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A STUDY ON ANTI-PUTREFACTION PROPERTY OF *DODONAE ANGUSTIFOLIA*

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ABSTRACT

The ethanolic extract of *Dodonae angustifolia* was used on carbon steel in acidic medium for anti-putrefaction study. The phyto constituents, antimicrobial activity were examined and GC-MS analyses were carried out for the plant extract. The anti-putrefaction property of *Dodonae angustifolia* was determined by weight loss method, for various concentrations of the extract with Zn^{2+} ion and in the presence of additives such as tartaric acid, citric acid and lactic acid in 0.5M HCl medium. The resistive film that protects the surface of the metal was confirmed by the electrochemical studies such as FT-IR, UV-Visible and fluorescence spectra. The film formation is confirmed by the surface morphology SEM analysis, which confirms the formation of complex between the metal cation, additive and the compounds present in the extract of *Dodonae angustifolia*.

KEYWORDS: Anti-putrefaction, Dodonae angustifolia, Carbon Steel, FT-IR, GC-MS, SEM.

INTRODUCTION

Now-a-days metals are used almost in all fields of technology, industries and home appliances. Corrosion (putrefaction) is a natural deterioration process which can be controlled but cannot be completely prevented. In past years, chemical inhibitors were used to control corrosion. Later it was found that the chemical inhibitors were hazardous and toxic. So eco-friendly, non–toxic chemical inhibitors were used. In recent days, green inhibitors from natural products have been used as inhibitors which are eco-friendly and non-toxic.[1-3] *Dodonae angustifolia* is a plant which belongs to the family '*Sapindaceae*'. It is widely distributed in Tropical, subtropical regions of Africa, America, Southern parts of Asia and Australia. The leaves of the plant are used as plasters for wounds.[4] The present objective is,

➢ to evaluate the anti-putrefaction property of *Dodonae angustifolia* (DL) by the inhibition efficiencies of DL-Zn^{2+}-Additives(citric, lactic, tartaric acids) systems in resisting the corrosion on carbon steel in acidic medium.

➢ to analyse the protective film formed on the metal surface by FT-IR, UV and fluorescence spectra.

➢ to study the surface morphology by SEM analysis.

Plant Profile

Table. 1. Plant Profile.

Heading	Details
Name	*Dodonaea angustifolia*
Botanical name	*Dodonaea angustifolia*
Family	*Sapindaceae*
Name in Tamil	Virali
Name in Hindi	Sanatta
Name in English	*Dodonaea angustifolia*
Distribution	It is found in tropical, subtropical and warm regions of Africa, America, Southern parts of Asia and Australia

Fig. 1: *Dodonaea angustifolia.*

MATERIALS AND METHODS

Metal Specimens

The carbon steel specimens with the composition (wt%) of S-0.026 , P -0.06, Mn- 0.4, C- 0.1 and balance iron are taken. The dimensions of the metal active surface are 1.2 X 4.1 X 0.2 cm which was used for weight loss measurements. The carbon steel specimens were mechanically polished, washed in double distilled water and degreased with acetone and used for the weight loss method and surface examination studies.

Extraction

The leaves of *Dodonae angustifolia* were collected from Pachaimalai hills. The leaves were washed thoroughly for about 7 times in the running tap water and it was taken and dried under shade. About 100g of the powder was soaked in 500ml of ethanol under cold percolation method. At regular intervals of time the extract was filtered and distillation was carried out to collect the crude extract. The extract was stored in an amper bottle and refrigerated.[5] The extract is taken for phytochemical screening to analyze the presence of phyto-constituents that are present in the plant extract.[6]

Antimicrobial Activity: An antimicrobial is a substance that kills or inhibits the growth of micro-orangisms such as bacteria, fungi or protozoans. Five bacterial and five fungal species are used to screen the possible antimicrobial activity for the ethanolic extracts of the medicinal plant *Dodonaea angustifolia*. Both gram positive and gram negative bacteria are chosen namely Escherichia coli (E. coli), Staphylococcus aureus (S,aureus), Pseudomonas aeruginosa (P. aeruginosa), Bacillus subtilis(B. subtilis), Proteus vulgaris (P. vulgaris). Similarly the fungal species that have been taken are Aspergillus niger (A. niger), Cochliobolus lunata (C. lunata), Alternaria solani (A. solani), Candida krusi (C. Krusi), *Candida* albicans (C.albicans). The anti-microbial screening of the ethanolic extract of *Dodonaea angustifolia* was investigated through disc diffusion method. The assay consisted ofboth anti-bacterial and anti-fungal evaluations.[6]

Weight-Loss Method

Determination of Corrosion Rate

Weight loss measurements were carried out using an ACCULAB Electronic top loading balance, with readability/sensitivity of 0.1 mg in 210g range. The specimens were immersed in beaker containing 100ml acid solutions without and with different concentration of *Dodonae angustifolia* leaves extract using hooks. Before it was immersed, the specimens were cleaned and the weight is recorded. After 72 hours, the test specimens were removed and then washed with double distilled water, dried and reweighed. The average mass loss of two parallel carbon steel specimens were obtained.[7]

