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Research Paper

Antimicrobial effects of *Azadirachta indica* leaves on corrosion causing microorganism (*Desulphovibrio* sp.)

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Abstract. The antimicrobial effects of *Azadirachita indica* crude leaves extract was investigated against *Desulphovibrio* species isolated from corroded water pipes in Minna, Niger State, Nigeria. Methanol and aqueous crude leaves extract of *A. indica* was prepared using cold maceration technique. Methanol crude extracts hindered corrosion of the metal in order of increasing concentration from 0.17 to 0.02%. Similarly, aqueous crude extract at 50 to 500 mg/ml inhibited corrosion of the metal from 0.15 to 0.02%. Phytochemical constituents of the crude extracts include saponins, anthraquinones, steroids, terpens, phenols, tannin, flavonoids, alkaloids and glycosides. Further investigations should be conducted to ascertain the chemical nature of the active compounds, including the minimal inhibitory and minimal bacteriocidal concentrations of the extracts.

Keywords: Antimicrobial, *Azadirachta indica*, corrosion, *Desulphovibrio* species.

INTRODUCTION

Corrosion is the deterioration of metal and non-metal as a result of its physicochemical interaction with its environment. Microbial influenced corrosion refers to microorganisms' involvement in the deterioration of metallic as well as nonmetallic materials. Microbiologically-influenced corrosion, or microbially-induced corrosion which is also known as bacterial corrosion, bio-corrosion, is not a new corrosion mechanism but it integrates the role of microorganism in the corrosion processes (Schwermer et al., 2008; Akpabio et al., 2011; Oyewole, 2011; Anichi and Abu, 2012).

Prerequisite for microbial influenced corrosion is the presence of microorganisms. If the corrosion is influenced by the activity of microorganism, further requirements are: (I) an energy source, (II) a carbon source, (III) an electron donor, (IV) an electron acceptor and (V) water (Beech et al., 2000; Javaherdashti et al., 2009).

Microorganisms present in biofilms can cause and influence corrosion because of their ability to utilize these metals as a source of carbon and energy requirement, this leads to deterioration and failure of steel pipes (Oyewole et al., 2011). The resulting metal deterioration is known as bio-corrosion or microbiologically influenced corrosion (MIC) (Beech and Gaylarde, 2008; Oyewole, 2011).

Microbiologically influenced corrosion is a serious industrial problem and affects diverse processes. It is a great challenge in petroleum, agricultural, food processing and water treatment industries. It has caused plants shutdown, equipment breakdown and products contaminations. It costs losses in the production, transportation and storage of water, petrochemicals, natural gas and oil in steel pipelines each year. The cost rate of corrosion in the world for one barrel of crude oil is 42 to 80%. This calls for the use of inhibitors in pipelines in order to protect the surface of metals used in oil and gas to prevent corrosion (Rajeev et al., 2012; Zarinabadi et al., 2012). Copper pitting corrosion in potable water plumbing systems is problematic because it can lead to fully-penetrating pinhole leaks, which burden consumers with the expense and frustration of repairing or replacing plumbing materials (Sarver and Edwards, 2012).

Bacteria frequently implicated in corrosion of metallic

piping are anaerobes that generate hydrogen sulphide, including sulphate reducing bacteria e.g. *Desulphorvibrio* sp., *Desulphotomaculum* sp. and *Desulphoromonas* sp. (Beech and Gaylarde, 2008; Oyewole, 2011).

Azadirachta indica (Neem) is a tree in the mahogany family Meliaceae. It is one of the two species in the genus Azadirachta, and is a native of India and Pakistan. Neem is a fast-growing tree that can reach a height of 15 to 20 m (49 to 66 ft), rarely to 35 to 40 m (115 to 130 ft). Its fruits and seeds are the source of neem oil. It is evergreen, but in severe drought it may shed most or nearly all of its leaves (Oyewole et al., 2011). Neem tree has been used for the treatment of ailments and for disinfective purposes.

MATERIALS AND METHODS

Collection of plant materials and processing

A. indica leaves were collected from the plantation of the Federal University of Technology, Minna. The fresh leaves were transported to Microbiology Laboratory, FUT Minna in a clean polyethene bag. The leaves were air dried for 2 days and ground into powder for further analysis.

