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Antioxidant, Antimicrobial activity (using Silver Nanoparticles) and Effect of Temperature on Polyphenol content in Spinach (*Spinacia oleracea*), Coriander (*Coriandrum sativum*) and Mint (*Mentha viridis)* extracts

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***Abstract*—**Polyphenols are secondary metabolites also called as polyhydroxyphenols are natural plant products present in leaves, flowers, bark and seeds. Polyphenols from plant sources are reported to have antioxidant, antimicrobial, anti-diabetic and many important properties. The aim of this study was to extract and determine polyphenol contents from green leafy vegetables like Spinach (*Spinacia oleracea*), Coriander (*Coriandrum sativum*) and Mint (*Mentha viridis*) using Folin-Ciocalteu method. To study the effect of temperature the samples were placed at low temperature for 10 days and for high temperature study, the samples were exposed to 100˚C and polyphenol content was analyzed. Antioxidant activity was determined by DPPH free radical scavenging. To study antimicrobial activity silver nanoparticles were prepared using *Ficus racemosa* leaf extract with polyphenols extracted from mint. The obtained nanoparticles size was analyzed using SEM. The Fresh methanolic extracts of polyphenols were high in coriander (4.26gm/100gms), mint (7.92gm/100gms) and in ethanolic extract of spinach (1.86gm/100gms). Polyphenols extracted from fresh samples was found to be high when compared to samples that were exposed to low and high temperature. It was found to have a strong Free radical scavenging activity due to higher phenolic contents in fresh samples. As concentration of polyphenol decreased free radical scavenging activity also decreased. Silver Nanoparticles produced from *Ficus racemosa* showed an absorption maximum at 430nm spectrophotometrically, indicates the formation of nanoparticles and the particle size was between 56.7 to 96.4nm analyzed using SEM. Antimicrobial activity was determined using paper disc method. The zone of inhibition was high in samples of *F.racemosa* NP with mint extract when compared to extracts obtained from fresh samples. This study shows that, polyphenol contents decreased when samples were stored at low and high temperature and also its antioxidant activity. Consumption of foods with less storage time and temperature exposure can retain the polyphenol contents to have a strong antioxidant activity. Preparation of nanoparticles using plant extracts for antimicrobial activity may increase its activity in an ecofriendly and be cost effective.

Keywords— *Antioxidant, Ficus racemosa, Polyphenols, SEM, Silver Nanoparticles*

I. INTRODUCTION

Polyphenols [1] are also called as polyhydroxyphenols which are characterized by the presence of large multiples of phenol structural units. Polyphenols are classified on the basis of the number of phenol rings they contain and of the structural elements that bind these rings to one another. They are divided into sub-groups like phenolic acids, flavonoids, stilbenes, and lignans [2]. Larger polyphenols are often concentrated in leaf tissue, epidermis, bark layers, flowers and fruits. Polyphenols can be found in fruits and vegetables, green tea, black tea, red wine, coffee, chocolate, olive and extra virgin, olive oil. Polyphenols are specific to particular foods like in citrus fruit (flavanones), soya (isoflavones), apples (phlridizin) and quercetin are found in vegetables, fruits , wine and tea [3]. These are exclusively synthesized from a common intermediate like phenylalanine, or a close precursor like shikimic acid through shikimic acid pathway or phenyl propanoid pathway for the phenolic acids.

An agent that kills or inhibits the growth of microorganisms are said to be antimicrobial in nature. Food poisoning is considered as one of the most common cause of illness and death in developing countries. Most of food poisoning reports are associated with bacterial contamination especially due to the members of gram negative and gram positive bacteria. Polyphenol containing plant extracts are considered as natural sources of antimicrobial agents, regarded as nutritionally safe and easily degradable [6]. Variety of diseases and pain are being treated or cured with

different plant sources. Many of the medicinal plants are known as best remedy sources as traditional medicine worldwide. Natural products of many plants are important therapeutic agents; hence many research groups are focused in isolation and characterization of biologically important components present in plants.

Green leafy vegetables are well known in India with health promoting properties due their nutrients and chemical compounds. Diets rich in vegetables and fruits provide protection against many chronic non communicable diseases like cardiovascular diseases, obesity and cancer. Spinach (Spinacia *oleracea* L), due to its nutritional content is considered as functional food. Presence of minerals, vitamins, bioactive and phytochemicals can promote good health. Many bioactive and phytochemicals can scavenge free radicals to prevent oxidative damage; genes regulation involved in inflammation, metabolism and proliferation and reduces the carving nature for foods by stimulating satiety hormones [7]. Coriander (*Coriandrum sativum* L), is a herb with medicinal and nutritional properties. Use of coriander bioactive constituents can be used to create drug formulations to be used as pharmacological and clinical agents in treating diseases like cancer and Alzheimer [8]. As traditional medicine, Mint (*Mentha viridis)* extracts are being used as antibacterial, antispasmodic, antiseptic, to treat cold, cramps, nausea and can reduce effect of benzo[α] pyrene which is a potent lung carcinogen [9] (Table 1).

