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Actilasioptera gagné, Aquatic Gray Mangrove, *Avicennia marina* Interactions using Phytochemicals Analysis on the Red Sea Coast, Egypt

Sanad H. Ragab[1*], Mohamed A. M. El-Tabakh[1], Yasser I. Hamza[1], Mohammed A. Mahmoud[1], Nashaat N. Mahmoud[2]

[1]Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt
[2]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt
[*]**Corresponding Author: sanadragab@azhar.edu.eg**

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ABSTRACT

Actilasioptera gagné (Diptera: Cecidomyiidae) is a gall inducer associated with the gray mangrove, *Avicennia marina* (Acanthaceae) which hasn't been previously recorded in Egypt. Mangrove forests are unique functional ecosystems having much social, ecological, economic and biological importance. This work aimed to study the relationship between the gall marker *Actilasioptera Gagné* and the gray mangrove, *Avicennia marina*, in addition to evaluating the quantifications of phytochemical and proximate compositions of *Avicennia marina* leaves with galls and without galls, as well as their antinutrients compositions on the Red Sea coast. The results of phytochemical compositions showed that total flavonoids, flavonols, steroids, saponins, tannins, alkaloids, and total phenolic contents were statistically significant at $P< 0.05$ and higher in *Avicennia marina* leaves with galls than without galls. The total flavonoids, flavonols, and phenolic acids in the *Avicennia marina* leaves with galls and leaves without galls were 181.93± 1.36mg RTE/ g DW, 51.10± 0.56mg RTE/ g DW, 20.27± 0.65mg RTE/ g DW, 8.77± 0.51mg RTE/ g DW, 70.42± 0.61mg GAE/ g DW, and 26.90± 1.25mg GAE/ g DW, respectively. In addition, total steroids, saponins, tannins, and alkaloids were significantly higher in the *Avicennia marina* leaves with galls than in leaves without galls. The high values of proximate contents were found in *Avicennia marina* leaves with galls than *Avicennia marina* leaves without galls. Furthermore, the highest levels of anti-nutrients were found in *Avicennia marina* leaves with galls: oxalates, phytates, and cyanogenic glycosides were 21.60± 0.52mg/ 100g, 1.102± 0.08mg/ 100g, and 6.98± 0.22mg/ 100g, respectively. *Actilasioptera Gagné* can infest and damage mangrove plant leaf, leading to the death of the plant leaf as a result of access of plant defense chemicals against insect pests.

INTRODUCTION

Mangroves are found along tropical and subtropical coasts in all continents except Europe **(Giri *et al.* 2011)**. They are a valuable source of several items including food, honey, medicines, lumber, and firewood, as well as services like recreation, ecotourism, and aesthetics **(Guannel *et al.* 2016; Owuor *et al.* 2019)**. Mangrove timber is utilized in the construction of various structures such as houses, furniture, and bridges, as well as for making tools like paddles and rafts. It is also used as fuel. Mangroves aid in safeguarding

seagrasses, coral reefs, and shrimp by trapping sediments from river runoff. They play a crucial role in "coastal blue carbon" by storing and sequestering significant quantities of carbon **(Estrada & Soares, 2017; Kelleway *et al.*, 2017; Rogers *et al.*, 2019)**. Mangrove forests are decreasing and at a risk of extinction despite their importance for the environment and economy **(Polidoro *et al.*, 2010)**. Therefore, comprehending the mangrove forest ecosystems and their interactions with other creatures is crucial for developing successful conservation efforts.

On the coastlines of the Red Sea in Egypt, there has been a remarkable decline in the population and coverage area of gray mangrove forests (*Avicennia marina*, Avicenniaceae). This decline is attributed to several factors including oil pollution, extensive camel grazing, increased tourism, the construction of new tourist facilities, and the impacts of climate change. Currently, mangroves forests are mostly recognized as protected areas in Egypt, and several conservation programs have lunched to propagate mangroves and restore their populations **(Moustafa *et al.*, 2023)**.

In 2021, one of the authors (S. H. Ragab) discovered leaf galls induced by gall midges (Diptera: Cecidomyiidae) on gray mangroves in Safaga, Egypt. The gall midge was morphologically examined and identified as a new species of *Actilasioptera Gagné*, not previously documented in Egypt. Gall-inducing insects are extremely skilled in altering the physical characteristics of their victim organisms. Phytochemical alterations in galled areas stimulate tissue development and differentiation, while simultaneously enhancing the plant's defensive mechanism against herbivore attacks. Plant-herbivore interaction coevolves, leading to a chemical arms race through the development of advanced chemical defense and detoxification systems in the plant **(Roy *et al.*, 2022)**.

Some plants have protuberances or structures resembling fruit on their leaves and branches. These structures are sometimes misinterpreted as the fruit or seed of the plant, but they are actually known as "galls." Galls are aberrant growths that develop on plant tissue due to increased cell division and altered differentiation caused by external stimuli from another organism. There are about 2000 gall-forming species, including insects, mites, nematodes, bacteria, fungus, and viruses. Galls may form in nearly all types of higher plants. Insect galls are generated when plant tissue is encouraged by insects eating or laying eggs **(Hirano *et al.*, 2020)**.