From the change in weight of specimens the corrosion rate was calculated using the following relationship,

Corrosion Rate = $[87.6 \times W] / [A \times T \times D]$ (mpy)

W = Loss in weight in mg

A = Surface area of the specimen (cm^2)

T = Time in hours

D = Density ($7.2g/cm^3$)

Corrosion Inhibition Efficiency (IE) was then calculated using the equation

$IE = 100[1-(W_2/W_1)]$ %

Where,

W_1 = Corrosion rate in the absence of inhibitor and

W_2 = Corrosion rate in the presence of inhibitor

Infra Red (IR) Spectroscopy

Infrared spectroscopy is a well developed technique to identify chemical compounds. The specimens were suspended by means of hooks in solution having with and without inhibitor for 72 hours. After 72 hours the specimen were taken out. Then the film formed on the metal surface was scratched off and taken for FT-IR spectral study.

UV- Visible Spectroscopy

The possibility of the formation of film on the metal surface was examined by mixing the respective solution and recording their UV-visible absorption spectra using Lambda 35 UV-visible spectrophotometer which is a PC controlled single beam scanning spectrophotometer. It covers wavelength range from 200 nm to 1000 nm with a setting accuracy of ± 1 nm.

Fluorescence Spectroscopy: Fluorescence spectra of solutions and also the films formed on the metal surface were recorded using Jasco F-6300 spectrofluorometer.

SEM Analysis: The carbon steel specimen immersed in blank solution and in the inhibitor solution for a period of one day was removed, rinsed with double distilled water, dried and observed in a scanning electron microscope to examine the surface morphology.

RESULT AND DISCUSSION

Qualitative Preliminary Phytochemical Screening

The results of the screening of the ethanolic extract of the leaves of *Dodonae angustifolia* (DL) are shown in Table (2). It illustrates that the active compounds such as alkaloids, carbohydrates, tannins, proteins, amino acids, glycosides, saponins, terpinoids, phenolic compounds and flavonoids are present. These active constituents are responsible for the anti-putrefaction ability of *Dodonae angustifolia* (DL).

Table. 2. Qualitative Preliminary Phytochemical Screening of *Dodonae angustifolia.* (DL).

Phyto-constituents	Inferrence	Phyto-constituents	Inferrence
Carbohydrates	+	Anthraquinone Glycosides	+
Reducing Sugar	+	Saponin Glycosides	+
Hexose Sugar	+	Cyanogenic Glycosides	-
Non-Reducing Sugar	-	Alkaloids	+
Proteins	+	Tannins	+
Amino Acids	+	Phenolic Compounds	+
Tyrosine	-	Flavonoids	+
Steroids	+	Terpenoids	+
Glycosides	+	Saponins	+

Antimicrobial Activity

Antibacterial Activity: The results given below are the anti-bacterial activity for the various concentrations 50, 100, 200 µg/mL of the ethanolic extract of *Dodonaea angustifolia*. It is observed that the ethanolic extract of the plant at 200 µg/mL of concentration has a high inhibition activity against the bacteria. On comparing with the standard tetramycin, the inhibitive property of the plant *Dodonaea angustifolia* has been analyzed.

Table. 3: Variation in Zone of Inhibition of *Dodonaea angustifolia* against the bacterial species.

Bacterial Species	Zone of Inhibition (mm)		
	50 µg/mL	50 µg/mL	50 µg/mL
E.coli	7	9	13
S.aureus	6	6	7
P. aeroginosa	6	7	11
B.subtitis	10	12	13
P. vulgaris	6	8	9

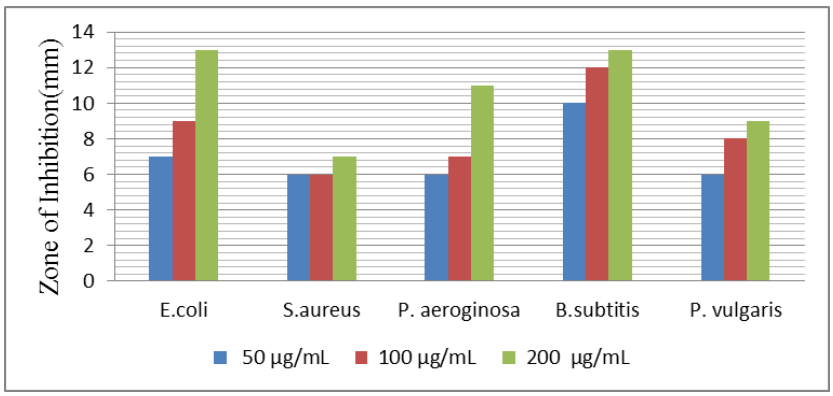


Fig. 2: Variation in Zone of Inhibition of *Dodonaea angustifolia* against the bacterial species.

Table. 4: Inhibition Efficiency of *Dodonaea angustifolia* at 200µg/mL of concentration.