Nanotechnology involves in designing, producing and applying strategies to create structures, devices or systems at nano scale by monitoring their size and shape**.** The nano material properties depend on their size, surface and morphology [10]. Silver Nanoparticles can be successfully synthesized from Silver nitrate solution ($AgNO_3$) through a simple green and natural route from the leaves of *Ficus racemosa* using Mint extract. Nanoparticle synthesized can be proven under UV – Visible absorption spectroscopy. These silver nanoparticles are clean, rapid, ecofriendly (non –polluted), non-toxic (safety for human health) and less expensive. These can be produced from organisms ranging from bacteria to fungi and also from plants. Nanoparticles have great application in medical field like gene therapy, cancer therapy and drug delivery, etc [11]. Silver Nanoparticle has applications in electronics, chemistry, energy, medicine, physics, and material science [12]. Silver Nanoparticles are being used in wound dressing, as catheters and for various household products due to their antimicrobial activity [13]. Plant extracts (*Ficus racemosa* and Mint extract) can be used for green synthesis of silver nanoparticles by reducing silver ions to silver Nanoparticles [14]. The synthesis of silver nanoparticles from plant extracts can be indicated by conversion of the aqueous ion solution to yellow brown. The color change is due to surface plasma resonance of the silver nanoparticles. Proteins, enzymes and phenols present in the plant extract are known to cause the reduction of silver ions and hence the formation of silver nanoparticles [15,16].

The aim of this study was to extract the polyphenols from spinach, coriander and mint leaves and determine its concentration, antioxidant, antibacterial activity using silver nanoparticles and to study the effect of temperature on polyphenol content.

II. METHODOLOGY

Preparation of plant extracts

Plant extracts were prepared by weighing 1gm of leaves (spinach, coriander and mint) and ground it with a motor and pestle using 50ml of different solvents [80% Ethanol (v/v), methanol and Acetone: water (1:1)]. The infusions were filtered through muslin cloth. The collected homogenate was centrifuged at 10,000rpm for 15minutes. The pellet was discarded and the supernatant was evaporated to dryness by placing the beakers containing the supernatant on a hotplate preheated at 40-50°C. Allow the samples to evaporate. Cool the container to room temperature. To the obtained residue 5ml of distilled water was added and the residue was dissolved thoroughly. The samples were stored at low temperature until use.

Preparation of plant extracts (Low temperature)

The leaves of spinach, coriander and mint were taken in a plastic container and then placed under refrigeration (4°C) for about 10 days. On 1^{st}, 3^{rd}, 5^{th}, 7^{th} and 10^{th} day samples were removed from storage condition and extracts were prepared as mentioned earlier and the content of polyphenols was determined by Folin- Ciocalteu method.

Preparation of plant extracts (High temperature)

The leaves of spinach, coriander and mint were exposed to high temperature (100°C) on a hotplate at different time intervals (5, 10, 15, 20, 25 and 30minutes) and polyphenols were extracted and its contents were determined by Folin's Ciocalteu method.

Estimation of polyphenols by Folin- Ciocalteu method

Folin-Ciocalteu reagent is formed from a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) which, after oxidation of the phenols, is reduced to blue oxides of tungsten (W_8O_{23}) and molybdene (Mo_8O_{23}), respectively. This reaction, which occurs under alkaline conditions, is carried out with sodium carbonate. Under these conditions, the electron is easily removed from the phenol molecule. The concentration of polyphenols in plant extracts was determined using (FCR) Folin-Ciocalteu colorimetric assay method. Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent under alkaline medium to produce blue colored complex (molybdenum blue). Leaf extracts from different solvents were diluted and different aliquots was pipetted out and

made up to 3.0ml with distilled water. 0.5ml of diluted Folin – Ciocalteu reagent was added and allowed to stand for 3minutes followed by addition of 2.0ml of 20% sodium carbonate (Na_2CO_3) A Blank devoid of leaf extract was simultaneously prepared with all the above reagents. The reaction mixture was vortexed and incubated at room temperature of 60 minutes. The absorbance was recorded at 650nm. A standard Calibration curve was prepared using known concentrations of catechol (10µg/ml) which was used to determine the sample polyphenol concentration. Total Phenol content (TPC) from leaf extract was expressed in mg/gm [17].