Insect gall is a result of a parasitic relationship between insects and plants. Insects benefit from host plants by obtaining food and shelter, while also being able to evade predators and harsh weather conditions. Plants suffer from this interaction, experiencing reduced growth, lower height, and smaller leaf area. Moreover, severe instances can lead to death **(Fay *et al.*, 1996)**. Typically, galls do not cause significant harm to plants and are formed as a means of self-protection to some degree. Galls, being aberrant proliferative tissues, contain much larger levels of chemical components such fat, protein, starch, and tannic acid compared to normal plant tissues. Galla chinensis are insect galls found on the Chinese sumac tree Rhus chinensis. They are utilized in Chinese traditional medicine due to their high tannin content, which may reach 70– 80%. Tannic acid is derived from these galls and is further processed into gallic acid and pyrogallic acid,

which has antidiarrheal, antiperspirant, hemostatic, and wound healing properties. Galla chinensis decoction is effective in treating peptic ulcer and inflammation **(Wang *et al*., 2022)**. Moreover, it may be utilized as an adjuvant treatment for diabetes mellitus and cancer **(Gao *et al*., 2018)**.

Understanding the insect fauna associated with Egyptian mangroves is important for conservation efforts. Previous studies have documented the relationship between gall inducers and host plant secondary metabolites, but this relationship has not been quantified in leaves with galls and without galls. In this work, we assessed the density of gall midge infestation on the gray mangroves and investigated the phytochemicals in galled and non-galled tissues to understand the impact of gall injuries on the mangroves.

MATERIALS AND METHODS

Gall collection and field survey

Leaves of *Avicennia marina* with galls and without galls were collected in January 2023 in Safaga, Egypt (Fig. 1). The gall length and diameter were measured in dry condition using a digital caliper, and notes on the gall morphology were taken. The morphological identification was performed by A. K. Elsayed (Saga University, Japan) based on the characteristics of the larvae and the pupal exuviae according to the guidelines of **Gagné and Law (1999)**. Some leaves were preserved dry to be used for the analysis of phytochemicals.

Seven plant leaves of *A. marina* were arbitrarily chosen for the study to assess the percentage of infestation of the gall midge. A random number of leaves were counted on each tree and categorized as galled and ungalled (no galls) leaves. Additionally, the number of galls per leaf was recorded to determine the average gall count per leaf.

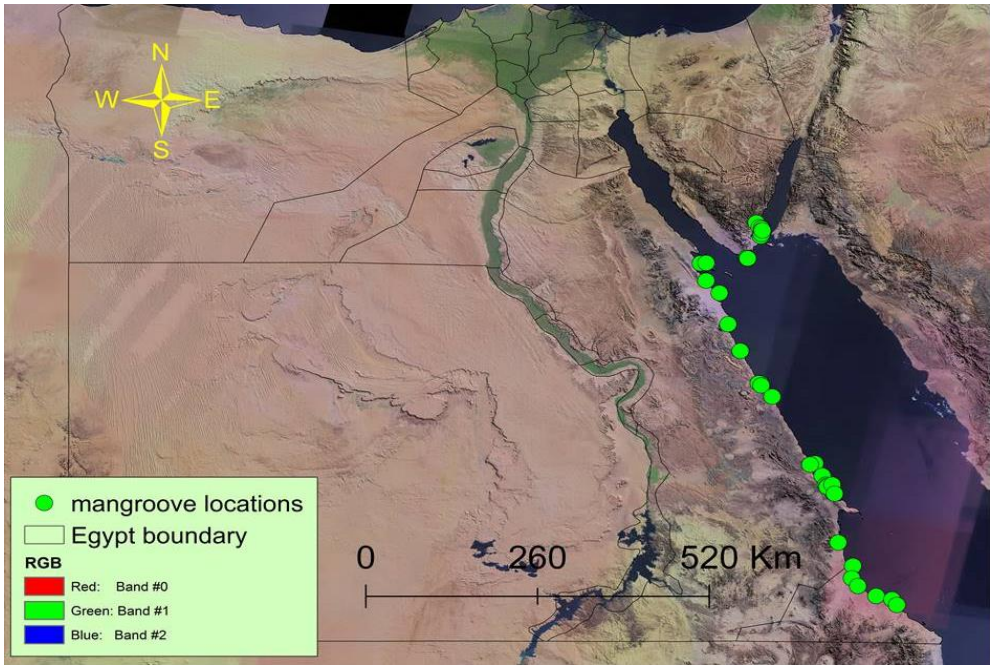

Fig. 1. Map showing distribution of mangrove in Egypt

Quantitative determination of phytochemicals and anti-nutrient measurements in galled and non-galled leaves

Determination of total phenolic content

The total phenolic acids in *Avicennia marina* leaves with and without galls were measured using the Folin-Ciocaleau technique as described by **Makkar (2003)**. 50µL of phenolic extract was distributed into multiple test tubes. Distilled water was added to each test tube up to the 1mL mark. To each test tube, including the blank, 0. mL of 1N Folin-Ciocalteu reagent was added and allowed to settle at room temperature for 5 minutes. Subsequently, 2.5mL of Na2CO3 (5%) was added to all test tubes, including the blank. The mixtures were then incubated for 40 minutes at room temperature in the dark. Absorbance at 725nm was measured using a spectrophotometer against a calibration curve of gallic acid dissolved in methanol. Total phenolic content was calculated as the mean ± standard deviation (n=3; each sample measured in triplicate) and reported as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/ g DW).

