



Physicochemical Properties, Antibacterial Activity, And Corrosion Inhibition Of Clove (*Syzygium Aromaticum* L.) Essential Oil

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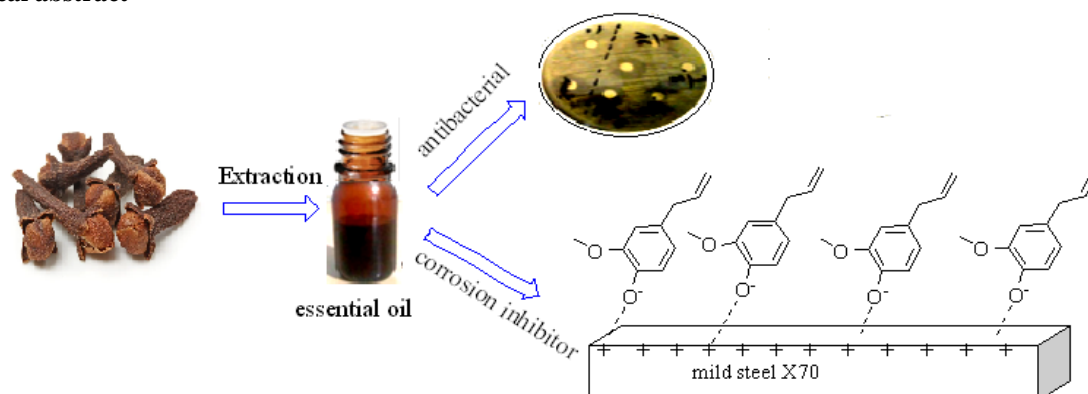
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Abstract:

This study investigated the properties of *Syzygium aromaticum* essential oil, including its physical and chemical properties, antibacterial activity, and corrosion inhibition effect on mild steel X70 in 1M hydrochloric acid. Weight loss method was used to study the corrosion inhibitory effect. Two extraction methods were used to compare their effects on the quality of essential oil: hydrodistillation and continuous extraction using a Soxhlet apparatus. The study found that the extraction yields for hydrodistillation and continuous extraction were 11.25% and 25.11%, respectively. Organoleptic characteristics (color, odor, and aspect), and some physicochemical properties of the extracted essential oil such as refractive index, acid index, ester index, and saponification value are given in this work. The study of antibacterial activity using the diffusion method shows that the clove essential oil has significant antibacterial activities against the bacterial strains tested. Moreover, the treatment with clove essential oil at a concentration of 1 g/l, and 2 hours of immersion time, prevented the corrosion of steel with an inhibition efficiency of 99.33% for the oil obtained by hydrodistillation and 99.5% for the oil obtained by continuous extraction using a solvent.

Keywords: essential oil, clove buds, antibacterial, corrosion, inhibitor

Graphical abstract



1. Introduction

In recent decades, the utilization of medicinal plants has experienced a significant upsurge, with a staggering 80% of the global population now turning to them for the treatment of diverse ailments. This surge is largely attributed to the adverse effects associated with conventional industrial medications and the lack of efficacious remedies for chronic conditions[1]. Cloves, the desiccated flower buds of the *Syzygium aromaticum* tree from the Myrtaceae family, are indigenous to Indonesia and boast a centuries-old history of employment for both culinary and medicinal purposes[2]. These flower

buds possess antiseptic and anesthetic properties that have been harnessed since ancient times, particularly in alleviating dental discomfort. Additionally, they have found application in kohl, an original ophthalmic ointment. Renowned for their anti-inflammatory and antibacterial attributes, cloves prove effective against a spectrum of urinary, digestive, and cutaneous infections[3]. Extensive studies have showcased the potency of clove extract against various bacteria, encompassing *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli* [4-7]. Furthermore, clove extract has demonstrated its proficiency in curtailing the corrosion of metals such as steel, copper, and aluminum[8, 9].