Antioxidant activity

DPPH (2, 2 –diphenyl 1- picrylhydrazyl) is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [18]. A decrease in DPPH radical absorbance was caused due to phenolic compounds due to their radical scavenging activity by donation of hydrogen atom as observed by its change in color from purple to yellow. (Meir et al., 1995). From the fresh samples (spinach, coriander and mint) polyphenol content was determined. Different concentrations (0.1 to 0.5 mg/ml) of polyphenol from the three fresh samples (spinach, coriander and mint) were pipetted out and made upto 1.0ml with 80%methanol. To this 4ml of 0.15mM DPPH in 80% methanol solution was added. The obtained mixture was vortexed and incubated at room temperature for 30 minutes in darkness [19]. The absorbance was measured at 520nm and the percent inhibition (%I) was calculated according to the following equation:

$$\text{DPPH Scavenging effect}\quad \%\ \text{Inhibition} = [(A_0\text{-}A)/A_0] \times 100$$

Where A_0 = Absorbance of DPPH,
A = Absorbance of DPPH + Leaf extracts

Synthesis of Silver Nanoparticles (NP) using *Ficus racemosa* leaves [20]

Three different concentrations of silver nitrate solution was prepared [10mM (0.17g of $AgNO_3$), 1.0mM (0.017g of $AgNO_3$), and 0.8mM (0.014g of $AgNO_3$)]. *Ficus racemosa* leaves were collected from our college campus and 5 grams of leaves was weighed accurately. *Ficus racemosa l*eaves extract was prepared by rinsing the leaves thoroughly with glass distilled water, air dried and cut into small pieces. Place the cut leaves in a beaker containing 20ml of glass distilled water and the mixture was boiled on a hotplate for 5 minutes, cooled to room temperature and filtered through funnel plugged with muslin cloth. For Reduction of silver ions, four different aliquots were prepared 1ml of *Ficus racemosa* leaf extract was added drop wise into i) 1.0ml of 10mM $AgNO_3$ [1:1 dilution], ii) 9.0ml of 10mM $AgNO_3$ [1:10 dilution], iii) 9.0ml of 1mM $AgNO_3$, and iv) 9.0ml of 0.8mM $AgNO_3$ aqueous solution into separate test tubes and stirred continuously by maintaining the tubes at a temperature between 50^0C – 60^0C. As soon as, *Ficus* extract was added into the silver nitrate solution, there was a color change observed from light yellow to yellowish brown due to formation of silver ion complex. This is due to excitation of surface plasma resonance (SPR) which indicates the formation of silver nanoparticles. The synthesized nanoparticles by *Ficus racemosa* leaf extract was centrifuged at 5000rpm for 15minutes and subsequently dispersed in 1ml of deionized water.

Plant extract- Polyphenol extraction from Mint to synthesize Nanoparticles – 0.1g of mint leaves were weighed and placed into a 5ml of 70% methanol in a test tube and maintained at 70^0C for 10minutes to release the polyphenol and maintained at room temperature. The contents were centrifuged at 2000 rpm for 10minutes. The extraction step was repeated twice. Both extracts were pooled and the volume was adjusted to 10ml with cold 70% methanol. One ml of the mint leaf extract was diluted to 100ml with distilled water. 1ml of this diluted solution was used for analysis.

Preparation of silver nanoparticles with mint extract

1.0ml of mint extract was added to 1.5ml of 1mM $AgNO_3$ followed by 1.5ml of *Ficus racemosa* extract and the tubes were incubated at room temperature for 30 minutes for the reaction to progress.

Spectrophotometric and SEM Analysis

The nanoparticles (NP'S) synthesized with the *Ficus racemosa* with mint extract fused with silver nitrate solution were determined in a UV – Visible spectrophotometer at wavelengths ranging from 200 to 600nm. The analysis was done in duplicates. From the spectrophotometric analysis, two peaks at 200 and 430nm were obtained which indicates the surface plasma resonance (SPR) and the formation of nanoparticles. Scanning Electron Microscopy (SEM) was performed for the samples which showed peaks at 430nm.

Determination of antimicrobial activity

Bacterial cultures were inoculated in nutrient broth and were incubated for 24hours at 37^0C.These microbial cultures were used as inoculum to test the antimicrobial activity of plant (mint) extract and *Ficus racemosa* silver nanoparticles. Sterilized petri plates were poured with sterilized nutrient agar medium. Allow the medium to undergo solidification. Petri plates for bacterial culture were inoculated with 0.1ml of 24 hours microbial suspension and uniformly spread by using a spreader. The plates were left for 5 minutes. Sterile paper discs were impregnated with plant extracts and *Ficus racemosa* silver nanoparticles individually and were aseptically placed on the surface of each of the inoculated plates in a peripheral position, using a sterile forceps and pressing gently to ensure even contact with the medium surface. Tetracyclin was used as a control. The inoculated

plates were incubated at 37^0C for 24hours. The zone of inhibition was expressed in mm, as the diameter of the clear zones around the discs [20].

