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Exploration of Antibacterial Compounds from the *Commiphora myrrha* Through GC-MS and Their Evaluation Against the Bacteria Isolated from *Corvus splendens*

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Abstract

The *Corvus splendens* (Indian house crow) normally fed on the garbage therefore, they have many pathogenic bacteria, which can infect the human and cause severe infection in human irrespective to the gender, age and region. The bacteria samples were isolated from the faeces, blood lever, and intestines of the crows in Luria-Bertani (LB) agar plates. The culture plates were incubated at 37°C until the colonies were appeared. The colonies were identified morphologically and through molecular marker using 16S-rDNA. The *Commiphora myrrha* plant extract was used to determine the antibacterial activities against the pathogenic bacteria isolated from crows. The plant was crushed with 70% methanol and filtered. The filtrates were dried and dissolved in 100% methanol. Antibacterial activities of plant extracts were determined against the pathogenic bacteria isolated from crows. The results showed that the plant extracts were very effective against the pathogenic bacteria and showed 100% antibacterial activities. The plants extracts were analyzed for the characterization of different antimicrobial compound through gas chromatography mass spectrometry (GC-MS). The undecane, tetrasiloxane, hexadecanoic acid, heptasiloxane, benzocyclohepten and many other compounds. The antibacterial activities of the plant extracts were might be because of their compound which had been reported previously as well as an antimicrobial compound.

Keywords: *Commiphora myrrha*, antibacterial activities, GC-MS, secondary metabolites

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1. INTRODUCTION

The importance of wild birds as a potential vector of disease has received new experimental attention, especially in relation to human health [1]. Understanding the spread of bacterial pathogens in wild birds can be a useful model for examining the spread of other organisms, whether among birds, or from birds to other species [2]. Gastrointestinal herbicides are limited to most types of wild birds, focusing on a few well-studied examples of animal-borne bacteria and/or commercial bird species [3]. However, most studies are limited by the small sample size, the frequent absence of longitudinal data, and the use of selective techniques to isolate specific pathogens [4]. Pathogenic species in the intestines are often attributed to the bird's habitats. However, little is known about the impact of this pathogen on the host [2].

Commiphora myrrha belongs to *Burseraceae* and used for the production of myrrh, a resin made from dried tree sap. The gum has been investigated for biological and medical properties and showed effects against ulcers and different types of gastric disorders help improve the digestive system, assisting and removing toxins and pathogenic bacteria. During the last several years, studies showed that *C. myrrha* components have anticancer activities and inhibit the proliferation of cancer cells. Plants produce these secondary metabolites for their protection against biotech and abiotic stressors [5]. However, the metabolic compounds can also be used against a variety of human disorders including infectious diseases [4]. In old age, the plant extracts were obtained through simple distillation but now as the scientific equipment has been developed; more refined products can be extracted [6]. *Different plant Cremaspora triflora, Hypericum roeperianum, Pittosporum viridiflorum, Heteromorpha arborescens, Calpurnia aurea, Bolusanthus speciosus, Maesa lanceolata, Morus mesozygia and Elaeodendron croceum were used assessed for the antibacterial activities against a wide range of pathogenic bacteria[5,6].*

In the present study, the plant metabolites were extracted in methanol and the chemical composition was determined through GC-MS. The extract was applied against the bacteria isolated from crows to determine the antibacterial activities against the pathogen spreads by local birds.

2. MATERIALS AND METHODS

2.1 Bacterial isolation from crows

The crows were collected from the Jeddah Municipality, KSA and were dissected to collect the bacterial samples. Bacterial samples were collected from the faeces, blood and institutes of the crows. The faeces, blood and intestinal tissues were brought to the lab. in antiseptic plastic and put in clean bench (Fig. 1). The Luria-Bertani (LB) agar plates (0.5% yeast extract, 1% NaCl, 1% tryptone and 1.5% agar) was prepared for bacterial isolation. The faeces, blood and intestinal tissues were spread on LB agar plate separately and the plates were incubated at 37°C until the colonies were appeared. The colonies were morphologically separated by selection of single colony.