Corrosion entails the material deterioration due to chemical or electrochemical reactions with the surrounding environment, resulting in gradual material loss at the interface with said environment. Wielding a pervasive impact across diverse industrial sectors, corrosion's annual economic toll amounts to billions of dollars[10, 11]. Hydrochloric acid ranks among the most prevalent agents in industrial applications, precipitating the degradation of metallic substrates via electrochemical and chemical pathways. Countermeasures against corrosion in this milieu include metallic coatings, anodic and cathodic protection, galvanization, and deployment of corrosion inhibitors[12]. In acidic settings, inhibitor utilization stands out as a superior strategy for safeguarding metallic materials.

While many synthetic compounds exhibit robust corrosion-inhibiting properties, a significant proportion also poses pronounced threats to human health and the environment[13]. Thus, the exploration of plant extracts as eco-friendly corrosion inhibitors is emerging as a prominent avenue of research. These natural extracts encompass a plethora of organic compounds (essential oils, flavonoids, alkaloids, tannins, etc.) that are not only readily available but also renewable.

Consequently, in light of mounting environmental concerns, oils and plant extracts are increasingly regarded as sustainable sources of green corrosion inhibitors, poised to replace the presently employed toxic chemicals[14-17]. The present study delves into the multifaceted properties of clove essential oil, encompassing its physicochemical attributes, antibacterial efficacy, and its potential to inhibit corrosion on mild steel X70 in a 1M hydrochloric acid environment.

The outcomes of this investigation are poised to furnish valuable insights into the potential roles of clove essential oil as an antimicrobial agent and a corrosion inhibitor. Moreover, the findings will aid in determining the optimal method for extracting essential oil from clove buds.

2. Experimental

2.1. Sample Preparation and Extraction

The dried clove materials were meticulously ground to achieve a fine powder consistency, facilitating optimal extraction.

- Hydrodistillation Extraction

Around 100 g of clove buds underwent a hydrodistillation process lasting approximately 3 hours. The resultant distillate was subjected to further extraction with dichloromethane solvent, employing a separating funnel. The organic phase was subsequently desiccated using anhydrous sodium sulfate. The use of a rotary evaporator operating under vacuum conditions facilitated the removal of the dichloromethane solvent. The resulting essential oil was then meticulously collected within light-resistant vials, maintaining a temperature range of 3-5°C for subsequent utilization[18].

- Continuous Extraction (Soxhlet Method)

Approximately 100 g of clove powder was subjected to extraction using dichloromethane through a soxhlet apparatus. The system was refluxed for 6 hours, until the solvent displayed discoloration. After reflux, the solvent was removed using a rotary evaporator.

The quality assessment of the extracted essential oil encompassed an evaluation of pertinent physicochemical properties, including solubility, density, refractive index, optical rotation, acid value, saponification value, and ester value[19]. All the evaluated parameters adhered to the standards stipulated by the French Association for Standardization[20].

- Phytochemical Screening

Phytochemical screening techniques offer insights into the diverse chemical constituents present within plant organs. To discern the chemical groups present in the aqueous extract of clove, a phytochemical screening was performed, following the methodologies outlined by Houghton and Raman [21].

Among the myriad chemical groups, some prominent ones include alkaloids, polyphenols (flavonoids, anthocyanins, tannins), saponins, steroids, coumarins, sterols, and terpenes.

2.2. Antibacterial Activity

Bacterial Strains

Four prevalent bacterial strains associated with human infections were chosen for this investigation. These strains, identified by the American Type Culture Collection (ATCC), were procured from the bacteriology laboratory of Hakim Saadane Hospital in Biskra, Algeria. The strains used were *Escherichia coli* (gram positive) (ATCC25922), *Staphylococcus aureus* (gram negatif) (ATCC25923), *Pseudomonas aeruginosa* (gram gram negatif) (ATCC27853), and *Klebsiella pneumonia* (gram negatif) (S912)

Diffusion Method

The antibacterial activity of essential oils was assessed using the agar diffusion method, also known as the aromatogram method, with the use of sterile cellulose discs [22]. The fundamental principle of this method is to ascertain the microorganisms' sensitivity to essential oils by measuring the diameter of inhibition zones.