III. RESULTS AND DISCUSSION

Total polyphenol content in Fresh samples

This study reveals that, polyphenols can be extracted using different organic solvents based on their solubility. Polyphenol extracted from spinach using 80% ethanol was high (18.66mg/gm) than in methanol and acetone: water. The polyphenol content from Coriander was high in methanol (42.26mg/gm) followed by 80% Ethanol (28.83mg/gm) and Acetone: water (1:1) (23.9mg/gm). Extracts from fresh samples of mint were found to be high in methanol (79.23mg/gm) followed by acetone: water (62.85mg/gm) and in 80%ethanol (47.8mg/gm) (Table 2). Polyphenols extracted using methanol and Acetone: water (1:1) from coriander and mint was high whereas polyphenols extracted using ethanol was found to be maximum only in spinach.

Effect of low temperature (4^0C)

When the samples were stored at low temperature (4^0C), the content of polyphenols in spinach decreased and increased on 7^{th} in ethanolic and acetone extracts (Fig 1). Polyphenol extracted from coriander using all the three solvents showed an decrease till 7^{th} day and increased on 10^{th} (Fig 2). Similarly, the polyphenols extracted from mint leaves (Fig 3) also showed the same results. It can be observed that when the green leafy vegetables were stored for short time period at low temperature (4^0C) showed a remarkable decrease in polyphenol contents in all the extracts. A slight increase in the polyphenol contents after 7^{th} or 10^{th} day may be due to degradation of these compounds during long storage at low temperature.

Effect of high temperature (100^0C)

The study of polyphenol content at high temperature at different time intervals (5, 10, 15, 20, 25 and 30minutes) showed a marked decrease in polyphenols when compared to fresh samples. There was a remarkable decrease in polyphenol contents approximately between 50 to 75% when samples were exposed to high temperature for 30minutes. It showed that polyphenol extracted from mint had maximum loss when compared to other samples [Fig: 4, 5, 6]

Polyphenols extracted from fresh samples was found to be high but the content of polyphenols decreased when the samples were stored or exposed at low and high temperatures.

Antioxidant activity

Polyphenols extracted from Fresh samples was found to have a strong free radical scavenging activity (Antioxidant) due to high phenolic contents. As concentration of polyphenol decreased free radical scavenging activity also decrease.

The result of antioxidant activity of plant extracts was due to inhibitory effects of these extracts against DPPH stable free radical. The IC_{50} concentration of ethanolic, methanolic and acetone: water extracts as well as ascorbic acid (positive control) were determined and expressed as mg/ml. The percent of inhibition at 0.5mg/ml concentration of different extracts was found to be 34.5% in ethanolic extract of spinach, methanolic extract (39.53%) [Fig: 7] and ethanolic extract (25.58%) of coriander [Fig: 8] and for mint methanol (49.25%), ethanol (37.2%) and acetone: water (44.1%) whereas ascorbic acid (positive control) it was 14.56% [Fig: 9]. The extracts that perform the highest antioxidant activity had the highest concentration of phenols. Plants with high phenol content can exhibit scavenging activity due to their hydroxyl groups. From the above results it shows that, mint extracted using all the three solvents showed maximum antioxidant activity. It can be observed that, the sample that showed high phenol content only showed high antioxidant activity. In few extracts like acetone and methanolic extracts from spinach and acetone extract of coriander antioxidant activity were not determined as the polyphenol content was less.

Synthesis of Silver Nanoparticles

Silver Nanoparticles were synthesized using *Ficus racemosa leaves* with methanolic extract of polyphenol from *Mentha viridis*, and its absorbance was measured spectrophotometrically between 200-600nm and its maximum absorbance was found to be at 430nm with 10mM $AgNO_3$[Fig: 10].

SEM Analysis

The size of the prepared Silver nanoparticles was analyzed using SEM and the particle size ranged from 56.7 to 96.4nm [Fig: 11.1 and 11.2].

Antibacterial activity

By using paper disc method, antimicrobial activity was compared between polyphenol extracted from *Mentha viridis* (fresh sample) and *Ficus racemosa* silver Nanoparticles. The largest inhibition zone was 10mm against gram negative bacteria (Fig 12) with 1:1 diluted *F. racemosa* with 10mM of $AgNO_3$ along with mint extract whereas 1:10 dilution samples did not show antimicrobial activity. Tetracyclin was used as control. Polyphenols from fresh samples did not show zone of inhibition against gram positive and negative bacteria. By comparing the results it can be observed that mint extract in nanoparticles were having anti-microbial activity than fresh samples.