Bacterial species	Tetramycin-standard	Ethanol-solvent	Zone length 200µg/ml (mm)	Inhibition efficiency (%)
E.coli	14	5	13	57
S.aureus	14	5	7	14
P. aeroginosa	15	6	11	33
B.subtitis	13	5	13	61
P. vulgaris	14	6	9	21

DL-E.coli **DL-S.aureus** **DL-P.aeroginosa** **DL- B.subtitis** **DL-P.vulgaris**

Fig. 3. Antibacterial activity by Disc Diffusion Method.

Antifungal Activity

The results given below are the anti-fungal activity for the various concentrations 50, 100, 200 µg/mL of the ethanolic extract of *Dodonaea angustifolia*. It is observed that the ethanolic extract of the plant at 200 µg/mL of concentration has a inhibition activity against the fungal species such as A. niger and C. lunata. On comparing with the standard ketoconazole, the inhibitive property of the plant *Dodonaea angustifolia* has been analyzed.

Table. 5: Variation in Zone of Inhibition of *Dodonaea angustifolia* against the fungal species.

Fungal Species	Zone of Inhibition (mm)		
	50 µg/mL	100 µg/mL	200 µg/mL
A. niger	7	9	11
C. lunata	-	-	8
A. solani	-	-	-
C. Krusi	-	-	-
C. dupliniesis	-	-	-

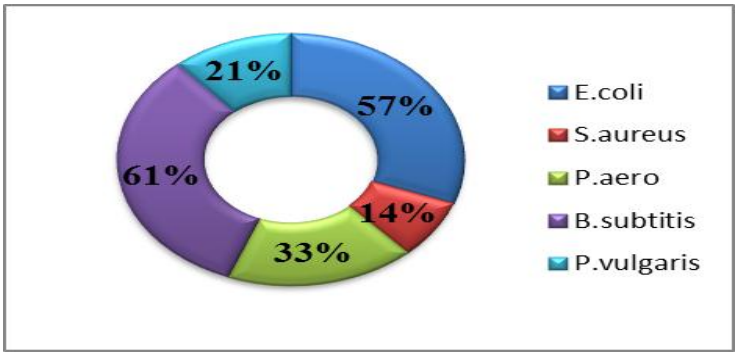


Fig. 4: Inhibition Efficiency of *Dodonaea angustifolia* at 200µg/mL of concentration.

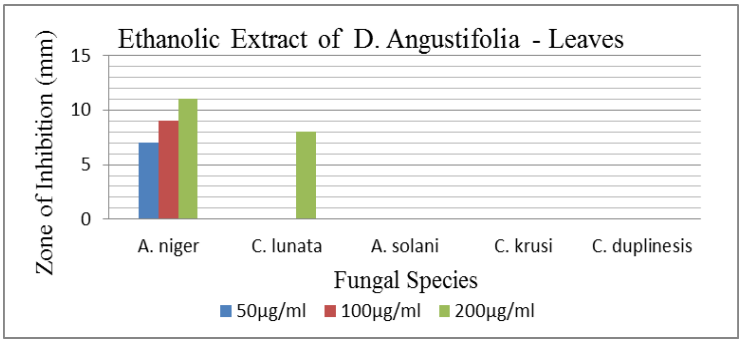


Fig. 5: Variation in Zone of Inhibition of *Dodonaea angustifolia* against the fungal species.

GC-MS Analysis

GC-MS chromatogram of the ethanolic extract of *Dodonaea angustifolia* showed 20 peakswhich indicates the presence of 20 active phyto-constituents in Fig.6. The 20 active constituents with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) which are present in the ethanolic extract of *Dodonaea angustifolia* are presented in Table - 6. On comparison of the mass spectra of the constituents with the NIST library the 4 predominant constituents were characterized and identified. The structure and nature of the compound are presented in Table - 7.

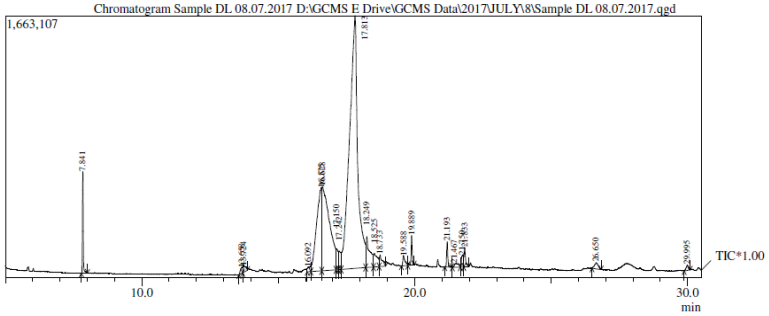


Fig. 6: GC-MS chromatogram of the ethanolic extract of the leaves of *Dodonaea angustifolia.*