For antibacterial tests to be accurate, young bacterial cultures in the exponential growth phase must be used. To achieve this, a few bacterial colonies were isolated from a plate using a platinum loop and then placed into a test tube containing 10 mL of nutrient broth agar (NBA). The mixture was then incubated for one hour at 37°C. Following incubation, well-isolated and identical colonies were transferred to 5 mL of sterile physiological water saline, using a platinum loop. The bacterial suspension was thoroughly homogenized, and its turbidity was visually assessed. The bacterial colonies were then spread onto agar plates using a swab. Sterile discs (Whatman paper n°3, with a diameter of 6 mm) were impregnated with a small quantity (10 µl) of essential oil previously diluted in dimethyl sulfoxide (DMSO) to various concentrations (undiluted, 1/2, 1/4, 1/8, 1/16, and 1/32 (v/v)). The discs were then placed onto the surface of the inoculated agar plates and incubated at 37°C for 24 hours[23].

Tetracycline (10 mg/disk) served as the standard antibiotic (positive control), and DMSO was used as the negative control. The manipulations were performed in triplicate to ensure method consistency. The sensitivity to different essential oils was categorized based on the diameter of the inhibition zones: non-sensitive (-) for diameters below 8 mm; sensitive (+) for diameters ranging from 9 to 14 mm; very sensitive (+ +) for diameters between 15 and 19 mm; and extremely sensitive (+++) for diameters exceeding 20 mm[24].

2. 3. Corrosion Inhibition

In this study, commercial mild steel strips with the following composition were utilized: 0.12% C, 1.68% Mn, 0.051 Cr, 0.012% P, 0.04% Ni, and the remaining portion comprised of iron. Specimens measuring 30 mm × 30 mm × 15 mm were employed for weight loss measurements and subsequent surface analysis.

The specimens were meticulously polished using varying grades of emery papers, subjected to degreasing with acetone, and then thoroughly rinsed with distilled water. A test solution of 1M HCl was prepared through the dilution of 37% analytical grade HCl with distilled water. For the present study, the concentration of clove oil used was 1 g/L, and a blank solution was also prepared for comparative purposes.

- Weight Loss Method

Each prepared specimen was initially weighed, and its mass, total surface area, solution properties (temperature, concentration, etc.), and test duration were documented. Subsequently, the specimen was immersed in a beaker containing 100 ml of the solution. At designated intervals, the specimen was retrieved from the solution, rinsed with water, and any resultant corrosion on the metal surface was gently removed using a soft toothbrush. After rinsing with distilled water and ensuring the specimen was dry, its mass was re-recorded, and the new measurement was logged into the dataset. The selected time values for our tests encompassed 2, 24, 48, 120, 144, and 168 hours.

3. Results and discussion

3.1. Chemical Screening

Phytochemical screening entails a qualitative analysis directed at discerning the presence or absence of various chemical groups within an aromatic and medicinal plant. These chemical families comprise flavonoids, quinones, alkaloids, tannins, saponins, triterpenes, and sterols. The methodology hinges on precipitation, solubility, and coloration reactions[25]. The outcomes of the chemical screening for the aqueous extract are presented in Table 1.

Table 1: Phytochemical screening of cloves

Active principle	Results
Saponosides	+
Flavonoids	+
Saturated sterols and terpenes	-
Tannins	+
Cardenolides	-
Anthracénosides	+
Glucosides	+
Alcaloïdes	+

The results presented in the above table highlight the presence of flavonoids, saponosides, tannins, anthracenosides, and glucosides within the *Syzygium aromaticum* plant. These plant-derived compounds have demonstrated antimicrobial effects against a diverse spectrum of bacteria and could potentially serve as corrosion inhibitors as well.

The absence of sterols, terpenes, and cardenolides in the *Syzygium aromaticum* plant is also noteworthy. These findings align with the observations made by Niranjana Das [26], who similarly reported the presence of flavonoids, tannins, and saponins within *Syzygium aromaticum*.

3.2. Organoleptic characteristics and Physico-chemical property

The following table (Table 2) succinctly encapsulates the organoleptic attributes of clove essential oil, encompassing its appearance, color, and aroma.