Quantification of total flavonoid content

The flavonoid content of *Avicennia marina* leaves with and without galls was measured using the aluminum chloride technique outlined by **Zhishen *et al.* (1999)**. 500µL of the *Avicennia marina* leaf extract was transferred into several test tubes. Pure water was added to each test tube to reach a total volume of 1mL. Next, 150µL of NaNO2 (5%) was added to all test tubes and allowed to incubate at room temperature for 5 minutes. Following this, 150µL of AlCl3 (10%) was added to each test tube and allowed to incubate at room temperature for 6 minutes. Subsequently, 2mL of 4% NaOH was added to each test tube, and the volume was adjusted to 5mL with pure water. The test tubes were thoroughly mixed and allowed to settle for 15 minutes at room temperature. Absorbance at 510nm was measured using a spectrophotometer against a calibration curve of rutin dissolved in methanol. The total flavonoid content was calculated as the mean ± standard deviation (n=3; each sample measured in triplicate) and reported as milligrams of rutin equivalent per gram of dry weight (mg RTE/ g DW).

Determination of total tannin content

The content of tannins was evaluated using the Folin-Denis spectrophotometer technique **(Makkar, 2003)**. 0.5mL of the plant sample was mixed with 0.5mL of distilled water and added to 0.1g of polyvinyl poly pyrrolidone under cold conditions to precipitate tannins. The tubes were then incubated for 4 hours at 4°C. After incubation, the tubes were centrifuged at 3000rpm for 10 minutes at 4°C to separate the supernatant containing non-tannin phenolics. Next, 100µL of the non-tannin phenolics extract was mixed with 0.5mL of 1N Folin-Ciocalteu reagent and diluted to 1mL with distilled water, including a blank. The test tubes were thoroughly mixed and allowed to stand for 5 minutes at room temperature. Then, 2.5mL of a 5% Na2CO3 solution was added to each test tube, including the blank. The tubes were agitated and incubated in the dark at room temperature for 40 minutes. The spectrophotometer measured the absorbance at 725nm against a calibration curve of tannic acid standard solution in methanol. The total tannin content was calculated as the mean ± standard deviation (n=3; each sample measured in

triplicate) and reported as milligrams of tannic acid equivalent per gram of dry weight (mg TAE/ g DW).

Determination of total flavonol content

The flavonol content of *Avicennia marina* leaves, with and without galls, was quantified using the aluminum chloride colorimetric method as described by **Miliauskas *et al.* (2004)**. A solution was prepared by mixing 2mL of aluminum chloride (AlCl3) with 6mL of sodium acetate (CH3COONa). Samples were then incubated in this solution at 20°C for 2 hours. After incubation, the absorbance of the samples was measured using a UV-Vis spectrophotometer at a wavelength of 440nm. The total flavonol content was calculated as the mean ± standard deviation (n=3; each sample analyzed in triplicate) and expressed as milligrams of rutin equivalent per gram of dry weight (mg rutin/ g DW).

Determination of total alkaloids

The total alkaloid content of *Avicennia marina* leaves, with and without galls, was quantitatively determined using the method outlined by **Harborne (1973)**. The dried plant powder samples (1g) were combined with a mixture of 70% ethanol and glacial acetic acid with a 4:1 ratio. The mixture was allowed to stand for a minimum of 6 hours and then filtered. The alkaloid present in the supernatant was precipitated by slowly adding strong ammonia solution. The alkaloids that formed a precipitate were filtered using a pre-weighed filter paper (Whatman 102) and then dried in an oven at 70°C until a consistent weight was achieved. The alkaloid content was determined and reported in milligrams per 100 grams of dry weight of the plant samples.

Determination of total saponins

Five grams of each sample were weighed and combined with 100 milliliters of 20% ethanol. The suspension was agitated and kept warm on a water bath at approximately 55°C for 4 hours. After filtration, the remaining substance was re-extracted using another 100mL of 20% ethanol. The combined extracts were concentrated on a water bath to a volume of about 40mL. The concentrated solution underwent a washing process using diethyl ether, followed by extraction with n-butanol. The n-butanol extract was then washed with a 5% aqueous sodium chloride solution. The resulting solution was initially boiled in a water bath and subsequently dried in an oven until a constant weight was achieved **(Otang *et al*., 2012)**. The concentration of saponins was determined and reported as milligrams per 100 grams of dry weight of the plant samples.

