Journal of Environmental Studies [JES]
*An International Journal*
https://jesj.journals.ekb.eg/
**doi:** *10.21608/jesj.2025.344766.1110*

# Phytochemistry, Allelopathy And Anticancer Potentiality of *Melaleuca alternifolia* (Maiden and Betche) Cheel and *Psidium guajava* L. (Myrtaceae)

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**Citation:** *El-Darier, S.M; Mabrouk, M.E; Zabady, N.A; Khattab,K.A (2025). Phytochemistry, Allelopathy And Anticancer Potentiality of Melaleuca alternifolia (Maiden and Betche) Cheel and Psidium guajava L. (Myrtaceae). Journal of Environmental Studies, Vol. 38(1): 10-23.*

**Article Information**

Received 15Decem. 2024,
Revised 20 Feb. 2025,
Accepted 23 Feb. 2025,
Published online. 1 June 2025

**Abstract:** *Melaleuca alternifolia* leaves ethanolic extract exert highest detection (++) of tannins, steroids, flavonoids and alkaloids. *Psidium guajava* ethanolic extract achieved strong detection (+++) of phenolics and flavonoids, while aqueous extract revealed high detection (++) of flavonoids. In petri dish experiment *P. guajava* leaves aqueous extract exert great inhibition effect on growth parameters of *Rumex dentatus*, *Solanum lycopersicum* (geaara 023) and *Solanum lycopersicum* (tomato extracted seed) under (control, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5%) concentration levels compared to *M. alternifolia* leaves aqueous extract. Pot experiment prepared to determine vegetative seedling storage protein profiling that showed a great decrease in genome template stability GTS (%) of *R. dentatus* under the effect of *P. guajava* leaves aqueous extract, however GTS (%) of *S. lycopersicum* (geaara 023) exert a great increase compared to *S. lycopersicum* (extracted seed) as crops under the effect of *M. alternifolia* leaves aqueous extract. Energy dispersive x-ray spectroscopy of *M. alternifolia* and *P. guajava* revealed that *M. alternifolia* leaves has high concentration levels of Cl, Ca, Al and Cu elements, whereas *P. guajava* leaves exert high concentration levels of Si, S, Fe and K elements. *M. alternifolia* ethanolic extract has a significant effect on lung (A549) and breast carcinoma (MCF7) cell lines with IC50 of about 31 and 98.9 µg/ml respectively. On the other hand, *P. guajava* ethanolic extract showed a significant effect on lung (A549) and prostate carcinoma (PC3) cell lines of about IC50 of 40 and 50.5 µg/ml respectively.

**Keywords:** Cell line, Carcinoma, Phytotherapy, Electrophoresis, Allelochemicals.

## Introduction

Myrtaceae family one of the most ecologically significant group of angiosperms that includes trees and shrubs this family comprises approximately of 140 genera and between 3800 and 5650 species valued for their edible fruits and use in traditional medicine (Mitra *et al*., 2012; Saber *et al*., 2023). *Melaleuca alternifolia* (tea tree) known for its medicinal values in treating wounds, fungal infections, sore throats and skin ailments due to their high levels of terpene hydrocarbon contents (Shah *et al*., 2019; Kairey *et al*., 2023). *P. guajava* (guava) valued for its nutritional and medicinal benefits, is used to manage stomach aches, diabetes, and diarrhea due to high levels of saponins, quercetin, flavonoids, terpenes, and tannins (Kumar *et al*., 2021; Liu *et al*., 2024).

Allelopathy has inhibitory and stimulatory effects in all plant processes such as seed germination, growth parameters and weed management by releasing some phytochemicals (Rice, 1984; Bachheti *et al*., 2020).

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Allelopathy is also considered one of the indirect factors of regular cropping difficulties in the agriculture sector, so recently agricultural production management plans and ecological restoration depending on applications of allelopathy (Cheng & Cheng, 2015). *M. alternifolia* aqueous extracts have shown inhibitory effects on root growth in *Brachiaria brizantha* (Queiroz *et al*., 2017) and have enhanced stress tolerance in crops (Yasin *et al*., 2021). Likewise, *P. guajava* has demonstrated allelopathic effects, such as weed control (Kapoor *et al*., 2019; Mabele & Ndong, 2019).

Cancer, defined as a complex disease arising from accumulated genetic mutations (Kumar *et al*., 2021). *M. alternifolia* shows anticancer activity against prostate and breast cancer cell lines (Clark *et al*., 2021). *P. guajava* extracts possess antiprostate cancer properties and contain pigments with antioxidant functions that may aid in cancer prevention during DNA repair, gene regulation and apoptosis (Chen *et al*., 2010; Nalkıran & Nalkıran, 2024).

The present study is an attempt to maximize the ecological and medicinal benefits such as allelopathic interactions, antibacterial and anti-cancer potentiality of *Melaleuca alternifolia* and *Psidium guajava* with a view to assessing their contribution to human livelihood.

## Materials and Methods

### Collection of Melaleuca alternifolia and Psidium guajava Leaves

Mature green leaves of both *M. alternifolia and P. guajava* were collected from El- Beheira Governorate, Egypt during Summer season. *M. alternifolia* collected from Karam nursery (Damanhour City) at 31°2'47" N and 30°28'14" E, while *P. guajava* were collected from Mahalla Nasr Village (Shubrakhit Center) at 31°01′39″ N and 30°42′46″ E.

### Preparation of Aqueous and Ethanolic Extracts of M. alternifolia and P. guajava Leaves

Collected *M. alternifolia and P. guajava* mature green leaves were washed with tap water, then with distilled water for further cleaning and dried in an electric oven at 45° C. The dried leaves were ground to a fine powder. 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 g were transferred to labeled bottles, and then 100 mL of distilled water were added to each bottle. The mixture was shaken then the bottles were left for 48 hours at refrigerator and then filtered through very fine mesh and pressed carefully for full extraction to get extracts of 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5%, the control (C) was (distilled water) (El-Rokiek *et al*., 2024), while ethanolic extract was prepared according to Sridhar *et al*. (2016).

### Germination Bioassay

Petri-dish experiment was applied to investigate the potential allelopathic effects of *P. guajava* and *M. alternifolia* on germination percentage (GP), radicle (RL) and plumule (PL) lengths of *R. dentatus*, *S. lycopersicum* (geaara 023) and *S. lycopersicum* (tomato extracted seed) as recipient species in pure culture. To accomplish this experiment 10 seeds of each recipient species were arranged in 9-cm diameter petri-dishes separately on disc of whatman No.1 filter paper under normal laboratory conditions. 10 ml of *P. guajava* and *M. alternifolia* leaves aqueous extract at Control, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5% and 5.0% were added daily to three replicates for thirteen days. The experiment was performed under normal laboratory conditions (20±2º C temperature, 75±2% relative humidity, and 14/10 h/dark photoperiod).