Table 2: Organoleptic characteristics of cloves essential oil

extraction process	aspect	color	odor
Hydro-distillation	Clear mobile liquid	Pale yellow	Spicy, characteristic of eugenol
Soxhlet	Clear mobile liquid	green	Spicy, characteristic of eugenol

The key findings pertaining to yield and physicochemical properties have been consolidated within Table 3.

Table 3: Physicochemical properties of cloves essential oil

properties	Extraction process	
	Hydrodistillation	soxhlet extraction
Yield	11.25%	25.11%
Density (20°C)	1.00964	1.01164
pH	6.27	4.89
Refractive index (20°C)	1.3775	1.3735
Optical rotation	- 4.75	- 4.6
Miscibility in ethanol	1/1	1/1
Saponification index (Is)	116.4	154.27
Acid index	17.95	49.36
Ester Index	98.45	104.91

The yields of essential oil obtained through hydrodistillation (EO1) and Soxhlet (EO2) extraction from clove buds are 11.25% and 25.11% respectively. It's noteworthy that the yield of essential oil obtained by Soxhlet extraction is higher than that obtained through hydrodistillation. Similar findings of higher yields of clove oil have been reported in several previous studies [27-29].

Based on the obtained results, it can be observed that the physicochemical parameters of clove bud essential oil align with those outlined by the French Association for Standardization [20].

The physicochemical analysis of clove essential oil reveals that the refractive index at 20°C falls within a specific range, where the overall clove essential oil exhibits values between 1.3775 and 1.3735. The refractive index serves as an identification tool and purity indicator for essential oils and various liquid compounds.

The refractive index primarily depends on the content of monoterpenes and oxygenated derivatives in the essential oil. A high content of monoterpenes corresponds to a higher refractive index. Some authors [30] suggest that the low refractive index of EO indicates its low light refractivity, which could enhance its suitability for use in cosmetic products.

As depicted in Table 3, the specific optical rotation $[\alpha]_D$ of clove essential oils extracted through hydrodistillation and Soxhlet extraction were -4.75° and -4.6° respectively, indicating slight optical activity or levorotation.

Regarding solubility in 98% ethanol, the outcome indicates that the essential oil is soluble at a 1:1 ratio.

3. 3. Anti-bacterial activity

The susceptibility of the four pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*, and *Klebsiella pneumonia*) to clove essential oil extracted through both hydrodistillation and Soxhlet extraction was demonstrated using the disc diffusion technique.

Tables 4 and 5 present the results of the bacterial sensitivity test to the extracted essential oils. The values indicated are the average of three measurements. The inhibitory action of the tested oils is manifested by the formation of an inhibition zone around the paper disc impregnated with the essential oils.

	Volume Concentrations (v/v)	Bacterial strains							
		Gram-positive bacteria				Gram-negative bacteria			
		S. aureus		E.Coli		P.aeruginosa		K.pneumonia	
		Inhibition zone (mm)	Relative inhibition zone (%)	Inhibition zone (mm)	Relative inhibition zone (%)	Inhibition zone (mm)	Relative inhibition zone (%)	Inhibition zone (mm)	Relative inhibition zone (%)
EO1	Pur	15	65.22	14	87.5	14	87.5	14	71.06
	0.5	14	60.87	13	81.25	11	68.75	12	60.91
	0.25	14	60.87	12	75	9	56.25	12	60.91
	0.125	10.5	45.65	11	68.75	9	56.25	9.5	48.22
	0.0625	9.75	42.39	10.75	67.18	8.5	53.12	9	45.68
	0.312	9.5	41.30	10	62.5	8	50	7.5	38.07
ATB Tetracycline	(10 mg/disk)	23	100	16	100	16	100	19.7	100

Table 5: Average of the diameter of inhibition zone (in mm) and Relative inhibition zone (%) for cloves essential oil extracted by soxhlet against the tested bacteria