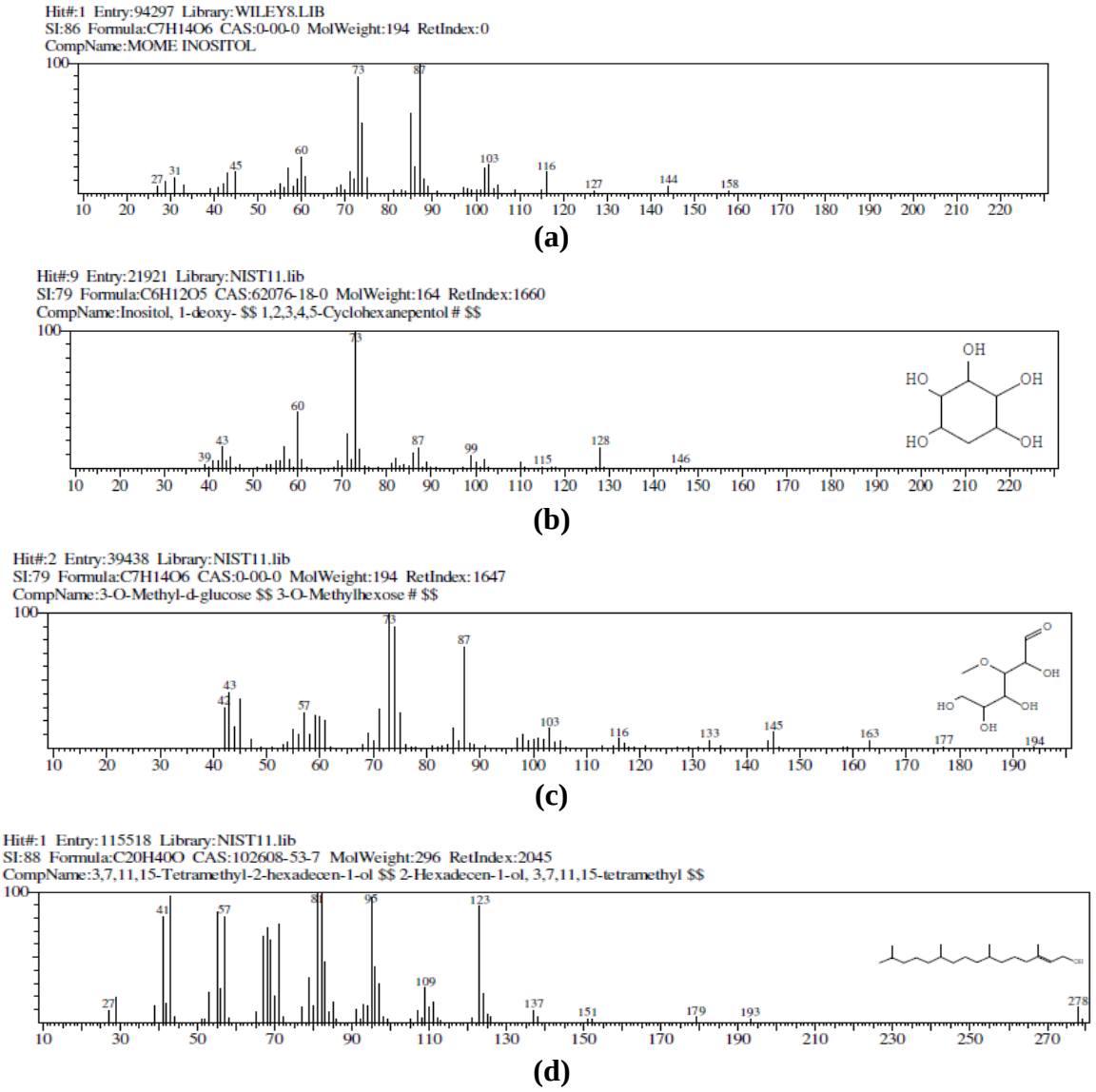


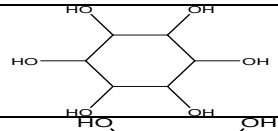
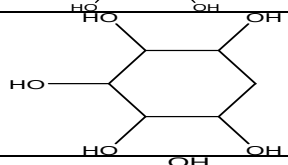
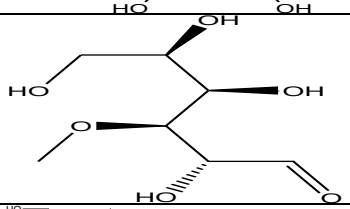
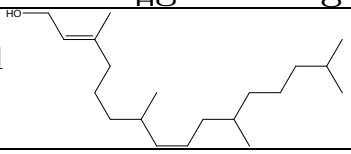
Fig. 7. (a,b,c,d): Mass Spectrum of predominant phytoconstituents present in *Dodonae angustifolia.*

Table. 6: Phytocomponents identified in the ethanolic extract of the leaves of *Dodonaea angustifolia* by GC-MS.