Fig. 1. Dissections of *C. splendens* for the isolation of bacteria from the intestine

2.2 *Compound extraction from plant*

The *C. myrrha* sample crushed using mortar and pestle into fine powder. Proper actions were taken to assure bioactive constituents were not distorted, lost and/or destroyed while drying and extraction of

plant metabolites. The metabolites from the plants were extracted using solvent-solvent extract method. The extraction was carried out by 70% MeOH.

2.3 GC-MS analysis of compounds from C. myrrha

The metabolites of *C. myrrha* was extracted in 70% MeOH according to the McShan et al [6]. The extract was dried and was suspended in 100% MeOH and filtered through 0.45 µm filter. The helium gas (He) was used as career gas at 1 mL/min flow rate and 2 µL sample volume was injected at the split ratio of 10:1. The column was temperature was set for 2 min at 60°C and then increased up to 160°C at a rate of 5°C /min for 5 min. In the electron ionization system, the ionization energy to detect the ions was 70 eV in electron impact mode. The spectrum of detected compounds in extract were compared with library present in NIST library.

2.4 Assessment of Antibacterial activities and MIC of plant extract

Antibacterial activity of the plant extract was carried out against the bacteria isolated from crows by disc fusion method as described by McShan et al [6]. Tetracycline (10 µg/mL) was used as positive control. The bacteria isolated from the mouth, gust, stomach and faeces were purified and spread in plants. Different concentrations of the plant extracts e.g., 0.001, 0.01, 0.1, 1.0 and 10 mg/mL were prepared and 100 µL of each was applied to culture plates. The plates were incubated at 37 ± 2°C for 48 ± 2 h and clear circles known as zones of inhibition were measured and the experiment was triplicated. Furthermore, minimal inhibitory concentration (MIC) of the compound was determined by adding different concentrations of compound in 5mL LB media. The respective bacterial species were inoculated and inoculated on shaker (200 ± 20 rpm) at 37°C and the growth determined at optical density (OD) 600 nm using spectrophotometer. The MIC was the concentration at which no visible growth was recorded at respective OD.

3. RESULTS AND DISCUSSIONS

3.1 Identification and characterization of compound through GC-MS

The extract was analysed by GC-MS to get the list of important antimicrobial compound. The metabolites of the plant determined through GC-MS revealed different bioactive compounds the such as tridecanoic acid, methyl ester, hexane, 3,3-dimethyl hexadecanoic acid, hexadecanoic acid, 15-methyl-, methyl ester, benzenedicarboxylic acid, bis(2-methylpropyl) ester and many other listed in table 1. The GC-MS data of the extract showed 18 peaks which indicated the respective compounds. The component metabolites that were present in the methanolic extract were identified according to their retention time and were confirmed by data available in the National Institute of Standards and Technology libraries.

All these compounds are important in the formulation of different medicines which showed very strong activities in previous studies [7]. Hexadecanoic acid, methyl ester is used as an antioxidant, anti-inflammatory, possess hypolipidemic properties and is also used as an antimicrobial agent [8]. The tridecanoic acid, methyl ester showed antioxidant and antibacterial activities [9]. The benzenedicarboxylic acid has been reported as strong antimicrobial agent against different pathogenic bacteria[10]. In addition, its antioxidant properties and anti-cancerous activities has also been reported [11].

