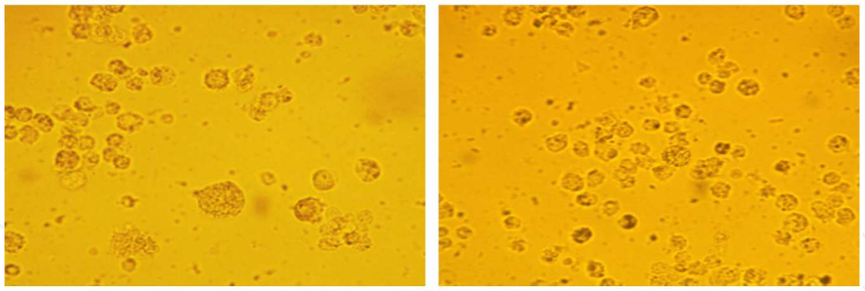
In the present study, anticancer activity of extracts of indigenous medicinal plants *E. globulus* and *T. cordifolia* grown in polluted and natural sources were investigated against human breast cancer cells MCF-7, whereas tamoxifen was used as experimental controls. A methodical evaluation of cytotoxicity effects revealed that the individual methanolic extract of *E. globulus* and *T. cordifolia* polluted and natural areas and their combined samples and tamoxifen showed dose-dependent cytotoxicity against MCF-7 cell lines. The IC_{50} values of combined plant extracts were found to be less than 100 μg/ml, indicating potent cytotoxic effects on breast cancer cell lines and further potential of these extracts for the isolation of biologically active phytochemicals. Most anticancer drugs are designed to eliminate rapidly proliferating cancerous cells and therefore they show cytotoxicity and induce apoptosis in cancer cells.

The phytochemical analysis of my study observed the presence of a large number of bioactive compounds in the methanolic extracts of these plants including terpenoids, alkaloids, phenols, and flavonoids, which exhibit various biological activities. These compounds hold great potential as drugs and are widely accepted among the public. This investigation provides evidence for cytotoxicity in MG(F)-7, which may be due to existing phytochemicals in the extract as mentioned previously. The sensitivities of cancer cells to cell death by flavonoids are in accordance with findings from previous reports in the literature. In another study, the presence of alkaloids with flavonoids in Onobishirta was reported expressing superior activity against cancer cells.

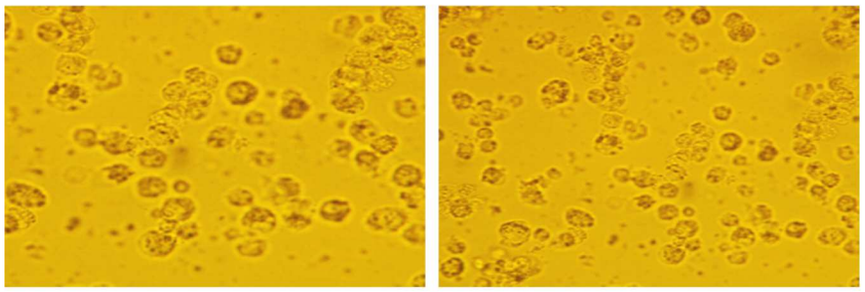
Our results are on par with the results that the phytochemicals present in *T. cordifolia* have potent cytotoxic and anticancer potential against MCF-7 cell lines [82]. Cancer cell lines used in the study exhibited differential sensitivity towards different plant extracts. The differential behavior of cell lines may be due to the different molecular characteristics of these cells. The present study clearly indicates that *T. cordifolia* extracts are very active against human breast cancer cell lines.

Polyphenols have been shown to possess antimutagenic and antimalignant effects. Moreover, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis.

MCF-7 breast cancer cell lines.



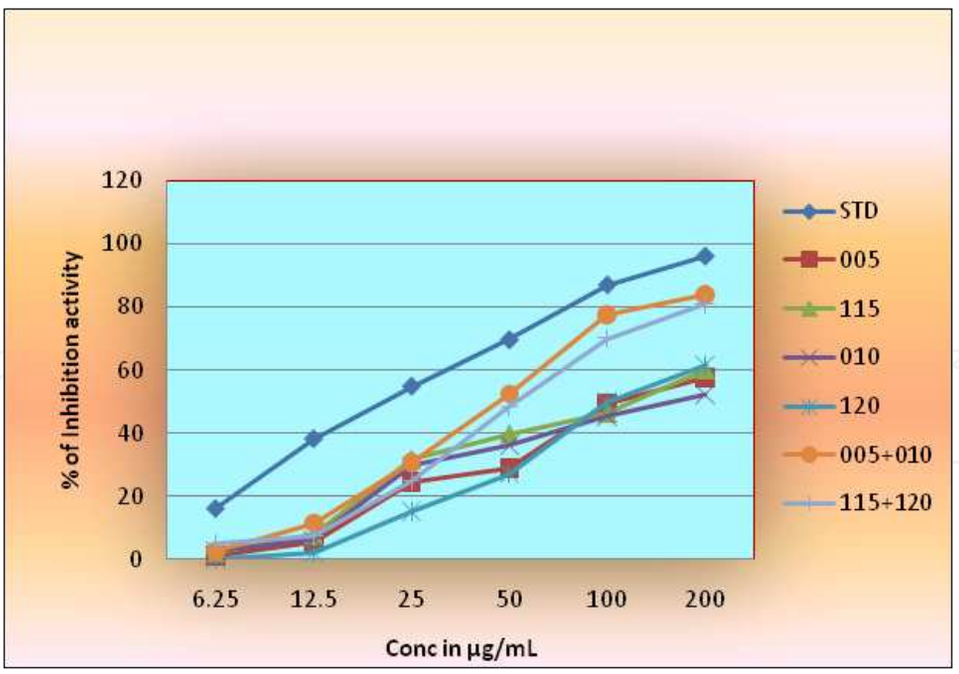
1. *E.globulus(N)* at 200ug/ml 2. *E.globulus(P)* at200ug/ml