Determination of total steroids

5 grams of the sample were measured and then boiled for 30 minutes in a 50mL hydrochloric acid solution to hydrolyze it. The solution was strained through Whatman filter paper and then poured into a separating funnel. An equal proportion of ethyl acetate was combined, stirred thoroughly, and separated into distinct layers. The ethyl acetate layer was retrieved and disposed of the aqueous layer or retained for further investigation if needed. The ethyl acetate layer was then evaporated for 5 minutes at 100°C using a steam bath, followed by extracting the steroids by heating with concentrated amyl alcohol until the mixture turned cloudy. The mixture was filtered using Whatman filter paper,

allowed to cool in a desiccator, and weighed again to determine the steroid concentration, reported in milligrams per 100 grams of dry weight of the plant samples. **(Harborne, 1973)**. The steroid concentration was determined and reported in milligrams per 100 grams of dry weight of the plant samples.

Determination of phytates

4.0 grams of each sample were immersed in 100 milliliters of 2% hydrochloric acid for 5 hours and subsequently separated via filtration. 25mL of the filtrate was transferred to a conical flask, followed by the addition of 5mL of 0.3% ammonium thiocyanate solution (NH_4SCN) as an indicator. Then, 53.5mL of distilled water was added to adjust the pH to 3.5. The mixture was titrated with ferric chloride solution ($FeCl_3$) until it turned into a brownish yellow tint that remained stable for 5 minutes **(AOAC, 1990)**. The phytate content was determined and reported in milligrams per 100 grams of dry weight of the plant samples.

Determination of oxalates

75 milliliters of 3.0 molar sulfuric acid were combined with 1 gram of each powdered sample, mixed periodically using a magnetic stirrer for about one hour, and subsequently filtered. A 25mL portion of the filtrate was obtained and titrated at 80°C with 0.05M $KMnO_4$ solution until a light pink hue emerged consistently for at least 30 seconds **(AOAC, 1990)**. The oxalate content was determined and reported in milligrams per 100 grams of the plant samples' dry weight.

Determination of cyanogenic glycosides

4.0 grams of each sample were immersed in a solution consisting of 40 milliliters of distilled water and 2 milliliters of orthophosphoric acid, and then left overnight at room temperature. This is to liberate all the bound hydrocyanic acid. The extract was distilled meticulously using paraffin as an antifoaming agent and broken chips as an anti-bump measure. 5mL of distillate was collected into a receiving flask with 40mL of distilled water and 0.1g of NaOH pellets. This mixture was then transferred to a 50mL volumetric flask and diluted with distilled water to the mark. 20mL of the solution was transferred to a conical flask, followed by the addition of 1.0mL of 5% potassium iodide solution. The resulting solution was titrated with 0.01M silver nitrate solution. Another solution was titrated until a little, yet lasting cloudiness was observed at the end point **(AOAC, 1990)**. The cyanogenic glycosides concentration was determined and reported as mg per 100g of plant samples on a dry weight basis. The proximate components were determined using the method of **AOAC (1990)**. The nutritional value of the samples was determined using the technique outlined by **Indrayan *et al*. (2005)**.

Statistical analyses

The data were coded and inputted into SPSS V.22 for statistical analysis. Chi-square test was conducted to compare the observed and expected frequencies of the infestation using MiniTab V 14. Principal component analysis (PCA) was used to analyze the infestation level and gall number per leaf in PAST V4.2. The data were displayed using R Studio version 2022.02.4 wherever feasible.

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RESULTS

Gall and biological aspects

Actilasioptera sp. forms galls of about 2.8– 6.2mm in diameter and 0.9– 1.6mm in height (n = 18) on leaves of *Avicennia marina*. Each galled leaf bears one to ten galls (Fig. 2). Galls are brown, smooth and mostly circular on the upper surface but pale to light brown on the lower surface. Each gall contains a single larva in a black carbonaceous chamber of which internal walls are covered with white hyphae of symbiotic fungus. The adult gall midge emerges from the lower surface, indicated by the pupal exuviae protruding from that side.