Table 1. The GC-MS profile of the metabolites of MeOH extract of *C. myrrha*

Peak#	Ret.Time	Area	Height	A/H	Name
1	6.235	160146	42764	3.74	Undecane, 3,8-dimethyl-
2	7.591	312359	92692	3.37	Azulene
3	10.592	31816	19545	1.63	Decane, 3,7-dimethyl-
4	11.9	85151	43639	1.95	Tetrasiloxane, 3,5-diethoxy-1,1,1,7,7,7-hexamethyl-3,5-bis(trimethylsiloxy)-
5	13.174	26246	17602	1.49	Hexane, 3,3-dimethyl-
6	13.997	60875	36248	1.68	N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine
7	14.657	22739	12867	1.77	Tridecanoic acid, methyl ester
8	15.456	65676	41386	1.59	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro-
9	15.815	56332	34165	1.65	Cyclohexasiloxane, dodecamethyl-
10	16.192	59062	27548	2.14	3-Heptyne, 5-methyl-
11	16.858	133585	66068	2.02	Hexadecanoic acid, 15-methyl-, methyl ester
12	17.292	108073	56030	1.93	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
13	17.438	39107	20989	1.86	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether
14	18.63	126476	66826	1.89	9-Octadecenoic acid (Z)-, methyl ester
15	18.862	90990	47331	1.92	Hexadecanoic acid, 15-methyl-, methyl ester
16	18.925	23704	14257	1.66	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether
17	20.268	20191	12280	1.64	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether
18	21.51	16131	10393	1.55	1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether

3.2 Isolation of bacteria from crow

The crows were dissected and four different types of bacteria e.g., *Pasteurella aviseptica, Erithacus rubecula*, *P. multocida* were isolated form different parts of such as mouth, gut, stomach and faeces (Fig. 2A, 2B, 2C and 2D). The methanolic extract of the plant was used to evaluate the antibacterial activities of different concentration e.g., 0.01, 0.1, 1.0 and 10 mg/mL of the plants were determined against the bacteria isolated from crows. The results revealed that 0.01 mg/mL concentration was not quite effective, however, the higher concentrations such as 0.1–10 mg/mL were very effective against all the bacterial isolates. Numerous bacteria such as *Pasteurella aviseptica, Erithacus rubecula*, *P. multocida* from were isolated from has been isolated from crows [12]. The *P. multocida*, was reported as pathogenic bacteria causing fowl cholera [13]

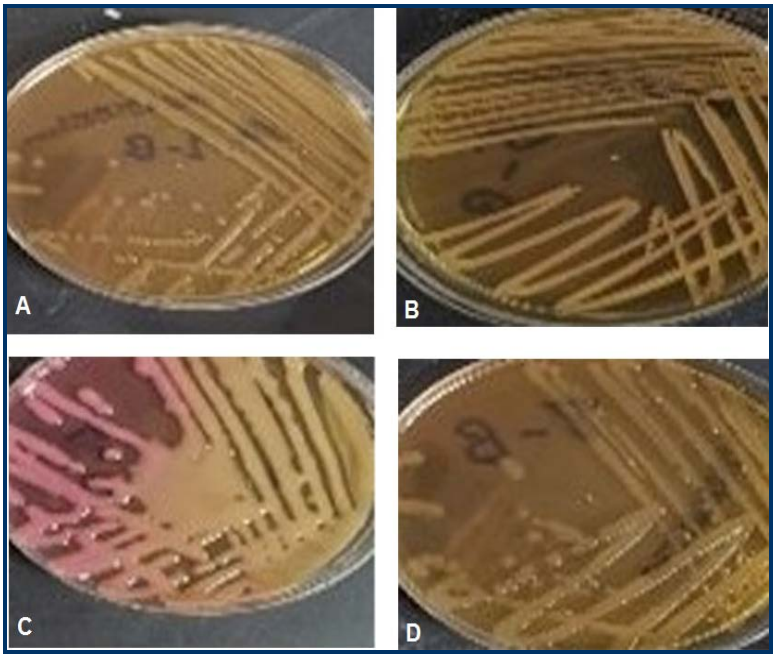

Fig. 2. Isolation of bacteria from such as mouth, gut, stomach and faeces of the crow