Isolation of *Desulphovibrio* species

Desulphovibrio s*pecies* was isolated from corroded water pipes at Minna, Niger State water treatment plant. The smears from corroded pipes were aseptically collected into a sterile McCartney bottles and brought into the Microbiology laboratory, Federal University of Technology, Minna. Five-fold serial dilution was done 10^3 and 10^5 dilution factor were inoculated on a postgate agar and incubated anaerobically for 5 days at 37°C.

Standardization of the test organism

Pure isolates were subcultured into Postgate broth (inoculums) and incubated anaerobically for 5 days at 37°C. One millilitre of the inoculum was sub cultured into boilers tubes (large tubes for culturing) each containing 10 ml of postgate broth.

Extraction

Methanol and distilled water was used for the extraction of the *A. indica* leaves. One hundred gram (100 g) of the ground dried leaves was mixed with 500 ml of methanol or distilled separately for the extraction. The mixture of ground plant leaves and distilled water was left for 3 days in the conical flask while that of methanol was also left for 3 days, after which the mixture were filtered using muslin cloth. The filtrate was evaporated in vacuole at 45°C. Phytochemical screening was done on the aqueous and methanol extracts in accordance with the technique described by Kuta et al. (2013).

Determination of anticorrosion effects of the extracts on the test organism

One millilitre of the test organism was inoculated into 10 ml of postage medium in boilers tubes. Varying concentrations of the extracts (50, 100, 200, 300, 400 and 500 mg/ml) were introduced into the medium; a control was also set up. One point five gram (1.5 g) of sterile water pipes was introduced into the medium containing different concentrations of the extract and was allowed to stand under aseptic condition for 30 days. The rate of corrosion was determined using weight loss method, and the reading was done at every 3 days interval for 10 times.

Statistical analysis

A result obtained from the study was subjected to analysis of variance (ANOVA).

RESULTS

The phytochemical components found in the crude methanol and aqueous extracts include saponins, anthraquinones, alkaloid, steroids, terpens, phenols, tannin, flavonoids, phlobotannins and glycosides (Table 1).

Methanol crude extract of *A. indica* had activity on *Desulphovibrio* species in order of increasing (50 to 500 mg/ml) concentration as indicated in Figure 1.

Aqueous crude extract showed activity on *Desulphovibrio* species in order of increasing (50 to 500 mg/ml) concentration as indicated in Figure 2.

DISCUSSION

Metal corrosion has remained a great challenge to water treatment plants and their distribution channels, including petroleum industries. Several efforts have been made to reduce metal corrosion and attendant economic loss but all prove abortive. In this study the antimicrobial effect of *A. indica* leaves extract on *Desulphovibrio* species (bacteria involved in metal corrosion) was investigated. The result revealed the presence of the following phytochemical components, that is, tannins, saponins, anthraquinones, steroids, alkaloid, terpens, phenols, and glycosides. The presence of compounds like these in *A. indica* leaves extract may have contributed substantially to the inhibitory action exhibited against the test organism

Table 1. Phytochemical components of aqueous and methanol crude extracts of *Azadirachta indica* leaves.

Phytochemical compounds	Aqueous extract	Methanol extract
Pholobotannis	-	+
Saponins	+	+
Anthraquinones	+	+
Steroids	+	+
Terpens	+	+
Phenols	+	+
Tannis	+	+
Flavonoids	+	+
Alkaloids	+	-
Anthranoids	-	-
Glycosides	+	+

Key: (+) = Positive, (-) = Negative.