Sl. No.	RT	Name of the Compound	Molecular Formula	Molecular weight	Peak Area %
1	7.841	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	2.12
2	13.685	Guanosine	$C_{10}H_{13}N_5O_5$	283	0.37
3	13.724	2-Amino-9-(3,4-Dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-Yl)-3,9-Di	$C_{10}H_{13}N_5O_5$	283	0.50
4	16.092	2-Dimethyl(ethenyl)silyloxytetradecane	$C_{18}H_{38}OSi$	298	0.41
5	16.575	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	11.57
6	16.628	Inositol, 1-deoxy-	$C_6H_{12}O_5$	164	16.84
7	17.150	1-Hexanol, 2-(hydroxymethyl)-	$C_7H_{16}O_2$	132	1.12

8	17.242	Carbonic acid, allyl octyl ester	$C_{12}H_{22}O_3$	214	1.10
9	17.813	Mome Inositol	$C_7H_{14}O_6$	194	55.50
10	18.249	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.13
11	18.525	Cyclohexanol, 2,3-dimethyl-	$C_8H_{16}O$	128	1.36
12	18.733	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	0.92
13	19.588	9-Octadecenoic Acid (Z)-	$C_{18}H_{34}O_2$	282	0.61
14	19.889	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.84
15	21.193	2-Hexadecen-1-Ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296	0.81
16	21.467	1,9-Nonanediol	$C_9H_{20}O_2$	160	0.44
17	21.750	9,12-Octadecadienoic Acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.71
18	21.833	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	0.75
19	26.650	trans-Geranylgeraniol	$C_{20}H_{34}O$	290	0.62
20	29.995	Isobenzofuran, 1,3,3a,4,5,7a-hexahydro-5-methyl-4-(1-propenyl)	$C_{12}H_{18}O$	178	0.29

Table. 7: The structure and nature of the predominant phytocomponents identified in the ethanolic extract of the leaves of *Dodonaea angustifolia* by GC-MS.

Sl. No.	Name of the Compound	Structure	Nature
1.	Mome inositol		Inositol
2.	1,2,3,4,5-Cyclohexanepentol		Inositol
3.	3-O-Methyl-d-glucose		Carbohydrate
4.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Phytol

Weight Loss Measurements

Effect of Inhibitor Concentration

The Weight loss method of monitoring corrosion rate is useful because of its simple application and reliability.[8] Inhibition efficiency of carbon steel with different concentration of *Dodonae angustifolia* (DL) extract in 0.5M HCl at room temperature are presented in Table(8). From the table, it is clear that the corrosion rate decreases with an increase in inhibitor concentration, i.e. the corrosion inhibition enhances with the inhibitor concentration. This behavior is due to the fact that the adsorption and coverage of the inhibitor on the carbon steel surface increase with the inhibitor concentration. The maximum inhibition efficiency of

79% was obtained at 50ppm of *Dodonae angustifolia* and 50 ppm of Zn^{2+} in 0.5M HCl at 72 hours immersion period in Table 4. The table also illustrates the inhibition efficiency and corrosion rate of the inhibitor DL- Zn^{2+}- additives (Citric acid, lactic acid and tartaric acid). The high inhibitive performance of *Dodonae angustifolia* suggests a higher bonding ability of inhibitor on carbon steel surface. It is observed that the inhibition efficiency increases further due to the addition of additive, it is found that the concentration of additives at 30, 50 and 50 ppm of citric acid, lactic acid and tartaric acid shows IE's of 91, 88 and 89% respectively. The above results are graphically represented in Fig.(8 & 9) respectively.

Table. 8: Inhibition efficiency and corrosion rate of carbon steel in *Zn^{2+}* ion and DL in 0.5M HCl of various concentrations. Immersion Period = 72 hours.

Sl. No.	Concentration (ppm)		IE (%)	CR (mpy)	Concentration (ppm)		IE (%)	CR (mpy)
	DL	Zn2+			DL	Zn2+		
1	0	25	46	4.6	10	0	42	3.6
2	0	50	72	2.3	20	0	49	3.2
3	0	75	67	2.8	30	0	55	2.8
4	0	100	78	1.9	**40**	0	**62**	2.3
5	0	125	76	2.0	50	0	57	2.7
6	0	150	36	5.4	60	0	60	2.5
7	Blank			8.5		Blank		8.5

Table-9: Inhibition efficiency and corrosion rate of carbon steel in DL-*Zn^{2+}* ion and DL-*Zn^{2+}* - Additives in 0.5M HCl Immersion Period = 72 hours.

Con. of DL (ppm)	Con. of Zn^{2+} ion (ppm)				Con. of DL- Zn^{2+} ion (ppm)	Con. of additives (ppm)	Citric acid		Lactic acid		Tartaric acid	
	25		50									
	IE%	CR (mpy)	IE%	CR (mpy)			IE%	CR (mpy)	IE%	CR (mpy)	IE%	CR (mpy)
10	72	2.14	75	2.09	**50:50**	10	87	1.25	75	1.95	53	4.67
20	75	1.81	78	1.89		20	89	1.08	80	1.17	20	7.97
30	73	2.30	77	1.98		30	**91**	0.88	82	1.70	87	1.23
40	74	2.19	76	2.00		40	87	1.28	86	1.38	81	1.87
50	78	1.85	**79**	1.81		50	88	1.21	**88**	1.54	**89**	1.08
Blank		8.56		8.56	Blank					9.93		9.93