	Concentrations	Bacterial strains							
		Gram-positive bacteria				Gram-negative bacteria			
		S. aureus		E.Coli		P.aeruginosa		K.pneumonia	
		zone	inhibition	zone	inhibition	zone	inhibition	zone	inhibition
Volume (v/v)	Inhibition (mm)	Relative zone (%)	Inhibition (mm)	Relative zone (%)	Inhibition (mm)	Relative zone (%)	Inhibition (mm)	Relative zone (%)	
EO2	pur	14	60.87	14	87.5	11	68.75	11.5	58.37
	0.5	12	52.17	13	81.25	10	62.5	11	55.84
	0.25	10	43.47	12	75	9	56.25	9	45.68
	0.125	9	39.13	11	68.75	8.5	53.125	8.5	43.15
	0.0625	8.75	38.04	8	50	8	50	8	40.61
	0.0312	7.5	32.6	7	43.75	7	43.75	7	35.53
ATB Tetracycline		23	100	16	100	16	100	19.7	100

The results indicated that the antibacterial efficacy varied based on the targeted bacteria, including *Escherichia coli*, *Staphylococcus*, *Pseudomonas*, and *Klebsiella pneumoniae*. Notably, all tested bacteria displayed sensitivity to both pure clove essential oil and to the volumetric concentration 0.5 and 0.25. Conversely, the tested bacteria exhibited relatively higher resistance to the volumetric concentrations (v/v) of 0.0625 and 0.312 of the essential oil extracted via the Soxhlet apparatus. This resistance was somewhat more pronounced compared to the oil extracted through hydrodistillation, leading to notably reduced inhibition zones (ranging between 7 and 8 mm). The observed outcomes underscored the antibacterial impact of the oil extracted via hydrodistillation against these bacteria.

Concerning *Staphylococcus aureus*, this bacterial strain demonstrated varying degrees of susceptibility to the two essential oils. *Escherichia coli* and *Pseudomonas aeruginosa* exhibited antibacterial activity that approached that of the positive control (Tetracycline, 10 mg/disk) when exposed to pure essential oil.

It's important to note that the essential oil extracted through hydrodistillation exhibited the highest level of antibacterial activity.

Comparing the relative inhibition zone of the essential oils studied with the antibiotic used as a positive control, EO1 exhibited relative inhibition rates of 65.22% against *S. aureus*, 87.5% against both *E. coli* and *P. aeruginosa*, and 71.06% against *K. pneumoniae*. EO2, on the other hand, demonstrated relative inhibition rates of 60.87% against *S. aureus*, 87.5% against *E. coli*, 68.75% against *P. aeruginosa*, and 58.37% against *K. pneumoniae*.

The minimum inhibitory concentration (MIC) of EO1 was relatively consistent for the three bacterial strains (*S. aureus*, *E. coli*, and *K. pneumoniae*), ranging from 0.0312 to 0.0625 (v/v). *Pseudomonas aeruginosa*, however, displayed some resistance to these concentrations, with an MIC of 0.25 (v/v). This resistance is attributed to the unique structure of *Pseudomonas aeruginosa*'s outer membrane, composed of two layers of lipoproteins and polysaccharides, rendering it highly resilient to the penetration of antibiotics agents.

While EO2 also demonstrated efficacy, it was not as potent as EO1. Its maximum relative inhibition rates were 87.5% for *E. coli*, 68.75% for *P. aeruginosa*, 60.87% for *S. aureus*, and 58.37% for *K. pneumoniae*. Notably, the MIC for EO2 was 0.25 (v/v) for all four bacterial strains.

The results of this activity showed that no zone of inhibition was observed for DMSO. which suggests that it is not involved in the antibacterial activity of essential oils. Indeed, DMSO is considered to be a sterile solvent, which does not promote bacterial proliferation.

3.4. Study of corrosion inhibitory efficiency on X70 steel

Aggressive solutions of HCl (1N) were prepared by dilution of 37% HCl acid with distilled water.

The samples were positioned on non-metallic supports and immersed in a 1M HCl solution, with a volume of 100 ml, aerated, and containing 0.1g of clove essential oil.