### Pot Experiment

Pot growth experiment was performed to test the allelopathic effect of 0.5%, 1.5% and 3.5% of *P. guajava* and *M. alternifolia* leaves aqueous extract on three replicates of *R. dentatus*, *S. lycopersicum* (geaara 023) and *S. lycopersicum* (tomato extracted seed) for three weeks with sandy clay soil (500 g in each pot) on seedling protein electrophoresis as molecular marker. The experiment was performed under normal laboratory conditions (20±2º C temperature, 75±2% relative humidity, and 14/10 h/dark photoperiod).

### Seedling Protein Electrophoresis

For assessing the allelopathic effect of *P. guajava and M. alternifolia* leaves aqueous extract at (0.5, 1.5 and 3.5) % concentration levels on protein content of *R. dentatus*, *S. lycopersicum* (geaara 023) and *S. lycopersicum* (tomato extracted seed) seedling compared to the effect of control (C) (distilled water), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using discontinuous buffer system according to Laemmli (1970).

### Phytochemical Screening

Phytochemical screening of *P. guajava* and *M. alternifolia* leaves was analyzed in order to estimate the presence of carbohydrates, phenolic compounds, saponins, protein and amino acid (Rajendrabhai, 2017), vitamin C (Kumar *et al*., 2011), tannins (Ramya *et al*., 2019), alkaloids (Khalifa *et al*., 2017), flavonoids (Baoduy *et al*., 2015), phlobatannins and steroids (Ejikeme *et al*., 2014). Total phenols, proteins and amino acids contents were determined by high

performance liquid chromatographic (HPLC). The quantity content of fatty acids and carbohydrates in the plant samples were performed by gas chromatography-mass spectrometry (GC-MS) using Agilent 6890N with mass detector 5973 inert and Agilent Technologies 6890 gas chromatograph (USA) with mass spectrometry detector 5973 and capillary column, respectively.

### Estimation Of Anti-Proliferative Activity

Three replicates of plant ethanolic extraction for cell line test (In Vitro) were determined by weighting 200 g of powdered sample of both *P. guajava* and *M. alternifolia* leaves were macerated in one liter of 99.6% denatured ethanol (1 Liter) for 24 hour at room temperature. The total volumes of solution were filtered then dried using rotary evaporator (buchirotavapor R114, Switzerland) under reduced pressure (Sergazy *et al*., 2021).

### Measurement Of Potential Cytotoxicity By Sulforhodamine B (SRB) Assay

Three replicates of *P. guajava* and *M. alternifolia* leaves ethanolic extracts were tested using the method of Skehan *et al*. (1990) at the National Cancer Institute, Cairo, Egypt.

### Five Human Cancer Cell Lines Were Used In The Current Study

HCT (Colon carcinoma), A549 (Lung carcinoma), Hep-G2 (Liver hepatocellular carcinoma), MCF7 (Breast carcinoma) and PC3 (Prostate carcinoma). Surviving fractions of cells throughout drug exposure was characterized graphically by $IC_{50}$ values (drug concentration that yields 50% less cells than the drug-free control (Mothana *et al*., 2009; Fithrotunnisa *et al*., 2020), while Growth Inhibition Percentage (GIP) was calculated according Mosmann (1983).

### Energy Dispersive X-ray Spectroscopy

*M. alternifolia* and *P. guajava* leaves were mounted onto a stub with double-sided adhesive tape to determine the elements in *M. alternifolia* and *P. guajava* leaves with JEOL JSM-5300 SEM EDS at Faculty of Science, Alexandria University by using method of Scimeca *et al*., (2018).

### Statistical Analysis

Some data of the present study were subjected to standard one way analysis of variance (ANOVA) using CoStat 6.303 (1998-2004) statistical analysis software manufactured by CoHort Software Company.

1- Germination percentage (GP)

Germination percentage (GP) = (Number of germinated seeds/total number of seeds) X 100

2- Inhibition percentage (IP)

The inhibition in seed germination as affected upon applying donor species extracts was calculated according to the formula of Cayuela *et al*. (2007).

Inhibition percentage (IP)=

[1- (allelopathic/control) 100]

3- Mean Germination Time (MGT)

Mean germination time (MGT) was calculated according to the equation of Battle & Whittington (1969).

$MGT = \Sigma (G \times T) / F$

Where,

T = the day on which germination count was made, G = the number of seeds germinated on the day of the count, F = final number of seeds which germinated in each replicate.

4- Seed Germination Index (SGI)

Seed germination index (SGI) was calculated according to the equation of Scott *et al*. (1984).

$SGI = \Sigma Ti\ Ni/S$

Where,

Ti = is the number of days after sowing, Ni = is the number of seeds germinated on day I and S = is the total number of seeds tested.

5- Seedling Vigor Index (SVI)

Seedling vigour index (SVI) was calculated according to the equation of Islam *et al*. (2009) and Elouaer & Hannachi (2012).

SVI= [Seedling length (cm) x germination percentage]/100

## Results

### Phytochemical Screening

Phytochemical screening of *M. alternifolia* and *P. guajava* leaves represented in Table 1. and Table 2. respectively.

**Table 1.** Qualitative analysis of phytochemical constituents of *Melaleuca alternifolia* leaves.

| Phytochemical classes | Tannins | Steroids | Flavonoids | Alkaloids | Carbohydrates | Glycosides |
|---|---|---|---|---|---|---|
| Aqueous extract | + | + | + | ++ | + | - |
| Ethanol extract | ++ | + | ++ | ++ | + | + |

+: Detected; ++: highly detected ; -: Not detected.

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**Table 2:** Qualitative analysis of phytochemical constituents of *Psidium guajava* leaves.

| Chemical Constituents | Aqueous | Ethanol | Chemical Constituents | Aqueous | Ethanol |
|---|---|---|---|---|---|
| Carbohydrates | + | ++ | Alkaloids | + | ++ |
| Protein | - | + | Flavonoids | ++ | +++ |
| Amino acid | + | + | Phlobatannins | + | + |
| Vitamin C | + | ++ | Steroids | + | - |
| Chloride | - | + | Phenolic compounds | + | +++ |
| Tannins | + | ++ | Saponins | - | - |

+++: Strongly positive; ++: positive; +: Trace; -: Not detected.