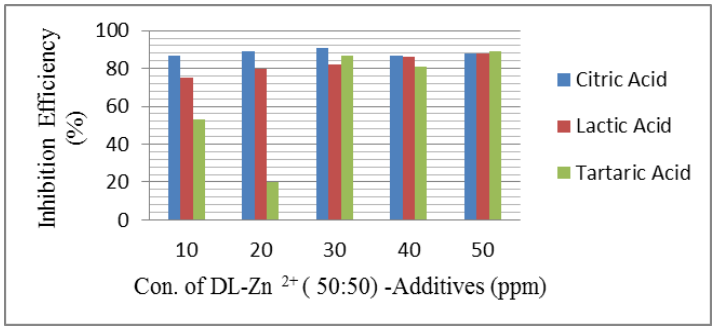


Fig. 8: Inhibition efficiencies of DL-Zn $^{2+}$ (50:50) -Additives (ppm) in various concentration.

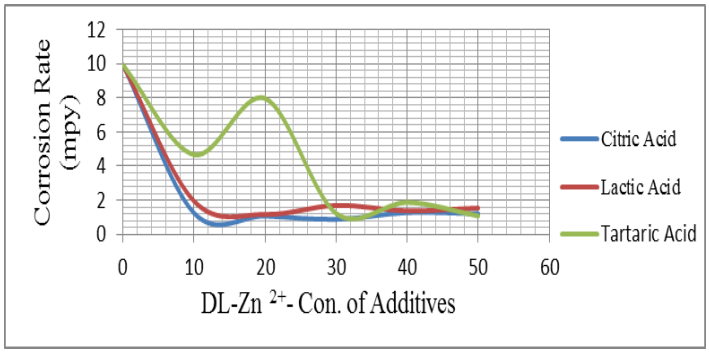


Fig. 9: Corrosion Rates of DL-Zn $^{2+}$ (50:50) -Additives (ppm) in various concentration.

Analysis of FTIR

FTIR is a technique used for identifying the functional groups associated with the adsorption of an inhibitor. The FTIR spectrum of the extract and the film formed on the surface of the metal immersed in 0.5M HCl in the presence of the inhibitor were taken. FTIR spectroscopy has been used to analyze the protective film formed on the metal surface.[9-11] The FTIR spectrum of the pure extract DL, DL-Zn $^{2+}$, DL-Zn $^{2+}$-Additives(citric, lactic and tartaric acids) are correlated in Fig.(10). For the pure extract the band observed at 3372.23 cm^{-1}. There is a decrease in the frequency from 3600.00 cm $^{-1}$ to 3372.23 cm^{-1} and the broadening of the band indicates the presence of intermolecular hydrogen bonding. Similar decrease pattern is observed for DL-Zn $^{2+}$, DL-Zn $^{2+}$-Additives(citric, lactic and tartaric acids), the bands were observed at 3357.46, 3384.50, 3371.65 and 3450.68 respectively. This decrease trend indicates the presence of intermolecular hydrogen bonding.

The bands at 1626.77 cm-1 and 1384.55 cm-1 which are due to the coupling of -C-O stretching and -C-O-H in-plane bending of the carboxylate anion are shifted to 1622.71cm-1 and 1404.94 cm-1 in DL-Zn^{2+}. Similar shift in bands were observed in DL-Zn^{2+}-citric acid(1611.85 & 1383.54cm^{-1}), DL-Zn^{2+}-lactic acid (1621.48 & 1459.62, 1402.14cm^{-1}) and

DL-Zn^{2+}-tartaric acid(1600.08 & 1440.75, 1385.34 cm^{-1}). The bands at 1031.98cm-1 and 848.30cm-1 (due to the ring oxygen) are shifted to 1066.59cm-1 and 859.51cm-1. Similar shift in bands were observed in DL-Zn^{2+}-citric acid(1113.41 &644.47^{-1}), DL-Zn^{2+}-lactic acid (1118.76 & 685.46cm^{-1}) and DL-Zn^{2+}-tartaric acid(1051.04, 1120.22 & 629.67cm^{-1}). This reveals that due to interaction between the metal and the active constituents there is a change in the chemical nature of the active constituents.[12]

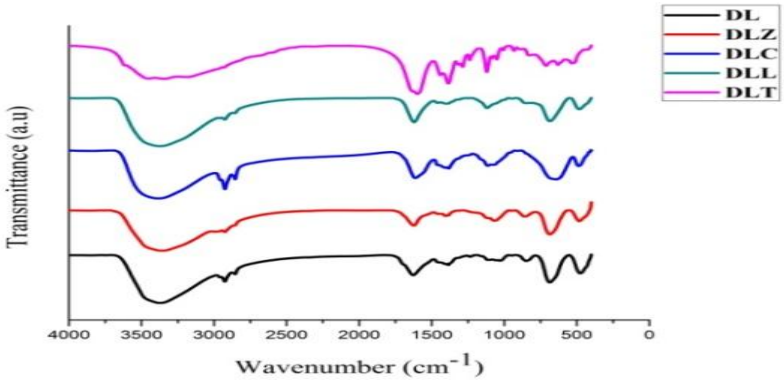


Fig. 10: FTIR Spectra Correlation Daigram.