Polyphenols can be extracted using different organic solvents and methanol showed maximum extraction when compared to 80%ethanol and acetone: water. High

Temperature drastically decreased polyphenol contents when compared to fresh samples whereas at low temperature, the contents decreased initially but increased after 10th day. DPPH is one of the best stable compound for determination of free radical scavenging activity for polyphenols. The study showed that there was free radical scavenging activity of polyphenols extracted from three samples, but absorbance were not determined in samples with less concentration. Many studies have reported the preparation of nanoparticles using *Ficus* species, this study also shows the standardized protocol for its preparation. The prepared nanoparticles were stable and showed absorbance maxima at 200 and 430nm, but the absorbance was between 2.0 and 3.0 even after dilution of extracts. So, a procedure for absorbance of NP less than 1.0 has to be standardized. Using these nanoparticles there was a slight increase in antimicrobial activity when compared to fresh extracts.

IV. CONCLUSION AND FUTURE SCOPE

Consumers usually purchase a fresh product driven by their visual appearance, while other components like quality, texture and aroma make the consumers to re-purchase again the same product. Natural plant sources rich in polyphenol content are correlated to have high antioxidant activity but number of days stored and exposure to high temperature (cooking) for long duration may decrease the polyphenol content leading to decreased antioxidant activity. So, the exposure of natural food sources either to low or high temperatures are having a drastic effect on the polyphenol contents. Consumption of foods close to their natural forms may retain their contents intact and when consumed can reduce diseases related to various factors. Mint has the highest polyphenols content with high free radical scavenging activity, which enables them to be used in various foods, pharmaceutical and other industries. Presently there is an increase in the number of diseases related to cardiovascular, nervous, renal dysfunction and other health issues due to stress and life style. So regular consumption of foods enriched with natural antioxidants can decrease the effect of free radicals. The biosynthesized SNP (Silver Nanoparticles) synthesized with *Ficus racemosa* leaves were stable are very promising as antimicrobial agents against gram negative bacteria. SNPs are environment friendly and cost effective. The future scope is to study the components of mint and to characterize the *F. racemosa* leaves so that it can help in the synthesis of nanoparticles that may be focused to apply in various fields.

Table 1. Uses of some commonly used plant sources as food

Botanical names	Common names	Family	Uses
Spinacia oleracia	Spinach	Amaranthaceae	1) To Scavenge reactive oxygen species (ROS) and to prevent macromolecular oxidative damage. 2) Curb food intake by inducing secretion of satiety hormones.
Coriandrum sativum	Coriander / Cilantro	Apiacea (umbelliferae)	1) Nutrition, medicine, beverages, flavouring agents, cosmetics and in industries. 2) It has biological activities like antimicrobial, antioxidant, hypoglycemic, anti-inflammatory and anti-convulsant and others.
Mentha Viridis	Mint , green mint	Lamiaceae	1) The organic compounds and menthol present in the spearmint acts as a natural antibacterial and antimicrobial helps to protect mouth and throat from infections. 2) It also provides dental and gum health It prevents halitosis (bad Breath) caused by bacteria below gums, and keeps the person healthy and great smelling.

Table 2. Polyphenol content

Solvent system	Total Polyphenol Content (mg/gm)		
	Spinach	Coriander	Mint
80% Ethanol	18.66	28.83	47.8
Methanol	8.19	42.26	79.23
Acetone:Water	5.238	23.9	62.85

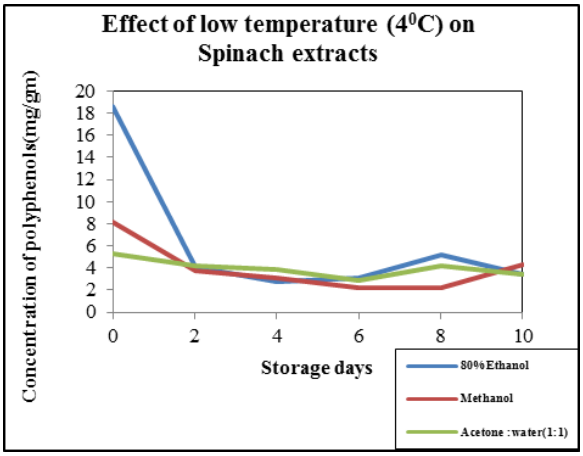


Figure 1. Change in total polyphenol content of spinach at low temperature (4⁰C)