## Allelopathic Experiment

### A) Germination Bioassay Experiment

Data concerning germination percentage (GP), radicle length (RL), plumule length (PL), inhibition percentage (IP), mean germination time (MGT), seed germination index (SGI), seedling vigor index (SVI) of *R. dentatus*, *S. lycopersicum* (tomato extracted seeds) and *S. lycopersicum* (geaara 023) seeds as a recipient species that affected by *M. alternifolia* and *P. guajava* leaves aqueous extract are illustrated and statistically represented in Figure 1., Figure 2. and Figure 3. respectively.

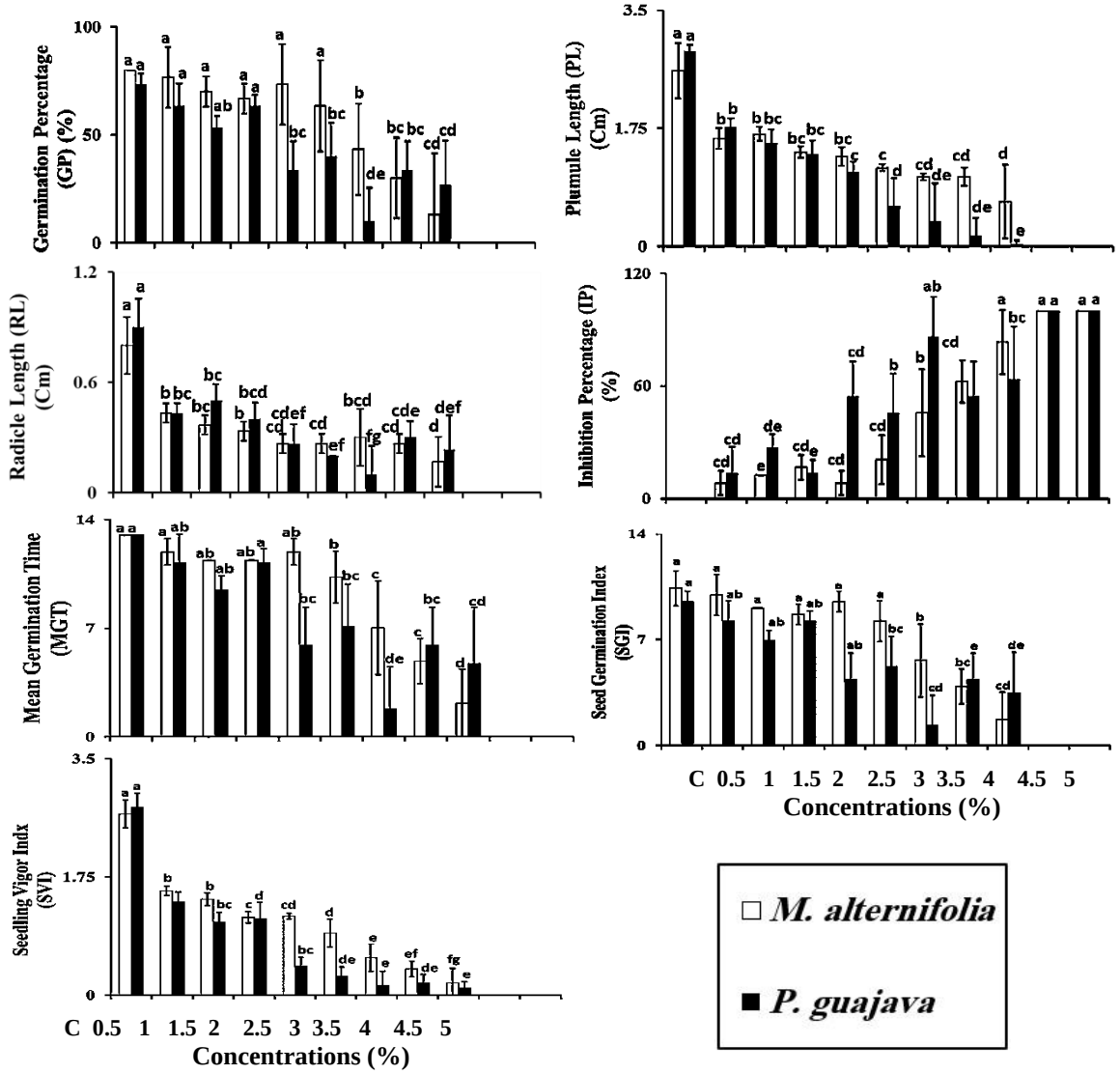


**Figure 1:** Variation in germination percentage (GP), radicle length (RL), plumule length (PL), Inhibition percentage (IP), Mean Germination Time (MGT), Seed germination index (SGI), Seedling vigour index (SVI) of *R. dentatus* seeds affected by *M. alternifolia* and *P. guajava* leaves aqueous extract. Different letters within each column indicate a significant difference at $p < 0.05$ according to one way ANOVA test. Error bars indicate standard error of means