3. *T.cordifolia(N)* at 200ug/ml 4. *T.cordifolia(P)* at 200ug/ml

N= NATURAL P= POLLUTED

Plate 7. Anticancer activity of methanolic crude plant extracts against MCF-7 breast cancer cell lines.



E.g=Eucalyptus globulus(N)=005, T.c=Tinospora cordifolia(N)=010,
E.g=Eucalyptus globulus(P)=II5, T.c=Tinospora cordifolia(P)=120, N=Natural, P=Polluted

Figure 8. Anticancer activity of *E. globulus* and *T. cordifolia* plant extracts on MCF-7 breast cancer cell lines.

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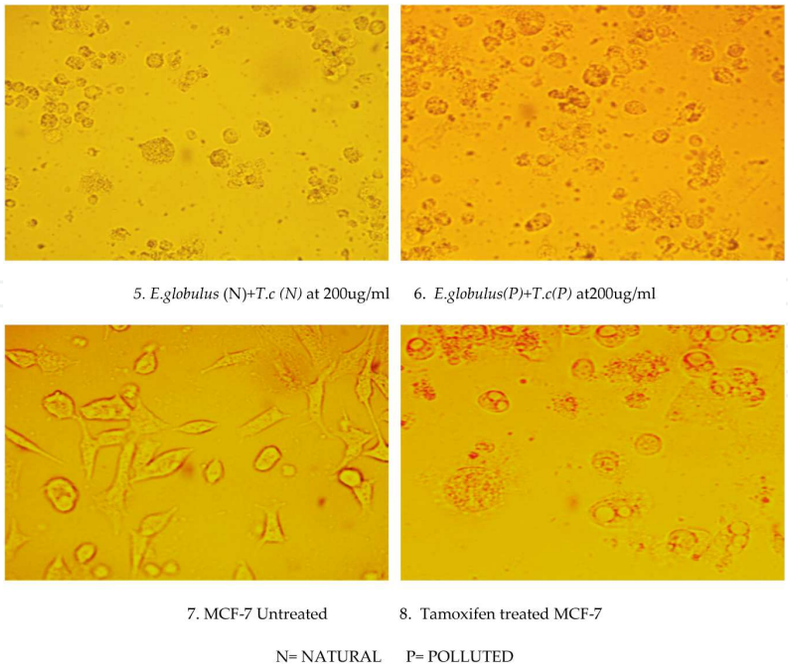

Plate 8. Anticancer activity of methanolic crude plant extracts against MCF-7 breast cancer cell lines.

The cytotoxic and antitumor properties of the extract may be due to presence of these compounds have shown very high efficacy in *T. cordifolia* extracts against Dalton lymphoma ascites (DLA) tumor model in Swiss albino mice in terms of survival as well as tumor volume control [82]. However, the exact mechanism is not clear. Available evidence suggests that DNA damage, inhibition of topoisomerase II, decline in clonogenecity and glutathione-S-transferase activity, activation of tumor associated macrophage, increase in lipid peroxidation, and LDH release are probable mechanisms behind the cytotoxic activity [83]. The arabinogalactan present in aqueous extracts of guduchi stem have also been shown to produce immunological activity. Many of the compounds mentioned above have been reported to be cytotoxic.

In this study the findings show there is much relationship between anticancer activity and phenolic composition. The extract of polluted plants that showed high anticancer activity also showed high phenolic composition. The finding suggests that the plants grown under polluted conditions reduced the number of viable cells compared with that of plants grown under natural conditions. As the geochemical analysis ruled out the toxicity of heavy metals in these plants as their concentrations fell below the permissible limits and the scope of using plants under abiotic stress and the taboo of avoiding these medicinal plants could be altered by further studies in these areas.