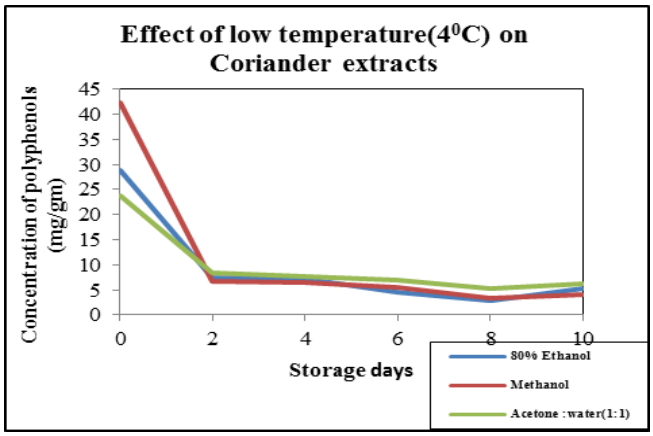


Figure 2 Change in total polyphenol content of coriander at low temperature (4^0C)

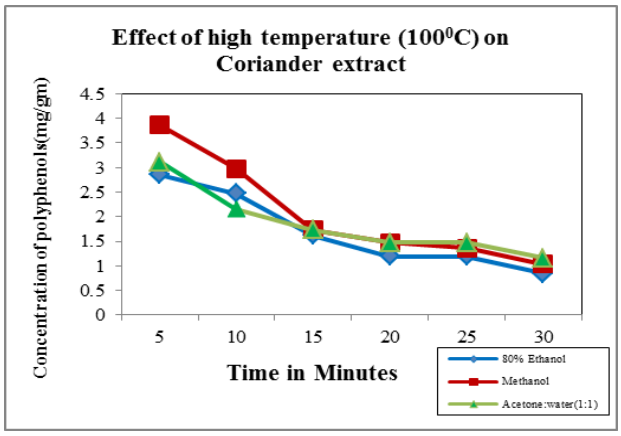


Figure 5. Change in total polyphenol content of Coriander at high temperature (100^0C)

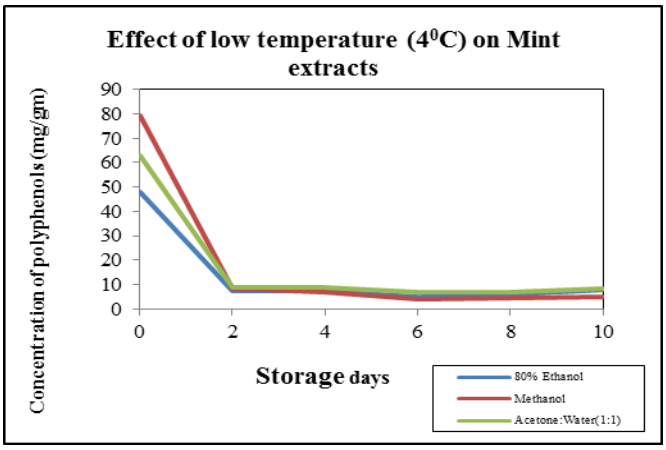


Figure 3. Change in total polyphenol content of Mint extract at low temperature (4^0C)

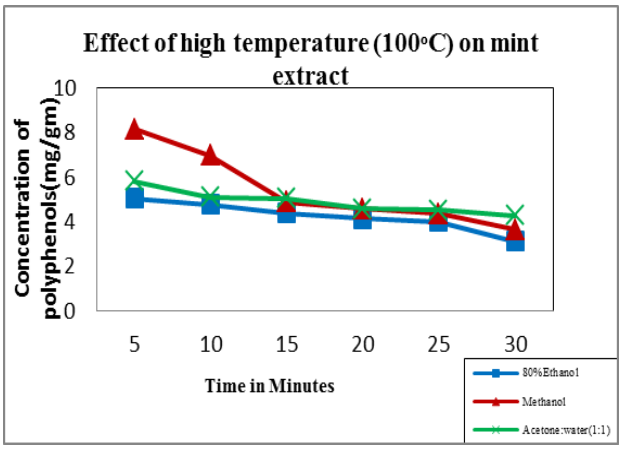


Figure 6. Change in total polyphenol content of Mint at high temperature (100^0C)

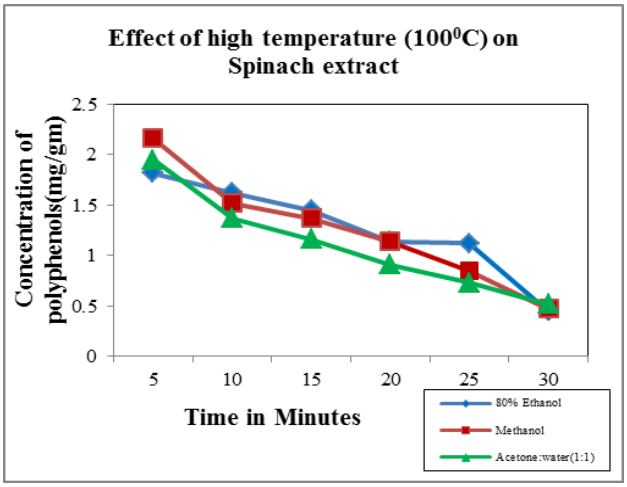


Figure 4. Change in total polyphenol content of spinach at high temperature (100^0C)