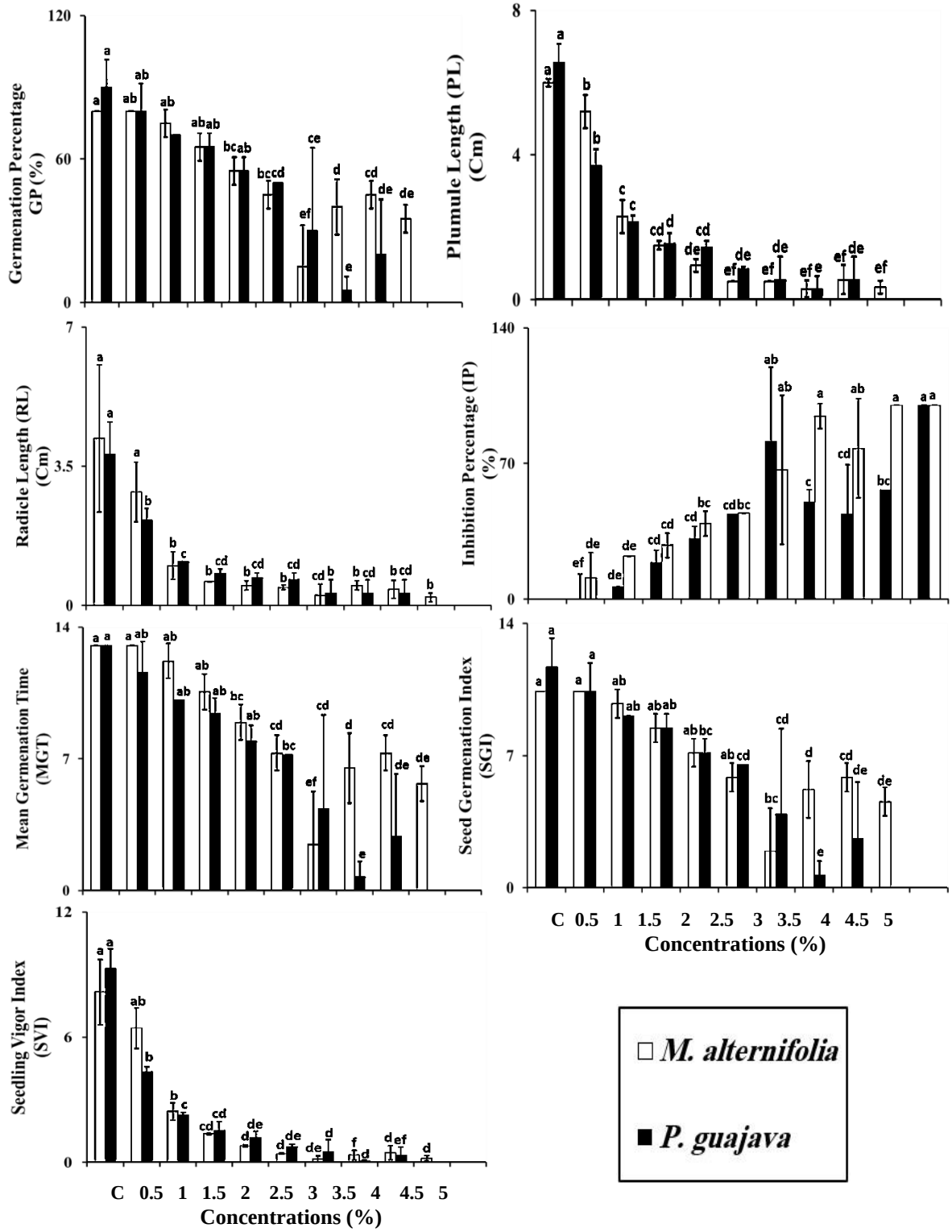


**Figure 2:** Variation in germination percentage (GP), radicle length (RL), plumule length (PL), Inhibition percentage (IP), Mean Germination Time (MGT), Seed germination index (SGI), Seedling vigour index (SVI) of *S. lycopersicum* (tomato extracted seed) seeds affected by *M. alternifolia* and *P. guajava* leaves aqueous extract. Different letters within each column indicate a significant difference at $p < 0.05$ according to one way ANOVA test. Error bars indicate standard error of means.

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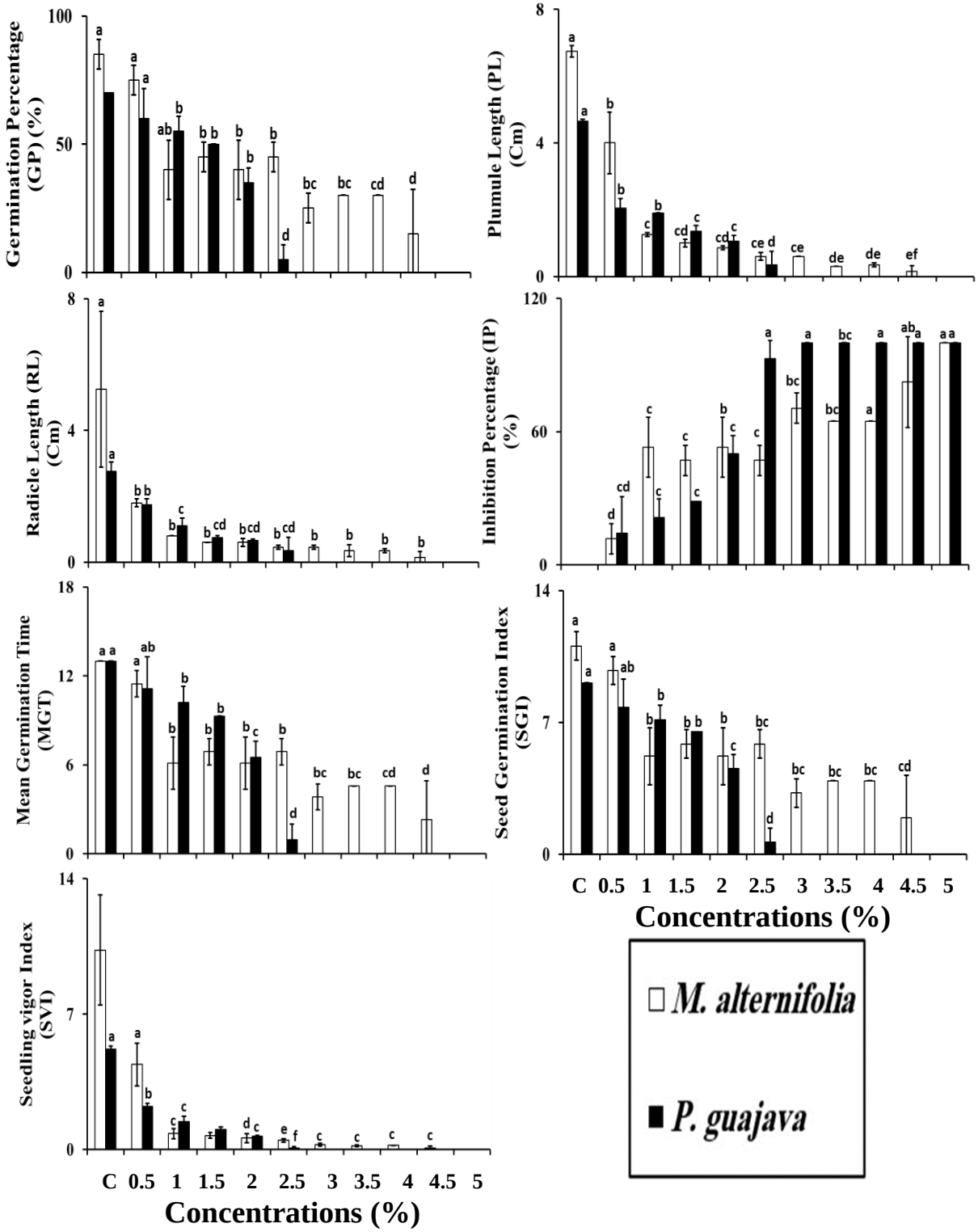



**Figure 3:** Variation in germination percentage (GP), radicle length (RL), plumule length (PL), Inhibition percentage (IP), Mean Germination Time (MGT), Seed germination index (SGI), Seedling vigour index (SVI) of *S. lycopersicum* (geaara 023) seeds affected by *M. alternifolia* and *P. guajava* leaves aqueous extract. Different letters within each column indicate a significant difference at $p < 0.05$ according to one way ANOVA test. Error bars indicate standard error of means.