5. Summary and conclusion

The present research work *"Phytopharmaceutical studies on selected medicinal plants subjected to abiotic elicitation (stress) in an industrial area of Malkapuram, Visakhapatnam district of Andhra*

Pradesh" was undertaken to study the effect of industrial pollution on selected medicinal plants that were thriving well and growing luxuriantly compared with the same plants grown in natural or less polluted conditions. Malkapuram industrial area in Visakhapatnam was selected as the polluted region and Paderu, the hilly and agency area, was selected as the natural or less polluted region. To fulfill the aims and objectives, I have carried out the work in six phases:

i. The plants, namely, *A. vasica* (Nees), *E. globulus* (Labill), *H. sauveolens* (L.) Poit, *R. communis* (Linn.), and *T. cordifolia* (Miers) were collected and selected for my study from industrial areas and the same from natural areas that are grown luxuriantly.

ii. To check out abiotic stress, namely, metal stress, the metal analysis of selected plants grown in both industrial/natural areas and the soil in which the plants were grown was carried out.

iii. Subsequently, to check the effect of metal stress on phytochemicals, the qualitative and quantitative analysis of selected plants and the correlation between metal analysis and phytochemicals was studied.

iv. To see the effect of phytochemicals, the antimicrobial activity of the same plants was done using 8 pathogens.

v. *E. globulus* (Labill) and *T. cordifolia* (Miers), two plants that showed high concentrations of phenols and flavonoids as well as better antimicrobial activity were checked out for their anticancer activity against MCF-7 breast cancer cell lines.

vi. The entire data of my research work was analyzed statistically by applying SPSS.

No comparative studies of these medicinal plants under abiotic stress have been carried out so far. Pollution and its effect on flora and fauna is a global issue today. The metal analysis, phytochemical, antimicrobial, and anticancer activities of medicinal plants under abiotic stress is rare and scanty. The salient findings of the present study are furnished below.

1. The metal analysis of soils from an industrially polluted area of Malkapuram and a natural area revealed that the metal concentration is higher in the industrially polluted area compared with that of natural soils collected from the Paderu region of Visakhapatnam district and the heavy metal concentrations in both areas are below the regulatory limits of soil adopted from the U.S. EPA (1993).

2. The metal analysis of plants resulted in high metal concentrations in industrial polluted plants than that of plants grown in natural conditions, and heavy metal concentrations falling below the permissible limits of WHO/FAO (1984). It is also evident even though the soils contained high concentrations of metals, that the plants did not contain the same as they absorbed selectively based on different factors.

3. The preliminary phytochemical analysis results were in positive correlation with the metal analysis where the industrial polluted methanolic extracts contained more

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phytochemicals in comparison with the methanolic extracts of plants grown in natural areas.

4. The total phenolics concentration showed a significant increase in the plants grown in polluted conditions and, in some, 2- to 3-fold increases than the plants from natural areas. The flavonoid concentrations are at par with phenolics but with less significance.

5. The antimicrobial activity of the methanolic plant extracts that contained a greater degree of concentration of phenolics and flavonoids showed a higher percentage of activity. The methanolic extract of *E. globulus* grown in industrial polluted area having 100% activity against pathogens followed by *A. vasica* Nees and *H. sauvelens* (L.) from polluted areas.

6. The anticancer activity of *E. globulus and T. cordifolia* were selected from five plants with a high concentration of phenols and flavonoids confirmed the activity of MCF-7 breast cancer cell lines. At 200 μg/ml, the methanolic extracts of plants from polluted areas showed 57%–67% cell inhibition and combined extracts showed 81%–84% of cell inhibition of MCF-7 breast cancer cell lines having IC_{50} values below 100 μg/ml, which gives scope and way for further work in this area.

7. The statistical analysis carried out for the entire data from my results by using SPSS has further strengthened the findings of my work.

It is therefore through my findings that the following suggestions and conclusions are proposed:

1. The abiotic stress on plants grown in industrial polluted area triggered increased productivity of phytochemicals that lead to promising antimicrobial and anticancer activity of methanolic plant extracts, paving a definite way for pharmaceutical industries.

2. To exploit the plants under stress for preparations of novel drugs by extracting larger quantities of therapeutic chemicals to meet the needs of a growing population with less usage of plant dry mass.

3. As all the heavy metal concentrations fell below the permissible limits of FAO/WHO in the plants from polluted areas and as it showed significant increase in the phenols and flavonoids, it is recommended that pharmaceutical industries exploit plants under stress for therapeutic measures than the plants from natural areas after a strict screening for toxic elements.

4. As 80%–90% of the population depends on herbal medicine due to its' cost, side effects, and drug resistance, these plants could be further screened for any toxicity and made available for therapeutic uses.

5. The anticancer findings suggested that the plants grown under polluted conditions reduced the number of viable cells than that of plants grown under natural conditions. As the geochemical analysis ruled out the toxicity of heavy metals in these plants as their concentrations fell below permissible limits and the scope of using plants under abiotic stress and the taboo of avoiding these medicinal plants could be altered by further studies in these areas.

6. As the percentage of cell inhibition by *E. globulus* and *T. cordifolia* are close to the percentage of antibiotic tamoxifen, it could be further proposed to test the efficacy of its anticancer activity with live cells.

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