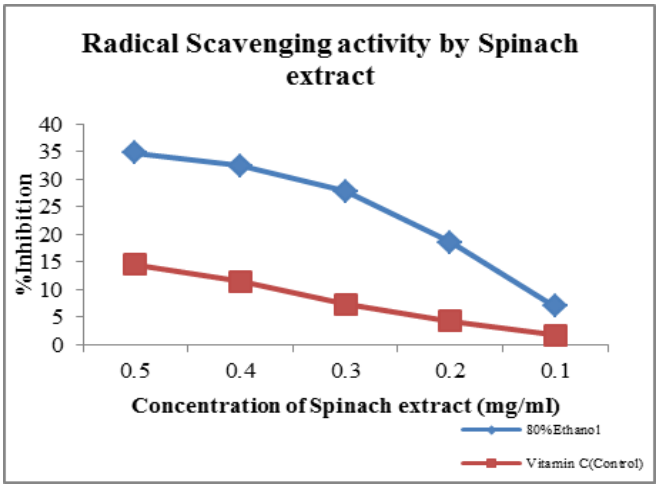


Figure 7. Radical Scavenging activity of Spinach extract

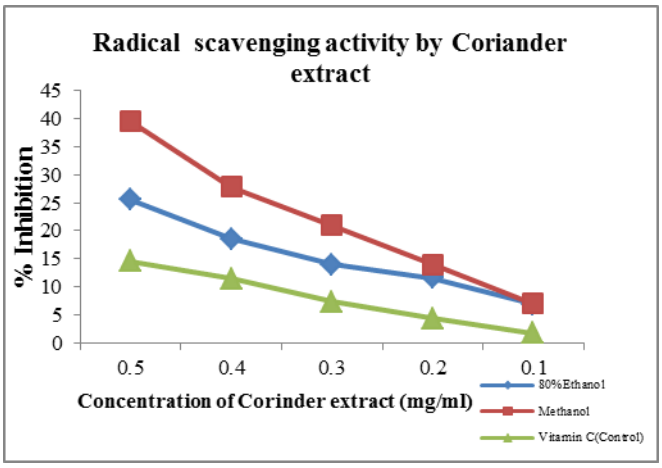


Figure 8. Radical Scavenging activity of Coriander extract

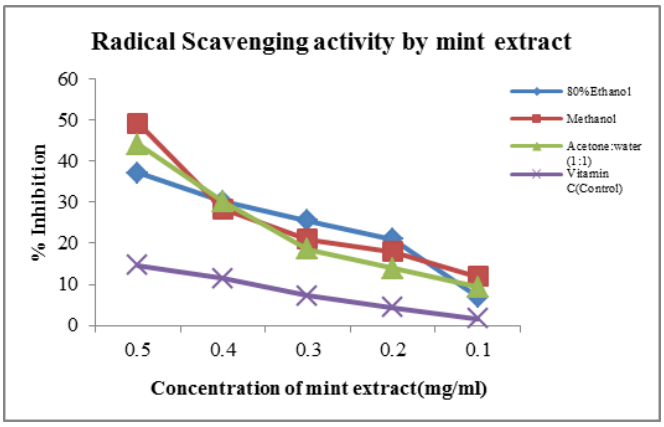


Figure 9. Radical Scavenging activity of Mint extract

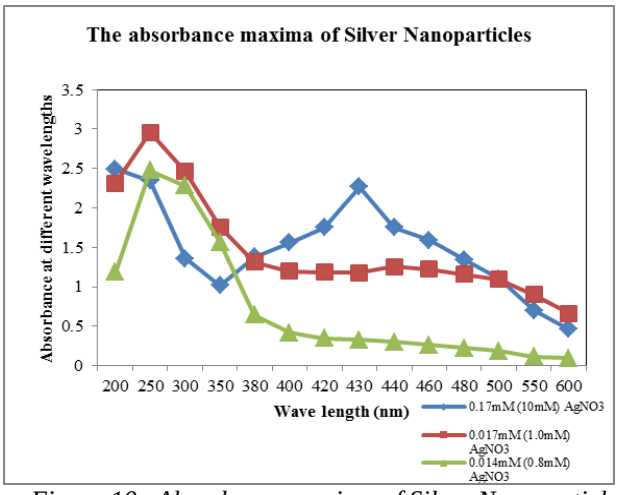
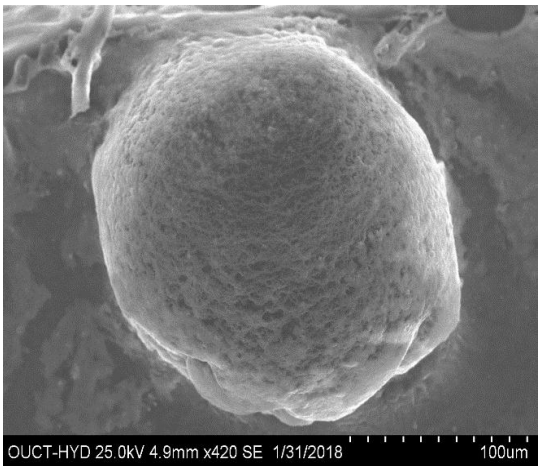


