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Detection of Bioactive Natural Products Produced from Bacillus cereus Using Gas Chromatography-Mass Spectroscopy and Evaluation of Its Efficiency of Antibacterial Activity

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Abstract:

Aims and Objective: This work involved characterizing the bioactive chemical composition of *Bacillus cereus* and establishing the efficacy of some plant extracts on the bacterium with regard to antibacterial, antifungal, and in-vitro antimicrobial activity.

Method: Bioactives which are chemical compounds that may also be termed as secondary metabolites were analyzed through a (GC-MS) technique. After that, the current investigation evaluated the antibacterial and antibacterial potency of the methanolic extract of *Bacillus cereus* both in vitro and in vivo.

Results: GC-MS analysis of *Bacillus cereus* revealed the existence of the: β Carotene , 1-Diisopropylsilyloxy-10-undene , 3-chloro-,(R*,R*)- , 6-Acetyl- β-d-mannose , Thieno[2,3-c]-furan-3-carbonitrile, 17-Octadecynoic acid , 3-(3-Carboxy-4-hydrox yphenyl)-D-alanine , N,N'-bis(2- hydroxyl-ethyl)- , Thieno [2,3-c]furan-3- carbnitrile, 2-amino-4, 6-dihydro-4,4,6,6- , 2- (9-octade-cenyl-oxy) ethyl ester , 1,8-Diethyl-3, 6-diazahomoadamantan-9-ol , Lactose , 1-[[3-Methyl-phenyl-]butyryl-4-[5-phenyl-4-oxo-2-oxzolin-2-yl] , 9,10-Seocholesta-5, 7,10(19)-triene3,24, 25-triol,(3β,5Z,7E)-, Ergsta-5, 22-dien-3-ol,ace-tate, (3β,22E)- , Aceta-mide , 1,4- Diacetyl-3-acetoxy-methyl-2, 5-methylene -l-rhamnit-ol , 2-[[2-[[2-[(2-pentyl-cyclo-propyl) methyl , Estra -1,3,5(10)-trien-17β-ol , [1,1'-Bicyclo-propyl]-2-octanoic acid , 5-Hydroxy-methylfufural , β-D-Gluco-pyranoside, methyl , Tetra-acetyld-xylo-nic nitrile , n-Hexa-decanoic acid , 9-Octa-decenoic acid , (2-phenyl-1 ,3-dioxo-lan-4-yl)-methyl ester , Octad-canoic acid , 9,10- Secocho-lesta-5,7,10(19)--triene-3,24,25-triol,(3β,5Z,7E)- and Pyrimidin-2-ol,4-(3,4-dimethoxy-phenyl)-6-phenyl, 2-(3-Hydroxy-propyl)-cyclo-hexane-1 ,3-dione, 1,2- Cyclo-pentanedione,3-methyl, α-D-Gluco-pyrano-side, O-α-D- gluco-pyranosyl-(1.fwdarw.3)-ß-D-fruc, Bicyclo-hep-tane-2- carboxylic acid isobutyl-amide, Cycloun-decanone , 6-Acetyl -ß- d- mannose, 5-Hydroxy-methyl- furfural, D-fructose , Octadecanal ,2 –bromo, L-Ascorbic acid , 6-octad-canoate. Antibacterial activity was assessed *Proteus mirabilis* was extremely sensitive to the metabolites of *Bacillus cereus* (7.9±0.04). Evaluation discovered antifungal activity *Bacillus cereus* metabolites were extremely effective (11.03±0.05) against *Cladosporium herbarum*. Against *Bacillus cereus*, *Cassia angustifolia* (Crude) (12.05 and 11.65 mm) was quite effective.

Keywords: Secondary metabolites, *Bacillus cereus*, Screening, Antimicrobial, GC/MS.