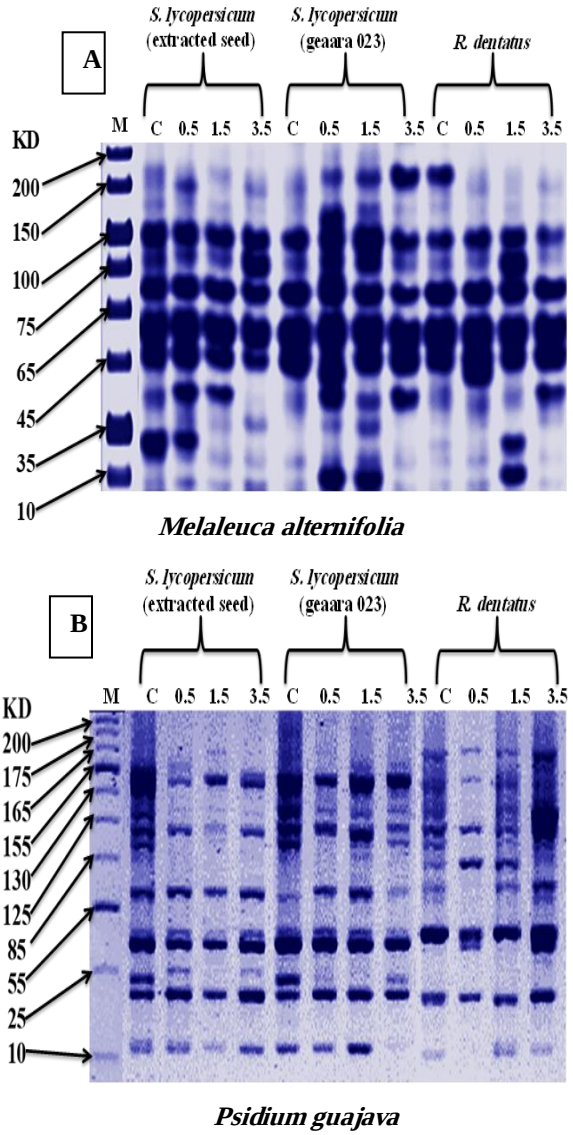
## B) Pot Experiment

### Seedling Protein Electrophoresis

The electrophenograms produced by SDS-PAGE (SDS-Polyacrylamide gel electrophoresis) of seedling proteins of *R. dentatus*, *S. lycopersicum* (extracted seed) and *S. lycopersicum* (geaara 023) affected by 0.5%, 1.5% and 3.5% of *M. alternifolia* leaves aqueous extract compared to control revealed a total of 22 bands come across all studied samples. The number of bands at 0.5% concentration level recorded values of about (12, 13 and 16), therefore the values varied to (12, 13 and 13) at 1.5% concentration level and lastly recorded values of about (14, 11 and 11) at 3.5%, compared to values of about (11, 13 and 13) at control level related to *R. dentatus*, *S. lycopersicum* (extracted seed) and *S. lycopersicum* (geaara 023) respectively. Protein profile exerts 3 common bands and absence of specific bands. The frequency of polymorphism at 0.5% concentration level recorded values of about (8% , 8% and 56%), while the values changed to (58%, 21% and 31%) at 1.5% concentration level and finally recorded values of about (43%, 36% and 27%) at 3.5% concentration level, compared to values of about (27%, 38% and 25%) at control level related to *R. dentatus*, *S. lycopersicum* (extracted seed) and *S. lycopersicum* (geaara 023) correspondingly Plate 1. A.

The electrophenograms produced by SDS-PAGE (SDS-Polyacrylamide gel electrophoresis) of seedling proteins of *R. dentatus*, *S. lycopersicum* (extracted seed) and *S. lycopersicum* (geaara 023) affected by 0.5%, 1.5% and 3.5% of *P. guajava* leaves aqueous extract compared to control showed a total of 18 bands come across all studied samples. The number of bands at 0.5% concentration level noted values of about (7, 10 and 9), therefore the values changed to (10, 7 and 13) at 1.5% concentration level and lastly recorded values of about (14, 11 and 10) at 3.5%, compared to values of about (11, 13 and 13) at control level related to *R. dentatus*, *S. lycopersicum* (extracted seed) and *S. lycopersicum* (geaara 023) respectively. Protein profile exerts 2 common bands and absence of specific bands. The frequency of polymorphism recorded null values at 0.5% concentration level, at 1.5% concentration level the frequency of polymorphism recorded values of about (20%, 14% and 23%) and finally recorded values of about (29%, 18% and 0%) at 3.5% concentration level, compared to values of about (0%, 25% and 14%) at control level related to *R. dentatus*, *S. lycopersicum* (extracted seed) and *S. lycopersicum* (geaara 023) respectively Plate 1. B.

**Plate 1:** Seedling protein electrophoresis attained from the seedling of R. dentatus, S. lycopersicum (tomato extracted seed) and S. lycopersicum (geaara 023) affected by 0.5%, 1.5% and 3.% concentration of A: Melaleuca alternifolia and B: Psidium guajava leaves.




## Energy Dispersive X-ray Spectroscopy (EDS)

The analysis of *M. alternifolia* and *P. guajava* leaves was performed by using EDS method **Figure 4**. Ten elements were identified; C, O, Al, Si, S, Cl, K, Ca, Fe and Cu. The identified elements in *M. alternifolia* were detected in in the following order C > O > Cl > Ca > K > Al > Cu > S > Fe > Si of about 70.1, 27.7, 0.63, 0.38, 0.32, 0.14, 0.13, 0.1, 0.07 and 0.06 respectively, while the identified elements in *P. guajava* revealed the following order C > O > Si > K > S > Fe > Al > Cu > Cl > Ca of about 67.9, 30.4, 0.5, 0.29, 0.2, 0.15, 0.11, 0.1, 0.09 and 0.08 correspondingly.