Figure 10. Absorbance maxima of Silver Nanoparticles



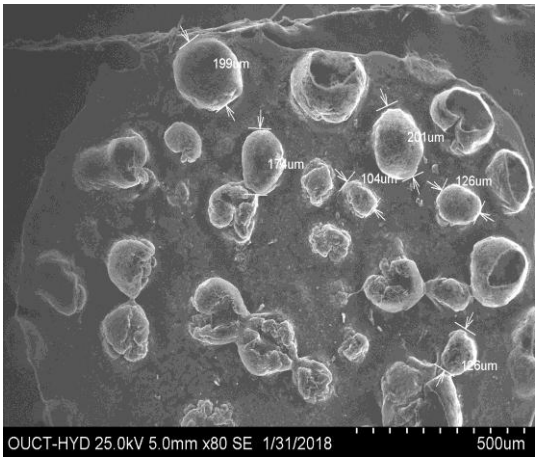
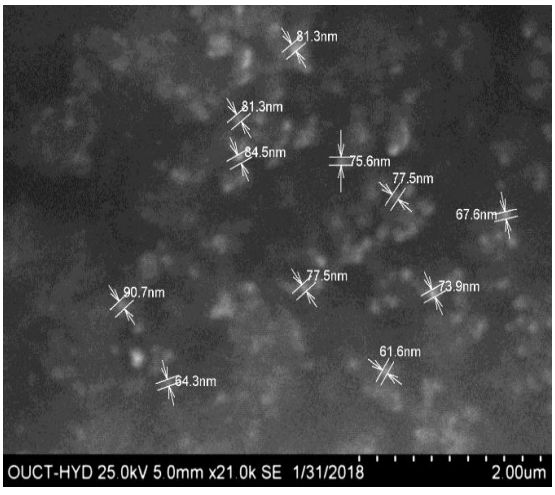


Figure 11.1 Ficus racemosa Silver Nanoparticles (1:10Ficus: $AgNO_3$)



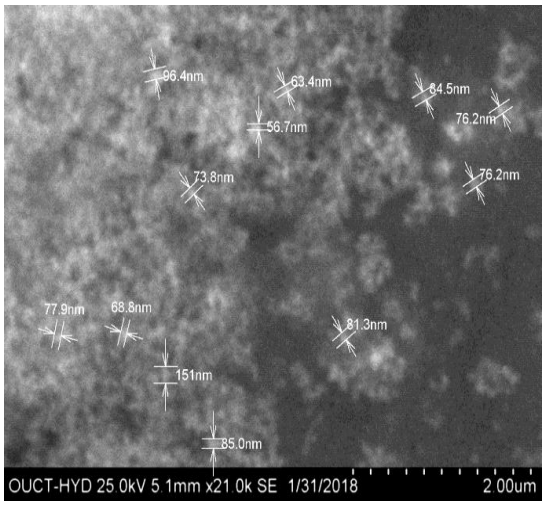


Figure 11. 2 Ficus racemosa with Mint extract Silver Nanoparticles (1:1 Ficus:$AgNO_3$)

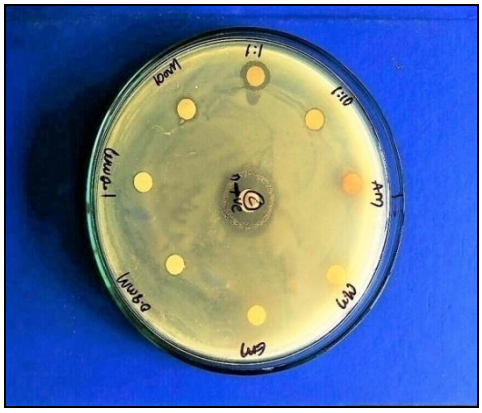


Figure 12. Antimicrobial Activity(AM-Acetone+mint, MM-Methanol+mint, EM-Ethanol + mint, 0.8,1.0,10mM of $AgNO_3$,1:1 and 1:10 are F. racemosa +$AgNO_3$ with mint)

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