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Introduction

The bacterium B. cereus is well endowed with this ability, and some of the antimicrobial substances that are formed include; Surfactins, Iturins and Fengycins. This ability has been demonstrated by many polyketide-derived macrolides as well as non-ribosomal peptides, dihydroisocoumarines, further linear lipopeptides with an antimicrobial action. For now, efforts are under way to establish potential outlets in both the agricultural and medical fields for B. cereus strains and the natural compounds from such a strain. However, the large scale synthesis of such chemicals remains negligible up to the present time because of the low yield of the fermentation process and the synthesizing complications [2,3]. That is why improvement of the genetically modified strains and the cultivation process is essential. Bacillus cereus is an aerobic, facultative anaerobic, motile, and spore-forming, aerobic or facultatively anaerobic, rod-shaped group of bacteria that are isolated from various substrates [4]. PDB. cereus has long been identified as the agent of GB crop and but recent data that have come forth reveal that this bacterium caused invasive infections that are not associated with the gastrointestinal system and some of which are fatal. This is for example in the case of B. cereus that in intestinal as well as in non-intestinal infection but is closely associated with the ability of the bacterium to produce tissue-degrading exoenzymes. These released toxins are emesis inducing toxin, protease, four immunological types of hemolysins and three enzymatic types of phospholipases [5]. The major difficulty when Bacillus cereus is identified in a clinical material is to get rid of a prejudice that the organism is always a contaminant. This is so because the following; Among them being stigma. Aside from having it linked to food poisoning, severe ocular infections and seizures, this bacterium is believed to be involved in several clinical syndromes. Some of these illnesses are include; anthrax-like progressive pneumonia, complete sepsis, and severe central nervous system infections that are quite fatal in immune compromised patients, intravenous users, and new born babies [6]. The same investigations prove its role in the nosocomial acquired bacteremia and in wound infections in postoperative patients. This is even so when the catheters or any other intravascular devices are inserted [7]. It is also well know that trauma may lead to primary cutaneous infection which may mimic Clostridial gas gangrene. These infections occur after an injury has occurred. : Where required B. cereus is known to produce a potent beta-lactamase which endows the bacteria with extremely high level of resistance to beta –lactam antibiotics [17]. Only at places and in those special circumstances only, It II scenarios were introduced based on new information as well as the information gathered through experiences which were considered wherever considered suitable. So, in order to achieve the intended purpose on the end of the article the reader should gain as much understanding and curiosity with the versatility and complexity of this highly fluid bacterial species [9]. Bacillus cereus is easily cultured, and most frequently isolated from soil, plant material, maize, vegetables, fresh water and sea water, and fomites. Furthermore, it may develop in the alimentary canal of such animals as invertebrates. These sources can contaminate soil and foods therefore, the temporary occupation of the human digestive system [10]. This work aims at; To identify chemical constituents present in Bacillus cereus To determine the effectiveness of extract of plants against Bacillus cereus in antibacterial, antifungal and in vitro antimicrobial activities.

MATERIALS AND METHODS

The name and impact of the metabolites

A successful Bacillus cereus strain was isolated and subcultures obtained from inoculating Nutrient Agar at 22 Cº for 48 hrs. Once the mixture was incubated at 4 degree celcius for 10 minutes the mixture was shaken at 130rmp for ten minutes [11]. After having obtained the metabolites through the liquid culture they were evaporated at a temperature of 45 Cº the use of a rotary evaporator..

Chromatographic qualitative and quantitative analysis of chemical constituents responsible for bioactivity of *Bacillus cereus* by GC/MS.

The GC-MS (Agilent 789 Aº) analytical system was used, along with a DB-5MS (30 m, 0.25 mm id, 0.25 um film from tweak, J&W Scientific Instrument, Folsom, CA), to conduct the analysis. After preheating the kettle bell and

setting the oven temperature to the measured value, the procedure was identical to the prior examination. Helium was utilized as the carrier gas at a flow rate of 1.0 mL/minute. The GC column's effluent was linked to the 250 Cº MS source via a transfer line. The ionization voltage was 70 eV, while the ion source temperature was 230 Cº. Between 41 and 450 amu was the scan range. The holding times of these components were compared to similar items in the WILEE MASS SPECTRAL DATA BASE Library in order to establish them [12].

Characterization of the inhibitory effects of Bacillus cereus secondary metabolites

A distal, five-millimeter diameter analogue was made from the agar using a sterile cork-borer, and 25 macroliters of the sample solutions which were Metabolites produced by Bacillus cereus was pipetted into the wells. The following test pathogens swabbed onto Muller Hinton agar plates included Escherichia coli, Staphylococcus epidermidis among others. The diameter of the wells was decreased to 0.5 millimeters. Subsequently, the plates were studied after 24 hours of incubation in 37C°. Methanol was used to control the solvent as indicated there is always a need for good and effective solvent control [13].