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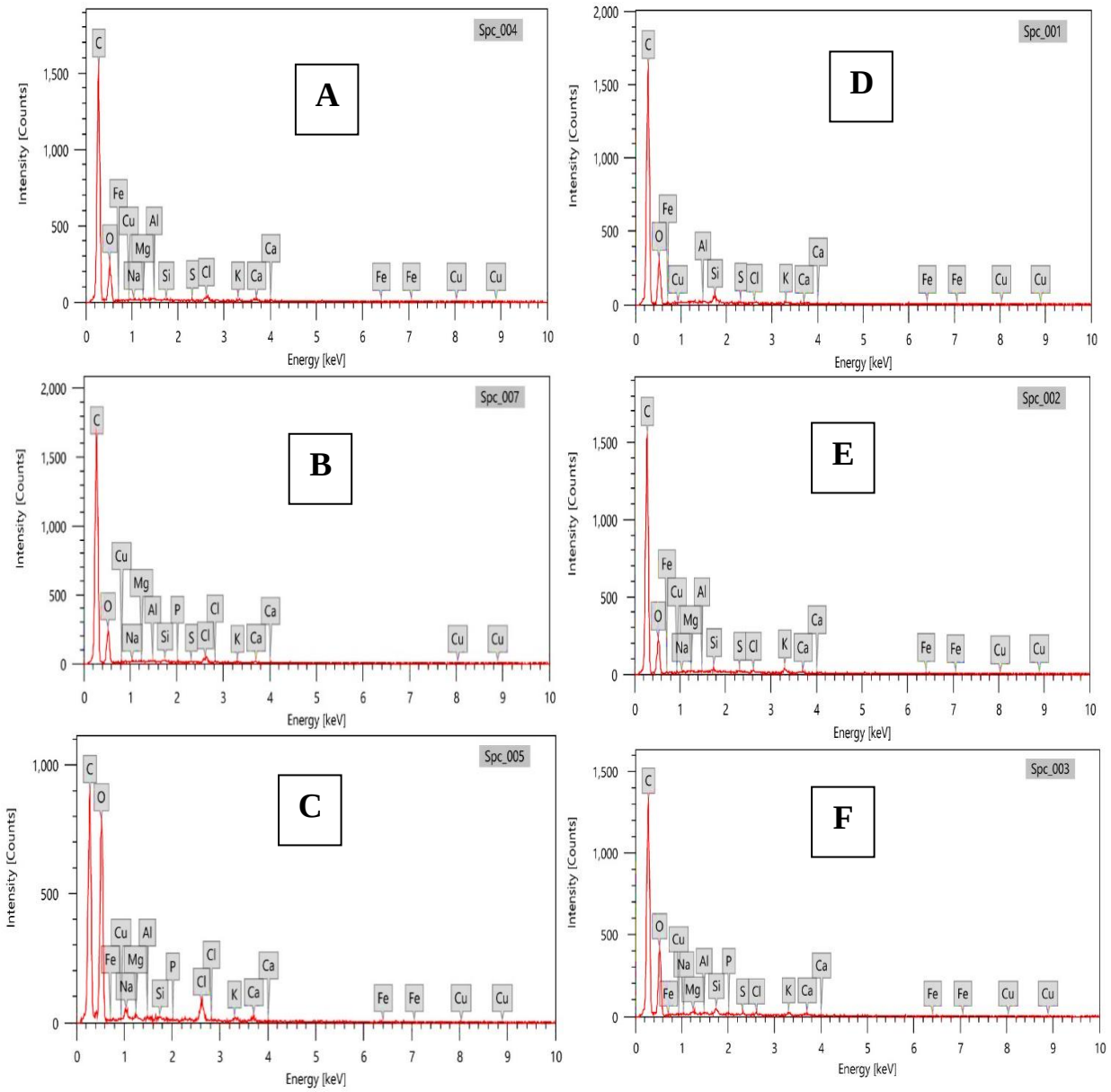


**Figure 4:** Analysis elements of *M. alternifolia* and *P. guajava* leaves by using Energy Dispersive X-ray Spectroscopy (EDS) method: (a-c) *M. alternifolia*; (d-f) *P. guajava*.

## Estimation of Anti-Proliferated Activity

The *in vitro* cytotoxic activity of *P. guajava* and *M. alternifolia* leaves ethanolic extract were determined on HCT (Colon carcinoma), A549 (Lung carcinoma), Hep-G2 (Liver hepatocellular carcinoma), MCF 7 (Breast carcinoma), and PC3 (Prostate carcinoma) carcinoma cell lines. The half-maximal inhibitory concentration IC50 value (concentration of active compound needed to reduce the cell viability to 50%) was determined from dose-response curves of percent growth inhibition against test concentrations. To assess the toxicity of the extracts, each of the five cell lines was treated with four different concentrations of *P. guajava* and *M. alternifolia*. Visual observations indicated that the viability of cancer cell lines was increasingly reduced while the concentrations of theextract were increased. *M. alternifolia* showed a significant effect on lung (**A549**) and **MCF 7** (Breastcarcinoma) cell lines with IC50 of 31 and 98.9 µg/ml respectively. While HCT (Colon carcinoma), **Hep-G2** (Liver hepatocellular carcinoma) and PC3(Prostate carcinoma) attained no effect of IC50. *P. guajava* showed a significant effect on lung (**A549**) and PC3 (Prostate carcinoma) cell lines with IC50 of 40 and 50.5 µg/ml respectively. While HCT (Colon carcinoma), Hep-G2 (Liver hepatocellular carcinoma) and MCF 7 (Breast carcinoma) attained no effect of IC50 Figure 5.