3.2 Assessment of antibacterial activity of plant extract

The plant extract was assessed for the antibacterial activity and the results revealed that the detected by GC-MS were very active. The antibacterial activities of the extract were determined to measure the zone inhibition (Fig. 3A, 3B, 3C and 3D). The larger zone of inhibition in the plats indicated efficacy of the plant extract. Medicinally important plants have a significant role in traditional and commercial medicine due to the chemical contents present in the plant extract. Previous studies reported the chemical profile of the plant extracts which were played very prominent role against the pathogenic bacteria such as *E. coli, Shigella, Pseudomonas* [12 2]. However, the strength of the activities of the different plant extract were different in different studies [14]. The differences in the antibacterial activity of extract might be due to the phytochemical components present in the extract [12 15 10]

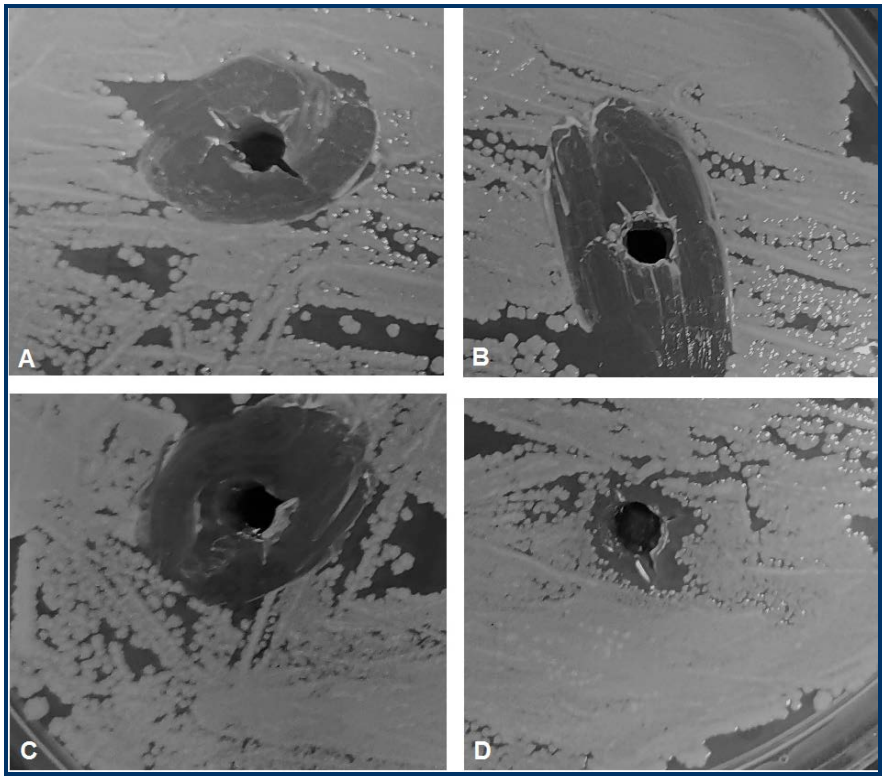

Fig. 3. Antibacterial activity of the plant extract against different bacterial strains isolated from crow

3.3 Assessment of MIC and MBC of plants extract

The plant extract was further analysed for to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the isolated bacteria. The effect of the plant extracts was shown in Figure 2, revealed that that bacterial strain was susceptible to the extract. The previous reports showed that a higher concentration of plant extract were effective against pathogenic fungi and bacteria of causing food spoilage[12]. Plant extracts rich with antibacterial compound were found to be effective against a wider range of bacteria pathogenic and non-pathogenic bacteria [15]. Many researchers have studied the efficacy of plant extracts and their active compounds as antimicrobial agents to control bacterial growth and food degradation [16].