Exploring the way through which Bacillus cereus bioactive compounds exercise their antifungal properties

The swabbing of the test organisms was on the Müller-Hinton agar plates. In ordered to treat the drilled wells 70 litres of this B. cereus extract was used. Fungal growth was assessed using the method of zone of inhibition on the test bacteria of 3rd year BDS dental students. To control the solvent methanol was used. In this regard, fluconazole and amphotericin B were employed as two study antifungal drugs. The tests were performed three times [14]. Molecular weights of the extracts were further determined after 48 hours of incubation using the antifungal activity standard procedure that entails determination of the inhibition-zone diameter.

The present in vitro experiment was performed to ascertain the antibacterial activity of the chosen medicinal plant extracts against Bacillus cereus.

Wells were punched on the agar using a sterile cork-borer elaborated with a five millimetre diameter, and 25 µl of the sample solutions of Gramineae poaceae to Rosmarinus oficinalis.

Statistical analysis

All the collected data were analyzed through mean value and by applying the analysis of variance (ANOVA) on an SPSS (Version 11.6) database.

RESULTS AND DISCUSSION

The GC-MS chromatogram of the twenty eight peaks of the compounds detected were: β Carotene , 1-Diisopropylsilyloxy-10-undecene , 3-chloro-,(R*,R*)- , 6-Acetyl-β-d-mannose , Thieno[2,3-c]furan-3-carbo-nitrile, 17-Octade-cynoic acid , 3-(3-Carboxy-4-hydroxy-phenyl)-D-alanine , N, N'-bis(2- hydroxyl-ethyl)- , Thieno [2,3-c]-furan-3- carbonitrile, 2-amino-4, 6-dihydro-4,4,6,6- , 2- (9-octadecenyl-oxy) ethyl ester , 1,8-Diethyl-3, 6-diazahomo adamantan-9-ol , Lactose , 1-[[3-Methyl-3- phenyl-]butyryl-4-[5-phenyl- 4-oxo-2 -oxazo-lin- 2-yl] , 9,10-Secocholesta-5, 7,10(19)-triene3,24, 25-triol,(3β,5Z,7E)-, Ergosta-5, 22-dien-3-ol,acetate, (3β,22E)- , Acetamide , 1,4-Diacetyl-3-acetoxy-methyl-2, 5-methylene-l-rhamnitol , 2-[[2-[[2-[(2-pentyl-cyclopropyl) methyl , Estra-1,3,5(10)-trien-17β-ol , [1,1'-Bicyclo-propyl]-2-octanoic acid , 5-Hydroxymethyl-furfural , β-D-Gluco-pyrano-side, methyl , Tetra-acetyl-d-xyl-onic nitrile , n-Hexa-decanoic-acid , 9-Octa-decenoic acid , Cyclo-undecanone , 6-Acetyl-ß- d-mannose, 5-Hydroxy-methyl furfural, D-fructose , Octade-canal ,2 –bromo, L-Ascorbic acid , 6-octadeca-noate. The impact of a Bacillus cereus secondary metabolite on four different harmful bacteria: The present investigation assessed the bioactivity of a methanolic Bacillus cereus extract in comparison to the gold standard antibiotics Rifambin and Cefotoxime against the five infections that were examined. *E. coli* (5.2±0.02, 3.4±0.01 and 3.7±0.01), *Staph. aureus* (5.9±0.03 , 3.5±0.01 and 4.7±0.01), *P. mirabilis* (7.9±0.04, 3.8±0.02 and 2.3±0.01), *Staph. Epidermidis* (6.7±0.03, 3.0±0.02 and 4.4±0.01). *Bacillus cereus* metabolites was very highly active against *Proteus mirabilis* (7.9±0.04).

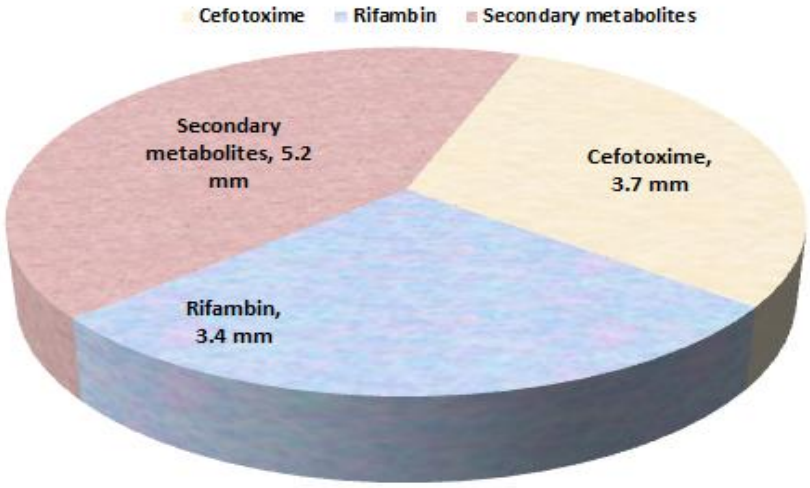

Figure 1. Metabolite products, Rifambin and Cefotoxime as anti-Bacterial activity against *Escherichia coli*