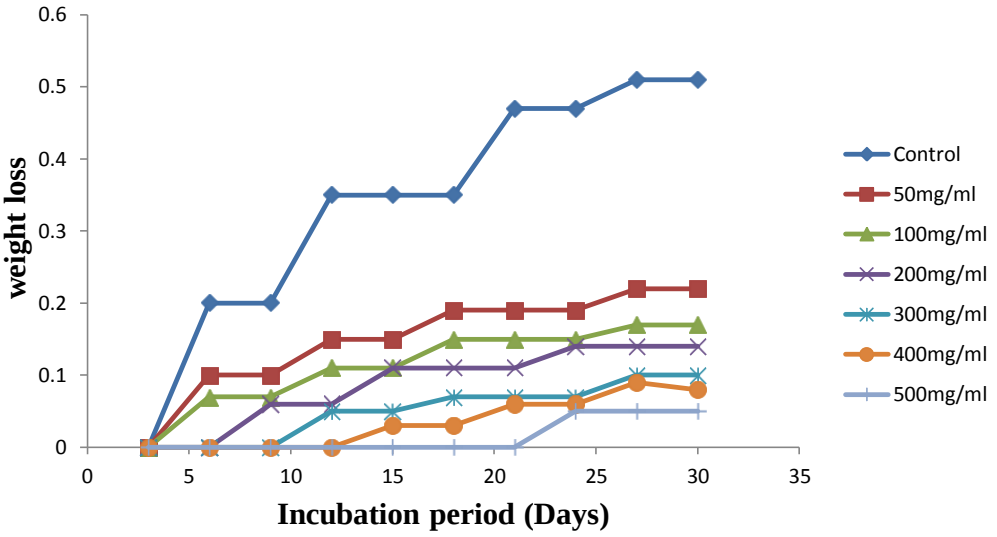

Figure 1. Effects of methanol extract of *Azadirachta indica* leaves on *Desulphovibrio* species.

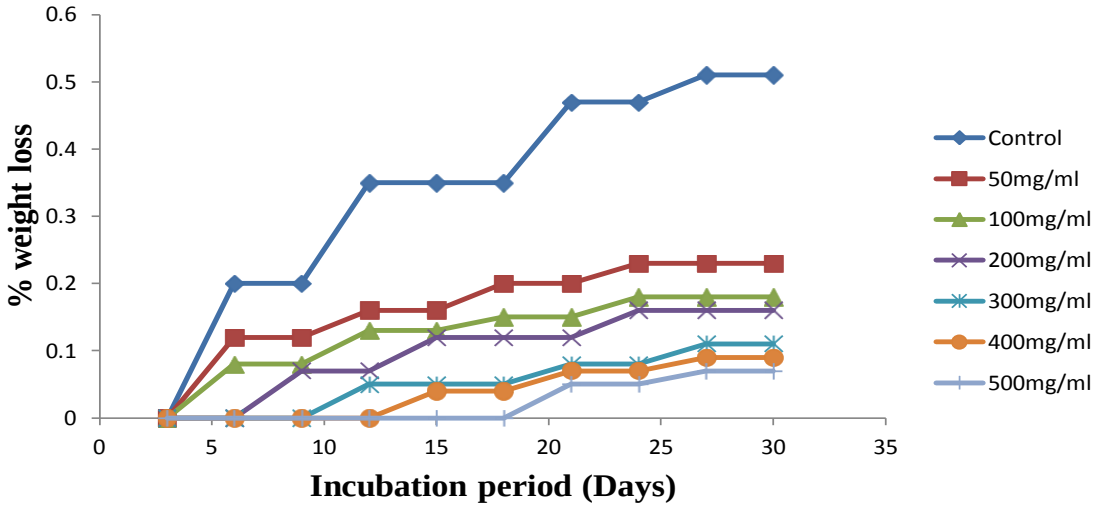

Figure 2. Effects of aqueous extract of *Azadirachta indica* leaves on *desulphovibrio* species.

(Table 1).

The antimicrobial action exhibited by the methanol and aqueous crude extracts in order of increasing concentrations (Figures 1 and 2) could be attributed to the concentration of the active compounds. In other words, it was observed that the higher the concentration of the crude extracts, the more activity was recorded against the test organism (*Desulphovibrio* species). Although the mechanism of action of the crude extracts that inhibited corrosion within 30 days is not known. The fact that the extract had activity on the test organism comparable to the control is an indication that the crude extract contains compounds that have adverse effects on the test organism and could be used as alternative chemical agent for the control of corrosion. Further investigation should be conducted to ascertain the chemical nature of active compounds, minimal inhibitory concentration and minimal bactericidal concentration.

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