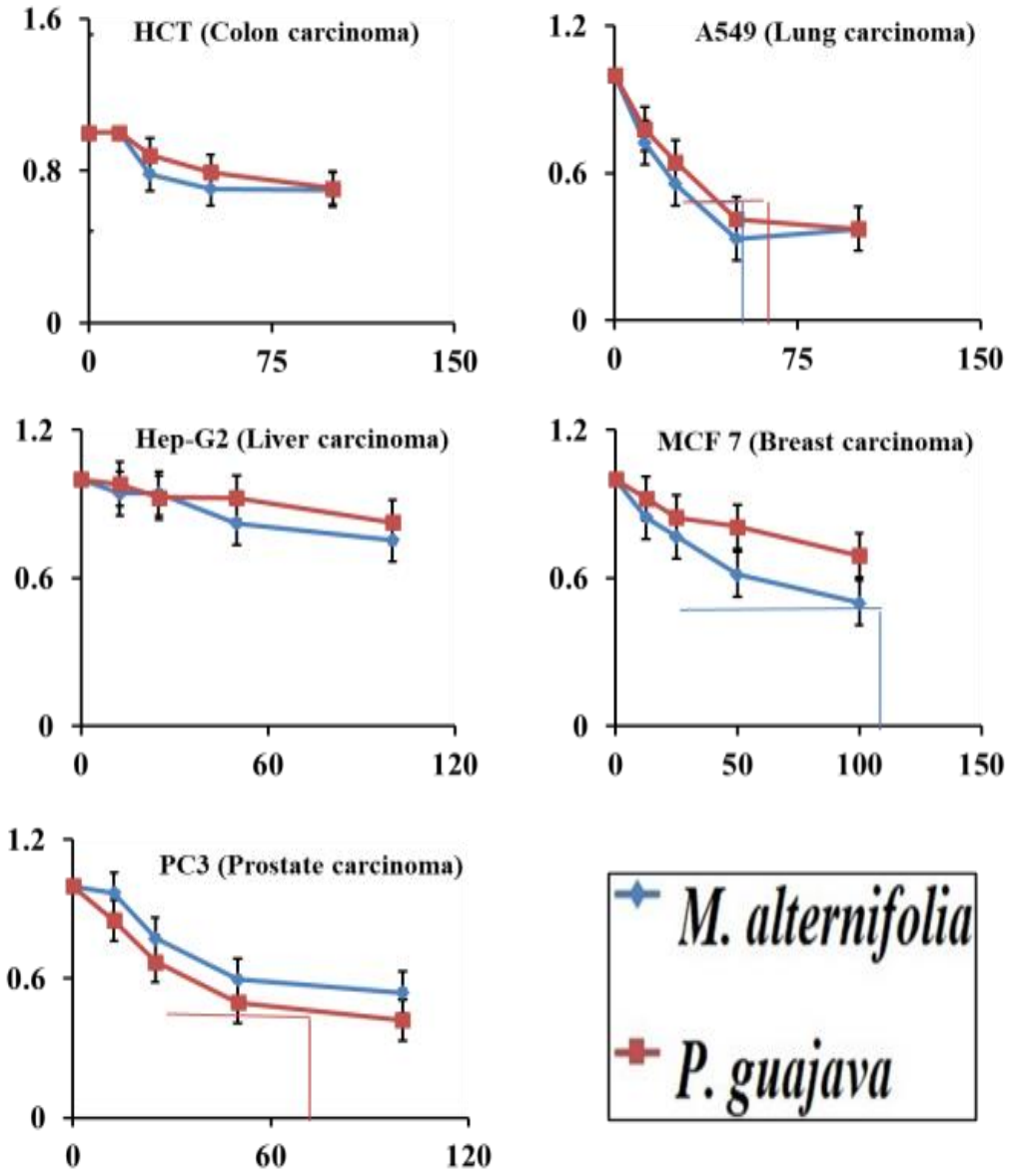


**Figure 5:** Variation in The half-maximal inhibitory concentration value ($IC_{50}$) through in vitro cytotoxic activity of *M. alternifolia* and *P. guajava* leaves ethanolic extract were determined on HCT (Colon carcinoma), A549 (Lung carcinoma), Hep-G2 (Liver hepatocellular carcinoma), MCF 7 (Breast carcinoma), and PC3 (Prostate carcinoma) carcinoma cell lines.

## Discussion

Myrtaceae is one of the dicotyledonous family belongs to order Myrtales and includes over 5650 species of about 130 to150 genera and represents the eighth largest flowering plant family that comprises of several genera of extraordinary ecological and economic importance worldwide (Shah & Baghel, 2017). *M. alternifolia* and *P. guajava* are members of Myrtaceae family and have great ecological and economical roles in addition to their medicinal values (Carson *et al*., 2006; Naseer *et al*., 2018; Saber *et al*., 2023).

*M. alternifolia* has therapeutic uses in traditional herbal medicine (Carson, *et al*. 2006; Refaey *et al*., 2024). In the present study phytochemical screenings of *M. alternifolia* and *P. guajava* leaves ethanolic extract exert high detection compared to aqueous extract. *M. alternifolia* leaves extract revealed presence of tannins, steroids, flavonoids and alkaloids that achieved high detection (++) compared to the others in both ethanolic and aqueous extracts. Concentration level of some secondary metabolites in *M. alternifolia* leaves disclosed presence of terpinenes with high concentration level, such as terpinen-4-ol, γ-terpinene, α-terpinene and methyl eugenol that acquires *M. alternifolia* various pharmacological activities such as anticancer, antioxidant, antimicrobial, and anti-inflammatory (Padovan *et al*., 2017; Shah & Baghel, 2017; Shah *et al*., 2019).

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*P. guajava* has many medicinal values (Otuoma *et al*., 2020; Kareem & Kadhim, 2024). *P. guajava* leaves ethanolic extract achieved high detection (+++) related to phenolic and flavonoids, while aqueous extract attained high detection (++) related to flavonoids that acquires *P. guajava* leaves various pharmacological activities such as antimicrobial, antimalarial, antimutagenic, anticancer, antitumor, anti-hyperglycemic and antinociceptive (Molla & Azene, 2017; Hashemi *et al*., 2018; Camarena-Tello *et al*., 2018; Lok *et al*., 2023).

Allelopathy occurred by releasing many phytochemicals into the environment which is responsible for suppressing germination and growth of neighboring plants by modification their metabolism or degradation their soil communities (Shan *et al*., 2023). In petri dish experiment *P. guajava* leaves aqueous extract exert great inhibition effect on some growth parameters such as GP, RL, PL, IP, MGT, SGI and SVI of *R. dentatus*, *S. lycopersicum* (geaara 023) and *S. lycopersicum* (tomato extracted seed) under all concentration levels (Control, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5% compared to *M. alternifolia* leaves aqueous extract that agree with Queiroz *et al*. (2017); Mabele & Ndong (2019) and Kapoor *et al*. (2019). The great allelopathic effect of *P. guajava* leaves aqueous extract due to presence of phenolic and flavonoids with excessive concentration according to Kawawa *et al*. (2016) and Motalebnejad *et al*. (2023).