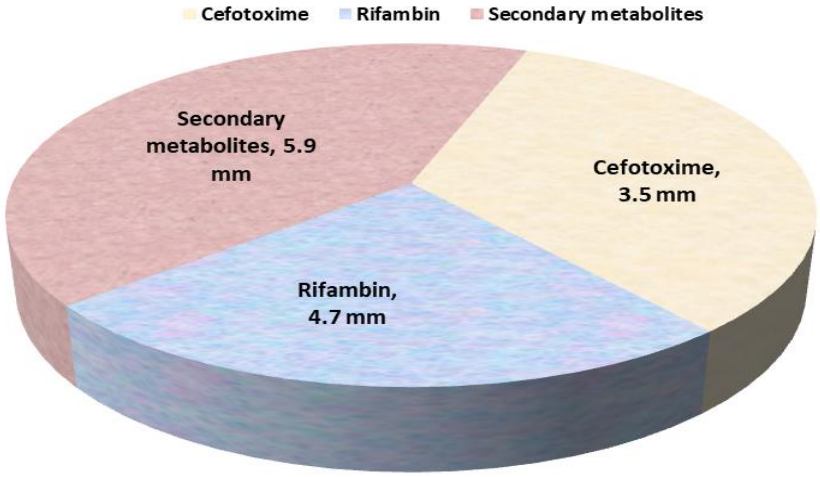

Figure 2. Metabolite products, Rifambin and Cefotoxime as anti-Bacterial activity against *Staphylococcus aureus*

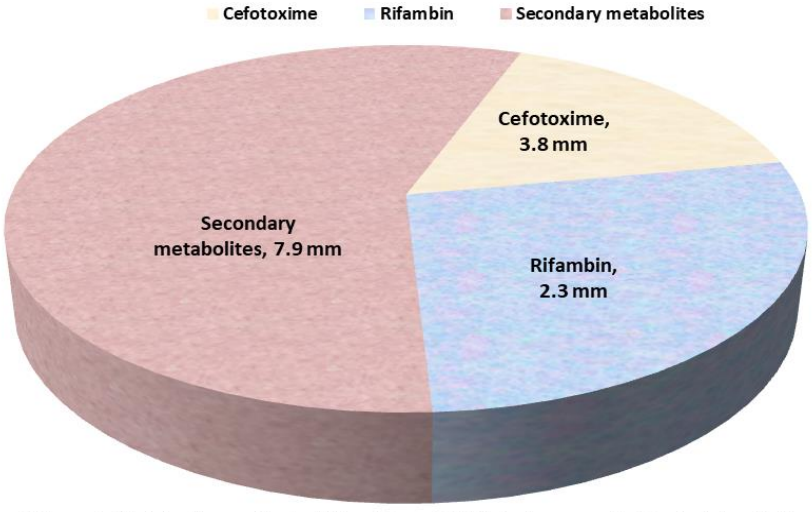

Figure 3. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Proteus mirabilis*

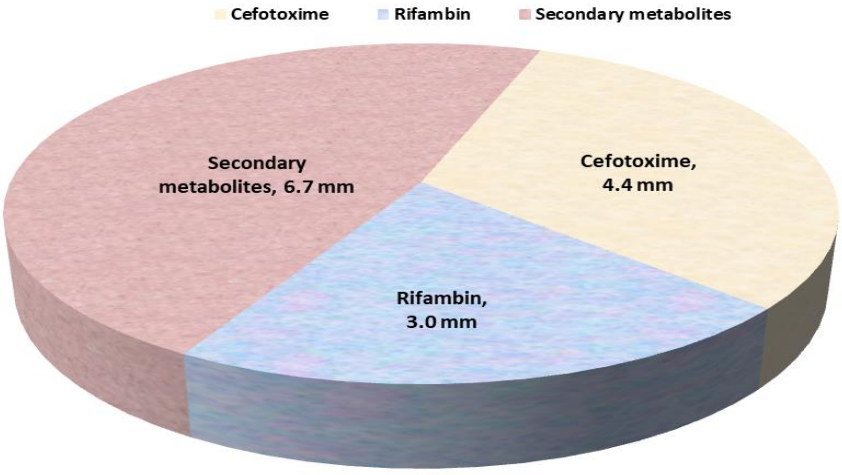

Figure 4. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Staphylococcus epidermidis*