Table 2. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extract against bacteria

Strains	MIC	MBC
Escherichia coli	100	700
Citrobacter freundii	70	400
Proteus mirabilis	100	400
Klebsiella pneumonia	70	400

4. CONCLUSIONS

Different genus of pathogenic bacteria were isolated from the crow which indicated that the crows could be a cause to spread the diseases. The plants extract was quite effective against the pathogenic bacteria isolated from the crows. The antibacterial activity of the plant extract was determined to be due to secondary metabolites present in plant extract which was detected by GC-MS analysis. The results revealed that the extract could be a better and alternative source of antibacterial compound.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Bradl HB. Adsorption of heavy metal ions on soils and soils constituents. Journal of Colloid and Interface Science. 2004;277(1),1-18.
2. Okamura M, Kaneko M, Ojima S, et al. Differential Distribution of *Salmonella Serovars* and Campylobacter spp. Isolates in Free-Living Crows and Broiler Chickens in Aomori, Japan. Microbes and Environments. 2018;33(1),77-82.
3. Ramonaitė S, Novoslavskij A, Zakarienė G, Aksomaitienė J, Malakauskas M. High Prevalence and Genetic Diversity of *Campylobacter jejuni* in Wild Crows and Pigeons. Current Microbiology. 2015;71(5),559-565.
4. Jalalpure SS, Salahuddin M, Imtiyaz Shaikh M, Manvi FV. Anticonvulsant effects of *Calotropis procera* root in rats. Pharmaceutical Biology. 2009;47(2),162-167.
5. Okamura M, Kaneko M, Ojima S, et al. Differential distribution of *Salmonella* serovars and *Campylobacter* spp. isolates in free-living crows and broiler chickens in Aomori, Japan. International Journal of Infectious Diseases. 2019;79,68.
6. McShan D, Zhang Y, Deng H, Ray PC, Yu H. Synergistic Antibacterial Effect of Silver Nanoparticles Combined with Ineffective Antibiotics on Drug Resistant *Salmonella typhimurium* DT104. Journal of Environmental Science and Health, Part C. 2015;33(3),369-384.
7. Ullah I, Khan AR, Park G-S, et al. Analysis of phytohormones and phosphate solubilization in *Photorhabdus* spp. Food Science and Biotechnology. 2013;22(1),25-31.
8. Abd Kadir SL, Yaakob H, Mohamed Zulkifli R. Potential anti-dengue medicinal plants: a review. Journal of natural medicines. 2013;67(4),677-689.
9. Ullah I, Al-Johny BO, Al-Ghamdi KMS, et al. Endophytic bacteria isolated from *Solanum nigrum* L., alleviate cadmium (Cd) stress response by their antioxidant potentials, including SOD synthesis by sodA gene. Ecotoxicology and environmental safety. 2019;174,197-207.
10. Hawas UW, Al-Omar MA, Amr AGE, Hammam AE-FG. Synthesis of some thiopyrimidine and thiazolopyrimidines starting from 2,6-dibenzylidene-3-methylcyclohexanone and its antimicrobial activities. Arabian Journal of Chemistry. 2012;5(4),509-515.
11. Ullah I, Khan AL, Ali L, et al. Benzaldehyde as an insecticidal, antimicrobial, and antioxidant compound produced by *Photorhabdus temperata* M1021. Journal of microbiology. 2015;53(2),127-133.
12. Tanaka J, Nakae T, Miyamoto H, et al. Complement-mediated bacteriolysis after binding of specific antibodies to drug-resistant *Pseudomonas aeruginosa*: morphological changes observed by using a field emission scanning electron microscope. Journal of Infection and Chemotherapy. 2010;16(6),383-387.
13. Benskin CM, Wilson K, Jones K, Hartley IR. Bacterial pathogens in wild birds: a review of the frequency and effects of infection. Biological reviews of the Cambridge Philosophical Society. 2009;84(3),349-373.

14. Kim Y-C, Glick BR, Bashan Y, Ryu C-M. Enhancement of Plant Drought Tolerance by Microbes. In: Aroca R, ed. *Plant Responses to Drought Stress: From Morphological to Molecular Features*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012: 383-413.
15. Rolfsson O, Palsson BO. Decoding the jargon of bottom-up metabolic systems biology. BioEssays : news and reviews in molecular, cellular and developmental biology. 2015;37(6),588-591.
16. Timmusk S, Behers L, Muthoni J, Muraya A, Aronsson A-C. Perspectives and Challenges of Microbial Application for Crop Improvement. Frontiers in Plant Science. 2017;8,49-49.