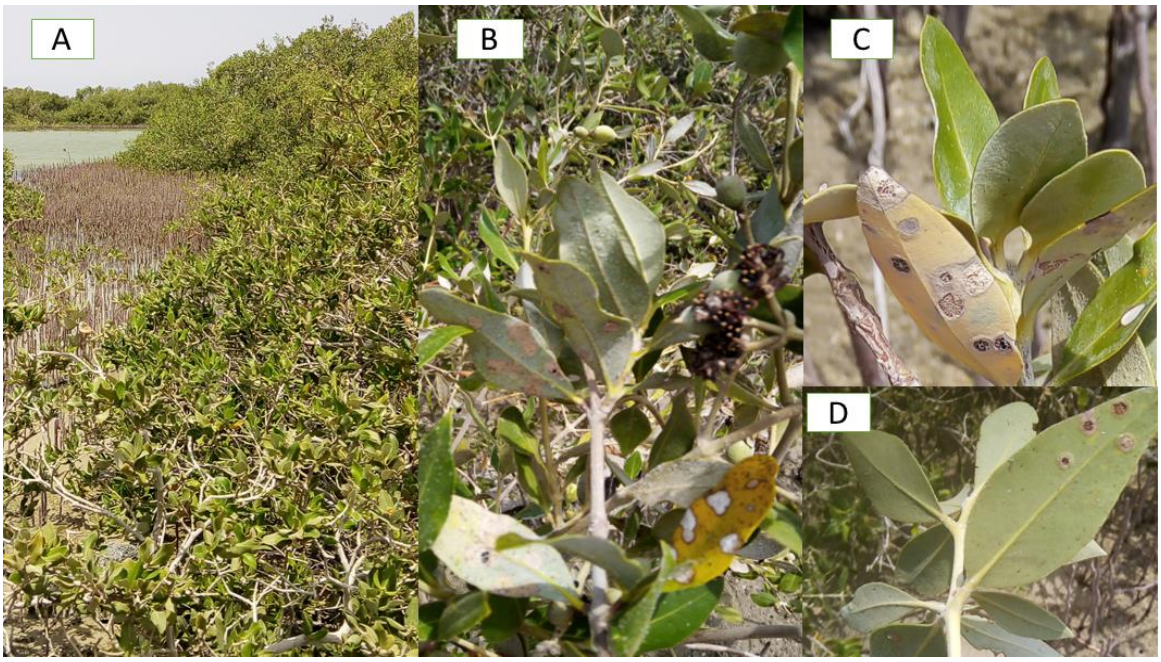

Fig. ٢. (A) *Avicennia marina* plant; **(A, B, C)** Some shapes of *Actilasioptera Gagné* gall associated with *Avicennia marina* plant

Number of infested *Avicennia marina* leaves per plant due to *Actilasioptera Gagné*

We observed some samples of infested leaves per plant among *Avicennia marina* plant to know percentage of infested leaves plant caused by *Actilasioptera Gagné*. The highest number of infested leaves were 66.7%, while the lowest infestation rates were observed in plant leaves, as shown in Table (1) and Fig. (3).

Table 1. Infested number in comparison to non-infested samples with significant

Sample no.	Infested	Non-infested	Total number	Percentage (%)	x^2	*P*-value
1	200	100	300	66.7	33.33	> 0.001
2	78	70	148	52.7	0.43	0.51
3	12	150	162	7.4	117.556	> 0.001
4	22	85	107	20.6	37.0935	> 0.001
5	133	228	361	36.8	25	> 0.001
6	1	99	100	1	96.04	> 0.001
7	0	100	100	0	100	> 0.001

Table (2) and Fig. (4) show that significant variation was observed among the varieties in relation to infested leaves plant-1 caused by *Avicennia marina*. The highest gall per leaf infection rate recorded was 66.7%, with the highest occurrence being 1 gall per leaf (54 instances). Instances where 8, 9, and 10 galls per leaf were observed, represented a 66.7% infection rate.

Table 2. Number of galls per plant leaf

Infestation	1 gall per leaf	2 gall per leaf	3 gall per leaf	4 gall per leaf	5 gall per leaf	6 gall per leaf	7 gall per leaf	8 gall per leaf	9 gall per leaf	10 gall per leaf
Sample with infestation 66.7 %	54	50	19	17	19	10	5	5	4	2
Sample with infestation 49.4 %	27	24	10	5	3	3	0	0	0	0
Sample with infestation 7.4 %	4	4	4	0	0	0	0	0	0	0
Sample with infestation 20.6 %	10	5	3	1	1	0	0	0	0	0
Sample with infestation 36.4 %	18	6	7	6	3	4	4	0	0	0
Sample with infestation 1 %	1	0	0	0	0	0	0	0	0	0
Sample with infestation 0.0 %	0	0	0	0	0	0	0	0	0	0