Bacillus cereus secondary metabolites and their antifungal activity

Effects of the methanolic extract of Bacillus cereus and standard antibiotics against six fungi and yeast isolates. *Alternaria alternate* (8.62 ± 0.05, 10.78 ± 0.06 and 8.06 ± 0.05), *Aspergillus flavus* (9.22 ± 0.03, 6.05 ± 0.03 and 5.17 ± 0.03), *Aspergillus fumigates* (2.00 ± 0.06, 3.03 ± 0.01 and 5.31 ± 0.03), *Candida albicans* (7.12 ± 0.03, 5.08 ± 0.03 and 4.06 ± 0.02), *Cladosporium herbarum* (11.03 ± 0.05, 6.83 ± 0.03 and 8.00 ± 0.04), *Fusarium oxyporum* (7.00 ± 0.04, 4.00 ± 0.02 and 1.05 ± 0.01), The anti-*Cladosporium herbarum* activity of *Bacillus cereus* metabolites was striking. (11.03 ± 0.05).

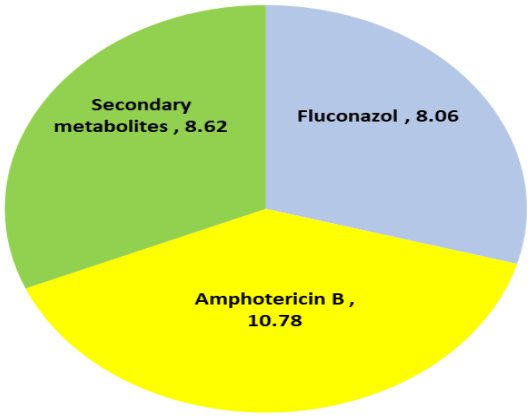

Figure 5. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Alternaria alternata*

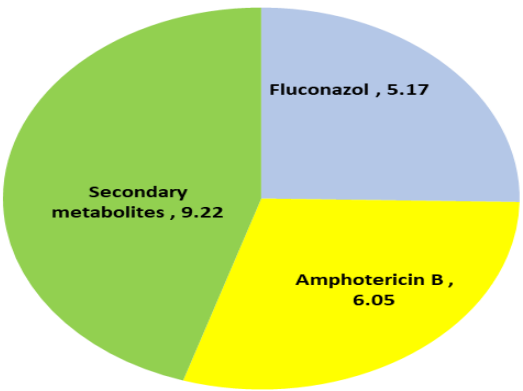

Figure 6. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Aspergillus flavus*

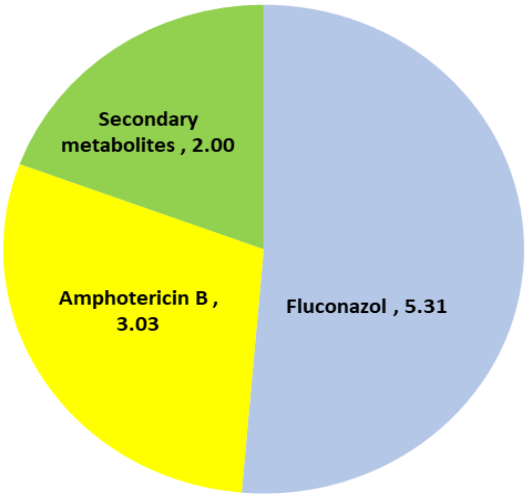

Figure 7. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Aspergillus fumigates*