Weight loss measurements serve as the primary approach to examining the corrosion inhibition of a metal in an electrolytic solution. This method is advantageous due to its simplicity and minimal equipment requirements. The approach involves quantifying the mass loss (Δm) of samples with a surface area (S) during immersion in a corrosive solution over a specified time period (t). The corrosion rate (V_{Corr}) is calculated using the following formula (1):

$$V_{Corr} = \frac{\Delta m}{S t} \quad (1)$$

Or V_{Corr} : Corrosion rate in (g / cm².h)

S: Surface subjected to the test in (cm²).

t: time of the experiment in (h).

Δm : the difference between the mass of the sample before and after immersion in (g)

The inhibitory efficiency (E %) of a compound is determined by comparing the corrosion rates of the electrochemical system in the presence and absence of the inhibitor. It is computed using the following formula (2):

$$E\% = \frac{V_0 - V_{inh}}{V_0} 100 \quad (2)$$

Where: V_0 and V_{inh} are respectively the values of the corrosion rate in the absence and the presence of the inhibitor.

Influence of immersion time on inhibitory efficacy

Considering the importance of this parameter, we plotted the curve of the corrosion rate (figure 1) and the inhibitory efficiency (figure 2) as a function of the immersion time. The choice of the concentration (1 g / l) of inhibitor is justified by the fact that at this concentration, the value of efficacy is maximized.

The curves of the variation of the corrosion rate as a function of time show the decrease in the corrosion rate as soon as the essential oil is added to the medium at different times (2, 24, 48, 120, 144, and 164 h). To this end, we can say that a large amount of essential oil adsorbs in the early stages. Afterward, the corrosion rate decreases slightly and stabilizes with increasing time indicating that the adsorption of oil on the steel surface forms a barrier and protective layer.