Table. 9: FTIR spectrum data interpretation.

Types of Vibration	Peaks(cm^{-1})				
	DL	DL-Zn^{2+}	DL-Zn^{2+}-C	DL-Zn^{2+}-L	DL-Zn^{2+}-T
O-H Str	3372.23	3357.46	3384.50	3371.65	3450.68
C-H Str	2925.47, 2855.41	2989.66, 2925.66	2955.52, 2925.08, 2855.04	2926.23	-
C-O str	1626.77	1622.71	1611.85	1621.48	1600.08
C=C Str (aro.)	-	-	-	-	933.18
C-OH inplane bending	1384.55	1404.94	1383.54	1459.62, 1402.14	1440.75, 1385.34
C-O (ring vib.)	1031.98	1066.59	1113.41	1118.76	1051.04, 1120.22
C-H bending(c-0 ring vib.)	848.30, 686.57	859.51, 686.46	644.47	685.46	629.67
C-O Str (aro.Ether)	1235.25	-	-	-	1287.57, 1237.79
M-O bond	-	484.18	486.97	482.31	535.28

Analysis of UV-Visible absorption spectra

The UV-Visible absorption spectra of the solution containing DL, 50ppm DL – 50ppm Zn^{2+} , (50:50)ppm DL –Zn^{2+}-30ppm Citric acid, (50:50) DL - Zn^{2+} -50ppm lactic acid and (50:50) DL - Zn^{2+} -50ppm tartaric acid are correlated in Fig. 11. A peak appears at 245.20nm (0.7547au), when Zn^{2+} ion is added a peak appears at 241.55nm (0.3785au), the intensity

decreases. This indicates that a complex formation occurs between DL and Zn^{2+}ion. It is observed that, when additives are added to DL- Zn^{2+} - additives (citric acid, lactic acid, tartaric acid) systems the peak appears at 202.05nm (0.1732au), 204.50nm (3.9830au), 205.10nm (4.0000au)the intensity decreases on comparing with the DL and DL-Zn^{2+} systems respectively. This indicates the complexation of DL - Zn^{2+} & Additives (Citric, lactic, tarataric acids).

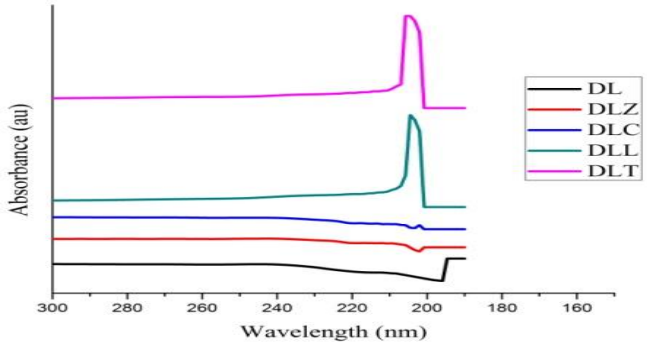


Fig. 11: UV-Visible Spectra Correlation.

Analysis of Fluorescence: Fluorescence spectrum is used to detect the presence of the inhibition complex formed on the metal surface. The λ_{ex} for the emission spectrum of the pure DL is found to be 516.51nm and for DL-Zn^{2+} the peak is obtained at 386.40nm. Fig 12 (c),shows the λ_{ex} for the emission spectrum of the 50ppm DL-50ppm Zn^{2+}-30ppm Citric acid, the peak is obtained at 336.99nm. There is a decrease in the intensity, which indicates the formation of protective film on the surface of the metal. The λex for the emission spectrum of the 50ppm DL-50ppm Zn^{2+}-50ppm Lactic acid, the peak is obtained at 367.19nm. Similarly, for Tartaric acid as additive the peak is obtained at 595.08nm for 50ppm 0f tartaric acid. There is a shift in the intensity on comparing with the pure DL fluorescence value indicates the formation of protective film on the surface of the metal.

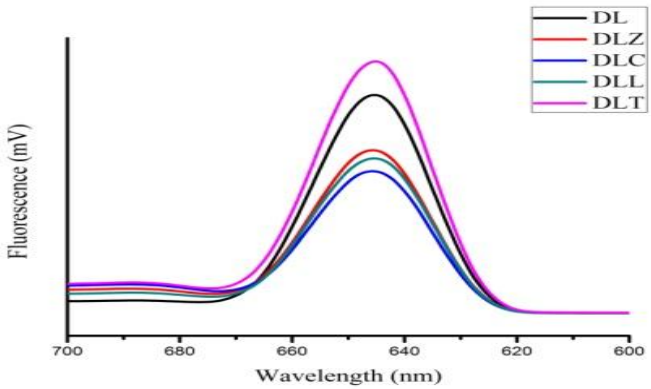


Fig. 12: Fluorescence Spectra Correlation.