Phenolics are recognized as one of the main promises allelochemicals in the ecosystem that is responsible for inhibition of seed germination and seedling growth (Madany & Saleh, 2015; Khan *et al*., 2017; Patanè *et al*., 2023), by inhabiting cell division and alter cells structures (Gomaa *et al*., 2014; El-Metwally *et al*., 2022) and suppress protein biosynthesis (Shahidi & Dissanayaka, 2023) and inactivate several enzymes (Singh *et al*., 2021). Flavonoids exert a great inhibitory effect on seed germination and seedling growth (Gomaa *et al*., 2014; Quy *et al*., 2024).

Phenolics and flavonoids with excessive concentrations are described as eco-friendly so used as bioherbicides for weeds management (Alghamdi *et al*., 2022; El-Metwally *et al*., 2022). High concentration levels of phenolics and flavonoids interfere with cell division, hormone biosynthesis and photosynthesis (Alghamdi *et al*., 2022), protein synthesis (Kuljarusnont *et al*., 2024), which causes significant inhibition of weeds. *P. guajava* leaves aqueous extract causes a great inhiation in seed germination and seedling growth of *R. dentatus*, whereas *S. lycopersicum* (geaara 023) and *S. lycopersicum* (tomato extracted seed) growing safely under low concentration levels of *M. alternifolia* leaves aqueous extract.

The analysis of *M. alternifolia* and *P. guajava* leaves was performed by using energy dispersive x-ray spectroscopy (EDS). *M. alternifolia* leaves revealed high concentration levels of Cl, Ca, Al and Cu elements, whereas *P. guajava* leaves exert high concentration levels of Si, S, Fe and K elements. High concentration levels of Si, S, Fe and K elements promote production of phenolics and flavonoids in *P. guajava* leaves that apparent as an ordinary defensive component against heavy elements toxicity (Shi *et al*., 2018; Vega *et al*., 2019).

Vegetative seedling storage protein profiling (VSPS) can be used for many objectives, such as supporting cropping polyculture ecosystems (Marzouk *et al*., 2017) weed management (Khattab & El-Darier, 2020). The study evaluates the VSPS in *R. dentatus*, *S. lycopersicum* (tomato extracted seed) and *S. lycopersicum* (geaara 023) seedlings affected by 0.5, 1.5 and 3.5% concentration of *M. alternifolia* and *P. guajava* leaves aqueous extract. *P. guajava* leaves aqueous extract decreased GTS (%) of *R. dentatus* as a weed in concordance with Khattab and El-Darier (2020), however GTS (%) of *S. lycopersicum* (geaara 023) increase more than *S. lycopersicum* (tomato extracted seed) as crops under the effect of *M. alternifolia* leaves aqueous extract according to Marzouk *et al*. (2017). *P. guajava* has phenolics and flavonoids with excessive concentration that decrease the incorporation of phosphorus into DNA and RNA and reduce the incorporation of certain amino acid into proteins that decrease protein synthesis rate according to Khattab & El-Darier (2020), so *P. guajava* leaves aqueous extract decrease GTS (%) more stronger than *M. alternifolia* leaves aqueous extract.

The current study intended to explore and manipulate the cytotoxic and anti-proliferative effects of *M. alternifolia* and *P. guajava* leaves ethanolic extract on five human tested cell lines HCT (Colon carcinoma), A549 (Lung carcinoma), Hep-G2 (Liver hepatocellular carcinoma), MCF7 (Breast carcinoma) and PC3 (Prostate carcinoma).

*M. alternifolia* leaves ethanolic extract exhibited a strong significant anticancer activity against lung carcinoma A549 ($IC_{50}$= 31 µg/ml), moderate significant anticancer activity against breast carcinoma MCF 7 (IC50= 98.9 µg/ml) and weak significant anticancer activity against HCT, Hep-G2 and PC3 carcinoma. Nalkıran & Nalkıran (2024) showed that the extracts of *M. alternifolia* induced the cell growth arresting apoptosis by down regulating NF-κb signaling in lung cancer cells. The anticancer activities of *M. alternifolia* are mainly due to its plentiful

steroidal saponins (Neychev *et al*., 2007). Clark *et al*. (2021) stated that the extract of *M. alternifolia* has many biological activities such as cytotoxic, anti-proliferative and proapoptotic activities against prostate cancer and breast cancer. *M. alternifolia* has high antitumor potential due to presence of high steroidal saponins act as potential candidates can be used for this purpose (Sobolewska *et al*., 2020; Elekofehinti *et al*., 2021; Cui *et al*., 2024).

According to Anggrelia *et al*. (2024) anticancer activities of *P. guajava* is due to excessive concentration of phenolics and flavonoids so considered as a free radical scavengers that help in gene expression regulation, DNA damage repair, cell proliferation and apoptosis (Luo *et al*., 2014; Kadhim & Kareem, 2024). In the present study *P. guajava* leaves ethanolic extract exhibited a strong significant anticancer activity against lung carcinoma A549 (IC50=40 µg/ml), moderate anticancer activity against prostate carcinoma PC3 (IC50=50.5 µg/ml) and aweak anticancer activity against HCT, Hep-G2 and Mc7 carcinoma that agree with Lok *et al*. (2023), who demonstrated that *P. guajava* ethanolic extract has a significant cytotoxic activity against lung, breast, colon, prostate, leukemia, kidney and ovarian cancer cells.

## Conclusion

In the present study we can conclude that, M. alternifolia and P. guajava leaves ethanolic extract have high concentration level of terpinenes and flavonoids respectively, which acquire them high pharmacological activities. M. alternifolia leaves revealed high concentration levels of Cl, Ca, Al and Cu elements, whereas P. guajava leaves exert high concentration levels of Si, S, Fe and K elements that promote production of phenolics and flavonoids which apparent as an ordinary defensive component against heavy elements toxicity.

P. guajava leaves aqueous extract exert strongly decrease in genome template stability GTS (%) of R. dentatus so may be used as a powerful bioherbicide tool in controlling this pernicious weed, while M. alternifolia leaves aqueous extract reveled increase in GTS (%) of S. lycopersicum (geaara 023) more than more than S. lycopersicum (extracted seed) as crops so may be used as a powerful tool in mixing polyculture ecosystems.

M. alternifolia leaves ethanolic extract revealed a strong significant anticancer activity compared to P. guajava leaves ethanolic extract against lung carcinoma A549 (IC50=31 µg/ml) and (IC50=40 µg/ml) respectively. M. alternifolia exerts moderate anticancer activity against breast carcinoma MCF7 (IC50=98.9 µg/ml), while P. guajava showed a moderate anticancer activity against prostate carcinoma PC3 (IC50=50.5 µg/ml).

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