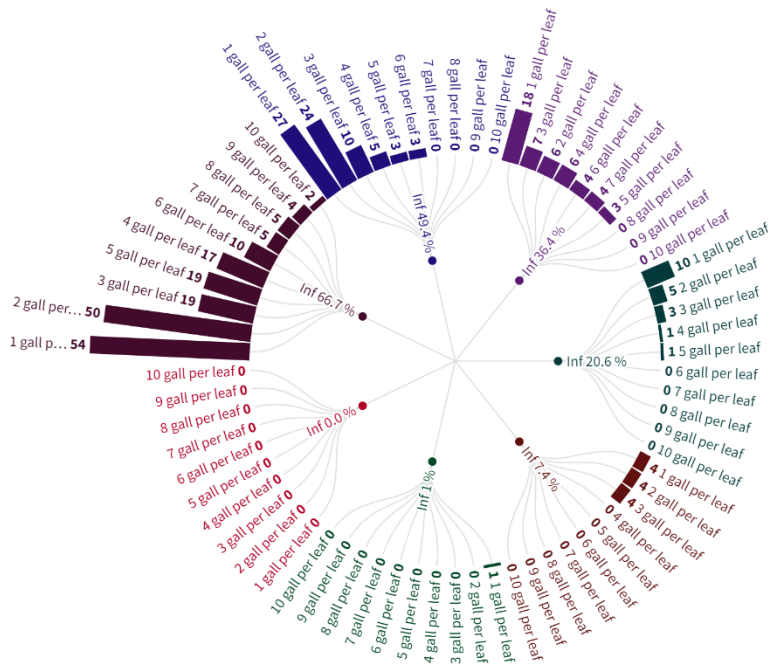

Fig. 4. Radial bar tree represent number of galls per plant leaf

In the principal component analysis (PCA) conducted to discern the variance and distribution of samples based on gall infestation, we observed distinct patterns. The graphical representation on the plane defined by component 3 and component 4 showcases a clear gradient in infestation levels. Specifically, samples with a 0.0% infestation rate are markedly separated from those with heightened infestation, notably the 66.7% group. For the classification based on the number of galls per leaf, these samples spread across the plot in a varied manner. For instance, the "3 galls per leaf" samples are positioned on the extreme right, contrasting with the "9 galls per leaf" and "5 galls per leaf" samples which lean towards the left. Interestingly, samples with intermediate infestation levels, such as 20.6, 36.4, and 49.4%, cluster together, suggesting similarities within these groups. Some overlapping labels were observed, hinting at closely related or overlapping data points in the PCA space. Overall, this PCA analysis underscores the potential differentiation of samples based primarily on their gall infestation level, paving the way for deeper exploration into the factors influencing gall formation and their respective infestation rates (Fig. 5).

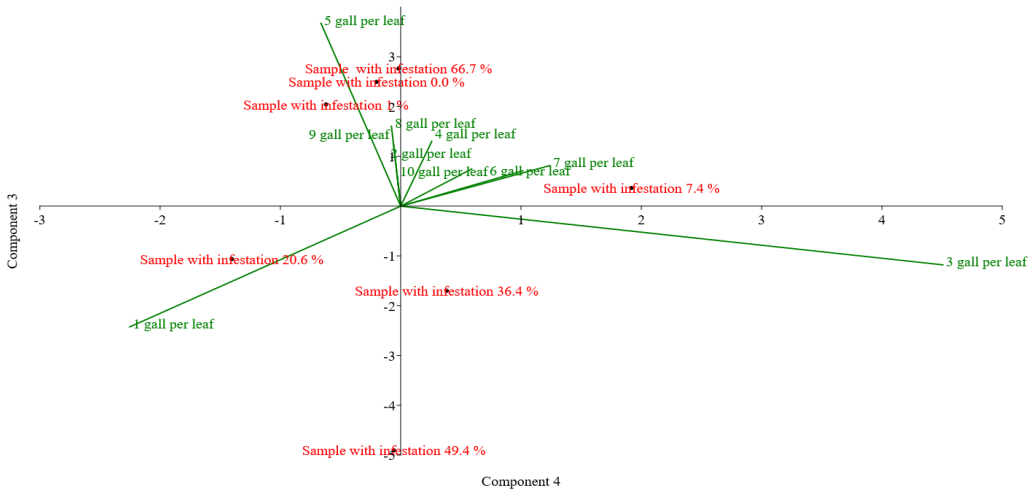

Fig. 5. PCA represent variance and distribution of samples based on gall infestation

Quantification of phytochemical, anti-nutrients, and proximate composition of *A. marina* leaves with galls and without galls

The results for the proximate analysis of *A. marina* leaves, with and without galls, are summarized in Table (3). Carbohydrate production through the photosynthetic process, which occurs inside leaves (plastids), requires the uptake of water and CO_2. The total carbohydrates of *A. marina* leaves with galls (24.61± 0.54g glucose/ 100g) were higher than that of leaves without galls (22.69± 0.46g glucose/ 100g). Bovine serum albumin (BSA) is a standard used to estimate total soluble protein, the percentage of total protein of *A. marina* leaves with galls (8.50± 0.32g BSA/ 100g) was significantly higher than that of leaves without galls (5.99± 0.0.28g BSA/ 100g). Additionally, the total lipid content of *A. marina* leaves with galls (0.072± 0.05mg/ 100 g) was higher than that of the *A. marina* leaves without galls (0.021± 0.02mg/ 100 g). The nutritive value was calculated by multiplying the protein, fat, and carbohydrate values by 4.00, 9.00, and 4.00, respectively, and then summing up the results. The nutritional value of *A. marina* leaves with galls was 133.088± 1.24kcal/ 100g, which was greater than that of *A. marina* leaves without galls at 114.903± 0.73kcal/ 100g.