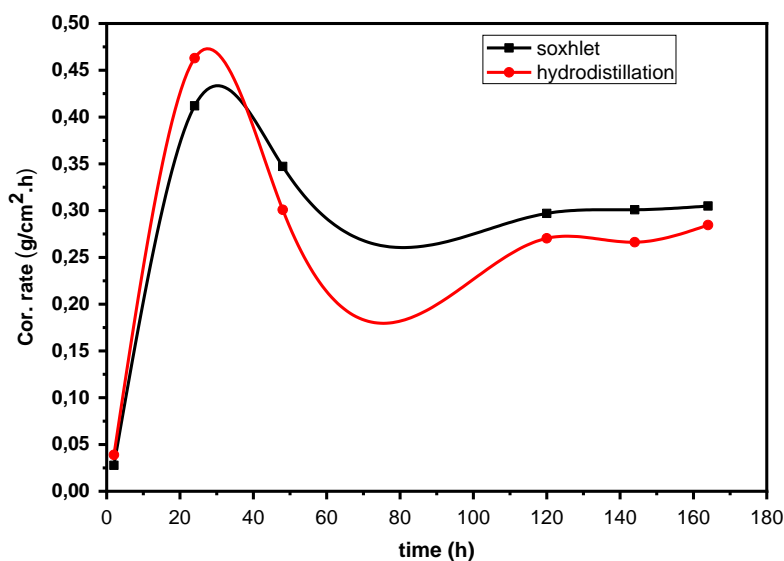


Figure 1: variation of the Corrosion rate as a function of the immersion time

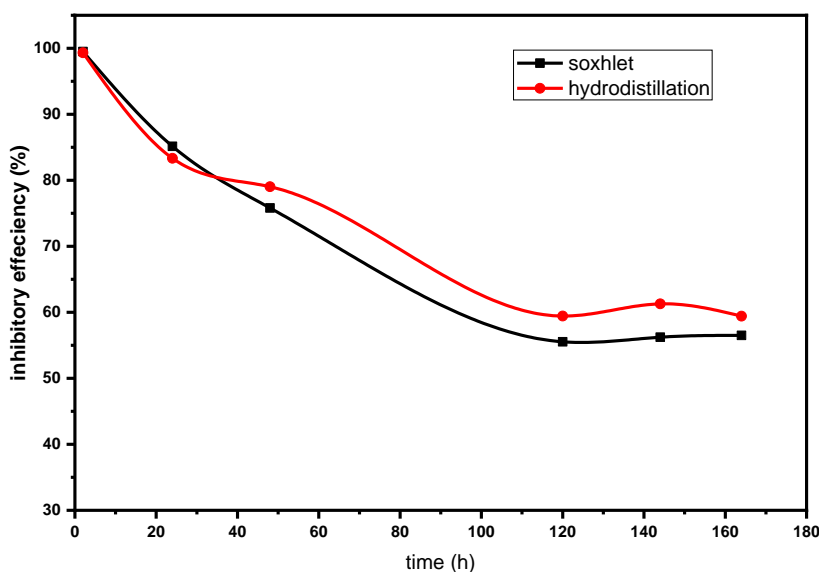


Figure 2: Curve of efficiency depending on time immersion

Figure 2 illustrates the fluctuation in corrosion inhibitory efficiency relative to the duration of inhibitor immersion. The outcomes unmistakably underscore the commendable corrosion-inhibiting attributes of clove essential oil on steel.

The interpretation of Figure 1 reveals a reduction in the corrosion rate in the presence of an essential oil (EO) inhibitor compared to its absence. This observation points to the extract's pronounced ability to inhibit steel corrosion in 1M HCl.

This phenomenon can be attributed to the escalated adsorption of the extract onto the steel surface, preventing its disintegration. The decline in corrosion rate (V_{corr}) with extended immersion time is attributed to the augmentation of metal surface coverage through the adsorption of inhibitor molecules [31]. However, the protective effectiveness (Figure 4) displays an initial enhancement at very short immersion times ($t = 2\text{h}$), followed by a subsequent decrease as immersion time prolongs.

At 2-hour immersion duration, the inhibitor showcased a peak protection efficacy of 99.3%. It is noteworthy that both weight loss and corrosion rate increased with prolonged immersion across the tested specimens.

The effectiveness of clove essential oil as a corrosion inhibitor for steel is attributed to its strong adsorption onto the steel surface. Clove essential oil molecules possess functional groups, such as hydroxyl (-OH) and methoxy (-OCH₃), that can form strong hydrogen bonds with the metal surface. This strong adsorption creates a protective layer that hinders the interaction between corrosive agents and the steel substrate.

As more clove essential oil molecules adhere to the steel surface, the adsorption intensity increases until it reaches a saturation point. At this point, the available adsorption sites on the steel surface are occupied, and further clove essential oil molecules cannot effectively attach. However, the presence of Van der Waals forces can induce interactions between the adsorbed clove essential oil molecules themselves. These interactions can lead to the formation of aggregates or clusters of clove essential oil molecules on the steel surface.

While Van der Waals forces are weaker than hydrogen bonds, they can still contribute to the stability of the protective layer. However, they can also have an unintended consequence. As the CEO molecules form aggregates, the effective coverage area of the inhibitor may decrease. This is because the aggregates may not be as tightly packed as the individual CEO molecules, leaving some gaps or bare patches on the steel surface.

Consequently, some inhibitor molecules may disengage from the surface, especially in the presence of strong mechanical stresses or aggressive corrosive environments. This disengagement can lead to a reduced effective coverage area of the inhibitor and a consequent diminution in efficacy.

4. Conclusion

The present study conducted a comprehensive investigation of *Syzygium aromaticum* essential oil, encompassing phytochemical analysis, physicochemical characterization, evaluation of antibacterial properties, and exploration of corrosion inhibition potential. The study yielded key conclusions as follows:

- Phytochemical screening revealed a rich profile of flavonoids, tannins, saponins, anthracenosides, glucosides, and alkaloids in cloves.
- Hydrodistillation and continuous extraction (Soxhlet) yielded approximately 11.25% and 25.11% essential oil, respectively.
- Antimicrobial activity assessment demonstrated notable efficacy against all tested strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*), with bacterial sensitivity increasing as essential oil concentration increased.
- The study of inhibitory efficiency using the mass loss method confirmed the role of clove essential oil as an adsorption inhibitor for mild steel corrosion in a 1 M HCl solution.

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