Scanning Electron Microscope (SEM) Analysis: The texture and pore structure of the inhibited and uninhibited surface in acidic medium are shown in Fig.13 (a-e). It is confirmed that the inhibitor has formed a dense film over the metal surface.

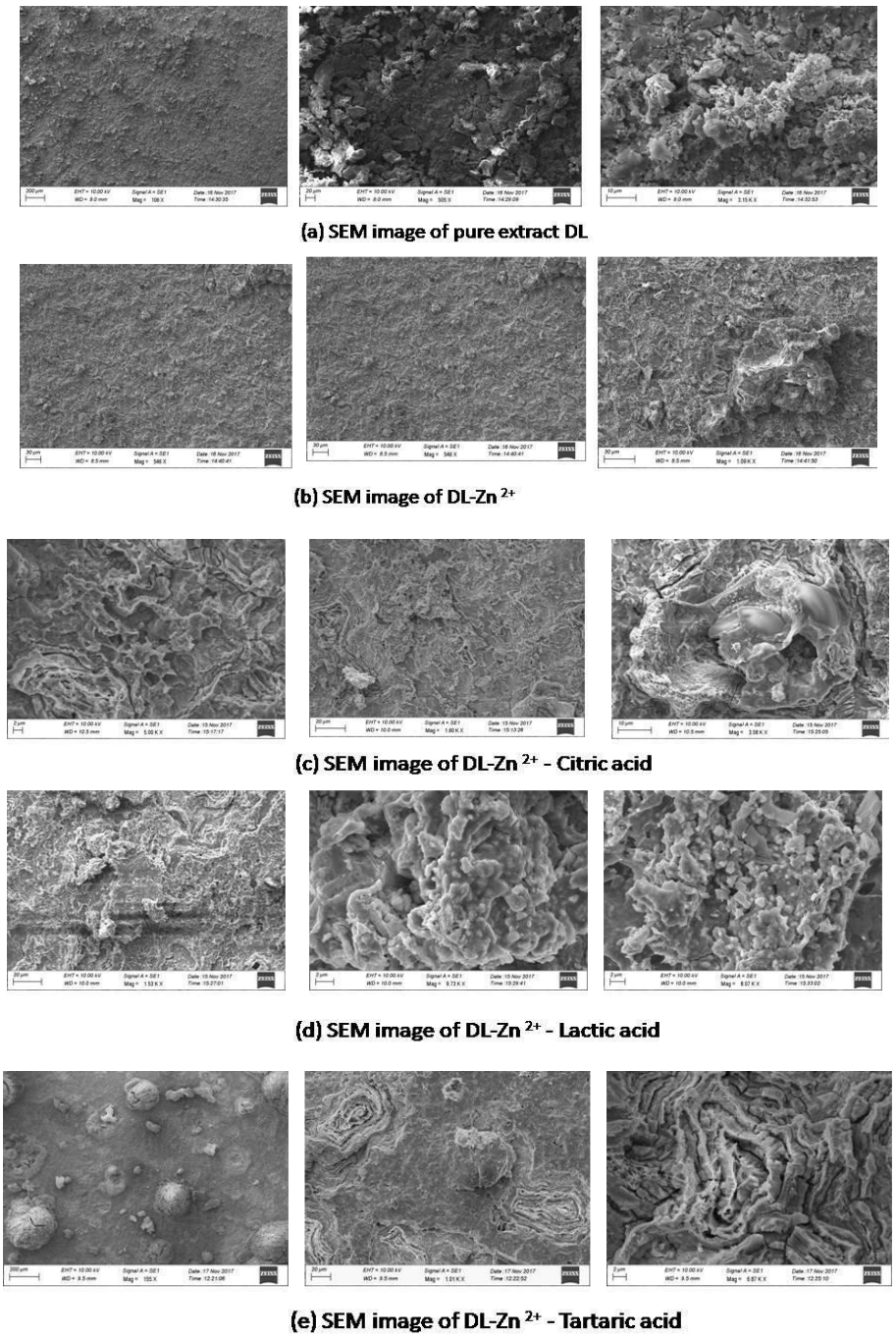


Fig. 13: SEM images of *Dodonae angustifolia* with Zn^{2+} ion and additives.

CONCLUSION

From the above study it is concluded that, *Dodonae angustifolia* has a good anti-putrefaction ability for carbon steel in 0.5 M HCl solution. The maximum efficiency was found to be 79%

at 50ppm DL + 50ppm Zn^{2+}. And the inhibitive efficiency was found to be increased from the maximum efficiency with the additives citric acid (91%), lactic acid (88%) and tartaric acid (89%). The shift in the peaks observed in FT-IR, UV-Visible spectra proves the formation of the film on the surface of the metal. The variation in the intensities observed in the fluorescence study results the formation of the film on the surface of the metal. The protective film formed on the metal surface is found to be denser by the SEM analysis. Thus, the SEM image finally confirms the formation of the protective film on the metal surface.

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