The phytochemical composition of *A. marina* leaves, with and without galls, is shown in Table (3). The total flavonoids, total flavonols, total steroids, total saponins, total tannins, alkaloids, and total phenolic contents were significantly higher in *A. marina* leaves with galls than in *A. marina* leaves without galls. The total flavonoids, flavonols, and phenolic acids in *A. marina* leaves with galls and leaves without galls were 181.93± 1.36mg RTE/ g DW, 51.10± 0.56mg RTE/ g DW, 20.27± 0.65mg RTE/ g DW, 8.77± 0.51mg RTE/ g DW, 70.42± 0.61mg GAE/ g DW, and 26.90± 1.25mg GAE/ g DW, respectively. The total alkaloid content in *A. marina* leaves, with and without galls, was 0.173± 0.06 and 0.103± 0.05mg/ 100g dry weight, respectively.

Saponin quantification in this research was significant ($P< 0.05$) in the leaves of *A. marina* with galls compared to those without galls, with values of 0.277± 0.06 and 0.089±0.06mg/v100g dry weight, respectively. Furthermore, the total tannins and steroids of *A. marina* leaves with galls (33.85± 0.41 and 6.68± 0.55mg TAE/ g dry weight) were significantly higher than that of leaves without galls (0.027± 0.02 and 0.017± 0.02mg/ 100g dry weight), respectively.

The antinutrients composition of *A. marina* leaves, with and without galls, are displayed in Table (3). Oxalates, phytates, and cyanogenic glycosides were significantly higher than those of *A. marina* leaves with galls compared to leaves without galls, recording values of 21.60± 0.52, 12.96± 0.42, 1.102± 0.08, 0.870± 0.0.08, 6.98± 0.22, and 4.49± 0.25, respectively.

Table 3. Quantification of phytochemical, anti-nutrients and proximate composition of *A. marina* leaves with galls and without galls

	Parameter	**Leaves of *A. marina* with galls**	**Leaves of *A. marina* without galls**	***P*-value**
Proximate composition	Carbohydrates (g glucose /100 g)	24.61±0.54	22.69±0.46	0.38
	Proteins (g BSA /100 g)	8.50±0.32	5.99±0.0.28	0.035
	Lipids (mg /100 g)	0.072±0.05	0.021±0.02	0.082
	Nutritive value (Kcal /100 g)	133.09±1.24	114.903±0.73	0.166
Phytochemical contents	Total flavonoids (mg RTE /g Dry weight)	181.93±1.36	51.10±0.56	0.006
	Total flavonols (mg RTE /g Dry weight)	20.27±0.65	8.77±0.51	0.02
	Total phenolic acids (mg GAE /g Dry weight)	70.42±0.61	26.90±1.25	0.035
	Total tannins (mg TAE /g Dry weight)	33.85±0.41	6.68±0.55	0.001
	Total alkaloids (mg /100 g Dry weight)	0.173±0.06	0.103±0.05	0.046
	Total saponins (mg /100 g Dry weight)	0.277±0.06	0.089±0.06	0.007
	Total steroids (mg /100 g Dry weight)	0.027±0.02	0.017±0.02	0.038
Anti-nutrient	Oxalates (mg /100 g Dry weight)	21.60±0.52	12.96±0.42	0.015
	Phytates (mg /100 g Dry weight)	1.102±0.08	0.870±0.0.08	0.016
	Cyanogenic glycosides (mg /100 g Dry weight)	6.98±0.22	4.49±0.25	0.009

Values mean ± SEM of three determinations.

DISCUSSION

The main dangers to Egypt's mangroves are coastal development, human exploitation for resources like firewood and lumber, and pollution causing a loss of biodiversity **(Afefe, 2021)**. Threats to the environment arise from natural and human factors, such as oil spills, human waste runoff, herbicide application, and coastal expansion, all of which can cause harm. Mangrove ecosystems are directly affected by fluctuations in salinity and increasing sea levels due to climate change **(Moustafa *et al.*, 2023)**. The mangrove has a new challenge from the gall inducer *Actilasioptera Gagné*, which infests it.

Keith and Antonius (2010) documented galls caused by *Actilasioptera Gagné* species on *Avicennia marina* leaves in the United Arab Emirates. Further research on the

connection between the genera Actilasioptera and *Avicennia marina* is needed in the UAE and other regions where the host plant is found. The present study examined the relationship between *Actilasioptera Gagné* and *Avicennia marina* focusing on phytochemicals.

This work is the first to objectively assess the correlation between gall inducer and host plant secondary metabolites in leaves with and without galls. Our findings indicate that gall inducers are linked to elevated levels of secondary metabolites, proximate, and anti-nutrients in the plants they infest. Effect sizes differed significantly according to the chemical class and sequence of gall inducer analyzed. Climate zones did not account for major variations in impact sizes. The results offer insights into the correlation between gall inducer and plant chemistry. *Actilasioptera Gagné* is a gall inducer on the gray mangrove leaves, causing detrimental effects on the plant's foliage. This finding is consistent with the findings of **Milewski *et al*. (1991)** and **Avila-Sakar *et al*. (2003)**.

Plant defense chemicals are composed of secondary metabolites, primarily terpenes, benzenoids, phenylpropanoids, flavonoids, or N-containing compounds. These compounds undergo modifications such as hydroxylation, glycosylation, methylation, acylation, and prenylation at different positions to create a wide range of chemical compounds **(Alseekh & Fernie, 2018)**. Our data showed that the insects had a preference for infected galls over several galls on a plant leaf. These defenses might include alkaloids **(Mattson, 1980)**. Some plants contain additional chemical defenses such as alkaloids and terpenes, which may exhibit varying patterns either independently or when combined.

Plants have developed a wide variety of secondary metabolites to protect themselves from herbivores and diseases. Secondary metabolites can impact insect herbivores either directly, through antifeedant and toxic effects when consumed, or indirectly, by attracting natural enemies **(War *et al.*, 2012)**. Insect gallers, among herbivores, have the ability to regulate the physical and chemical characteristics of their host plants due to the close contact they have.

This suggests that insect galls may boost the feeding efficiency, supporting the feeding theory proposed by **Price *et al*. (1987)**. In contrast, the outer layer of gall often contains elevated levels of phenolics and tannins compared to regular plant tissue **(Taper & Case, 1987; Abrahamson *et al*., 1991)**; this finding aligns with our results. Secondary metabolite levels showed a considerable rise in galled plant tissues. This might be attributed to the varying functions of these compounds in plant defense. The buildup of these substances in the exterior gall tissue is believed to provide protection to the gall insect against predators, other herbivores, and infections **(Cornell, 1983; Hartley & Lawton, 1992)**.

These secondary metabolites exhibit biological activity, such as: Polyphenols are a group of active compounds known for their strong antioxidant properties. They possess antiviral and anticancer properties and play a role in preventing cardiovascular disorders and dementia **(Fraga *et al*., 2019)**. They are extensively utilized in the domains of

cosmetics, food, and medicine **(de Lima Cherubim *et al*., 2020)**. Flavonoids can prevent cell degeneration and aging, inhibit cancer cell development, regulate blood pressure and cholesterol, and provide preventative benefits against cardiovascular and cerebrovascular illnesses **(Wen *et al*., 2021)**. Tannin possesses antibacterial and antiviral properties and can serve as an antidote for alkaloid and heavy metal toxicity. It exhibits potent reducing capabilities, capable of scavenging superoxide free radicals in the body, hence retarding the aging process **(Tong *et al*., 2022)**.

Carbohydrates are vital constituents of animal and plant cells. The liver performs physiological activities such energy storage and delivery, protein preservation, and inhibition of ketone body synthesis **(Jiang *et al*., 2021)**. Glycosides provide diverse pharmacological effects that can be utilized in the treatment of neurological disorders **(Bécquer-Viart *et al*., 2021)**. Plant protein possesses many characteristics and nutritional benefits, and it is readily digested and assimilated by the human body. It serves several roles, including immunological modulation, antioxidation, and antifatigue properties **(Fang *et al*., 2022)**. *Avicennia marina* leaves with galls included increased levels of carbohydrates, proteins, lipids, and nutritional value compared to *Avicennia marina* leaves without galls, impacting photosynthesis and plant development. Our findings support the research conducted by **Krapp and Stitt (1995)** and **Jeannette *et al.* (2000)**, which showed that the build-up of non-structural carbohydrates in leaves can inhibit photosynthesis. Certain plant defensive compounds, including alkaloids, flavonoids, phenols, saponins, tannins, glycosides, and terpenoids, have shown an enhanced efficacy in combating insect pests, as supported by **Paudel *et al.* (2017)**.

Avicennia marina leaves with galls showed an increase in flavonoids and phenolics, as observed by **Lane *et al*. (1987)**. A previous study on lupin (*Lupinus angustifolius*) root-derived flavonoids demonstrated that compounds like maackiain, medicarpin, vestitol, pisatin, phaseollin, phaseollinisoflavin, and 20-methoxy phaseollinisoflavin exhibited a significant feeding deterrent activity against the grass grub beetle and the African black beetle (*Heteronychusarator*). Phytochemical analysis revealed an elevation in alkaloids with anti-insect properties and animal toxicity. This finding is consistent with that of **Paudel *et al.* (2017)**, who confirmed that certain compounds, such as α-solanine and α-chaconine from potatoes, belong to multiple compound categories like steroidal alkaloids and saponins. An elevation in anti-nutrients and changes in the proximate composition of *Avicennia marina* leaves with galls have been found to hinder insect growth, making *Actilasioptera Gagné* detrimental to *Avicennia marina* **(Lane *et al*., 1987; Paudel *et al*., 2017; Chaubey, 2018)**.

Galls can impact indirect plant defenses by triggering the release of volatile chemicals that attract herbivores' natural enemies in response to herbivore eating. Indirect defenses have been demonstrated to enhance plant fitness **(Fritzsch & Turlings, 2001; Tooker & Hanks, 2006)**. Feeding by chewing or sucking insects or piercing-sucking mites often triggers volatile reactions in plants, as studied by **Turlings *et al*. (1990)**, **Du *et al*. (1996)** and **Dicke (1999)**. However, the indirect defensive response of plants to gall inducers has not been thoroughly investigated.

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