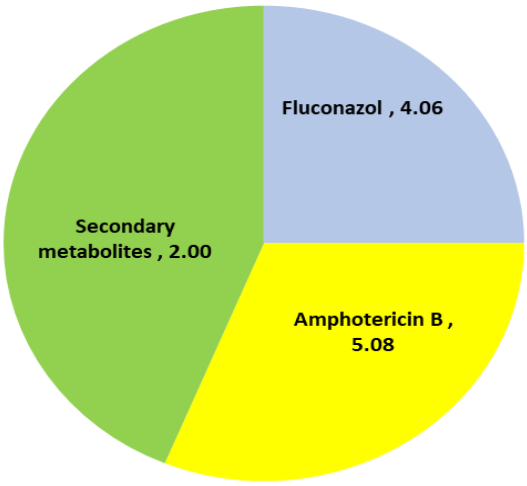

Figure 8. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Candida albicans*

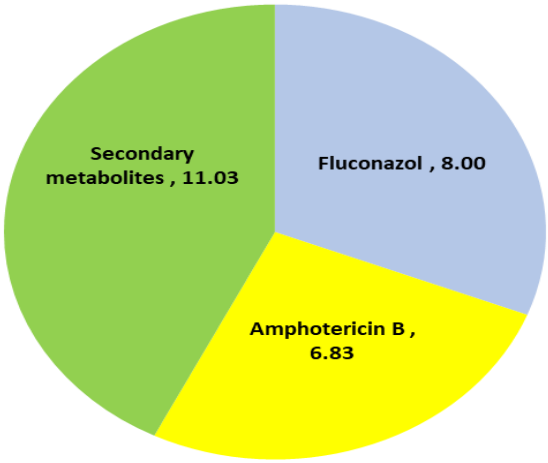

Figure 9. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Cladosporium herbarum*

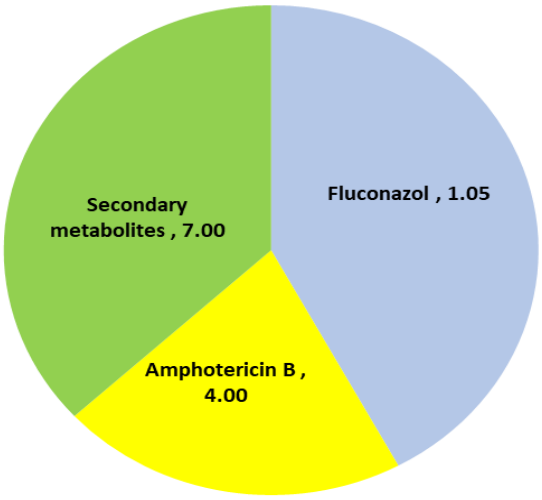

Figure 10. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Fusarium oxyporum*

Table 1. Percentage inhibitory zone of different test bioactive compounds as well as standard antibiotics of plants against Bacillus cereus.

S. No.	Plant extract	Diameter of zones of inhibition (mm) After 48 hr.		Mean Standard Deviation
		Replicate 1	Replicate 2	
1.	*Linum usitatissimum* (Crude)	7.09	7.80	7.45±0.14
2.	*Anastatica hierochuntica* (Crude)	6.64	6.07	6.36±0.17
3.	*Cassia angustifolia* (Crude)	12.05	11.65	11.85±0.13
4.	*Mentha viridis* (Crude)	7.0	7.5	7.25±0.14
5.	*Artemisia annua* (Crude)	6.2	5.6	6.40±0.19
6.	*Quercus infectoria* (Crude)	8.1	8.5	8.30±0.21
7.	*Citrullus colocynthis* (Crude)	5.5	5.8	5.65±0.19
8.	*Althaea rosea* (Crude)	7.5	6.4	6.95±0.20
9.	*Coriandrum sativum* (Crude)	6.5	6.0	6.25±0.19
10.	*Melia azedarach* (Crude)	5.3	4.6	4.95±0.17
11.	*Origanum vulgare* (Crude)	7.5	7.0	7.25±0.17
12.	*Urtica dioica* (Crude)	4.5	4.8	4.65±0.14
13.	*Equisetum arvense* (Crude)	6.5	6.3	6.40±0.18
14.	Amphotericin B	6.5	6.8	6.65±0.19
15.	Fluconazol	8.0	8.0	8.00±0.21
16.	Control	0.0	0.0	0.0

In *vitro* antimicrobial activity of plant extracts on *Bacillus cereus*

Inhibition zone diameter (millimeters) Following a 48-hour period, two repeats were *Linum usitatissimum* (Crude) (7.09 and 7.80 mm), *Anastatica hierochuntica* (6.64 and 6.07 mm), *Cassia angustifolia* (Crude) (12.05 and 11.65 mm), *Mentha viridis* (7.0 and 7.5 mm), *Artemisia annua* (Crude) (6.2 and 5.6 mm), *Quercus infectoria* (Crude) (8.1 and 8.5 mm), *Citrullus colocynthis* (Crude) (5.5 and 5.8 mm), *Althaea rosea* (Crude) (7.5 and 6.4 mm), *Coriandrum sativum* (Crude) (6.5 and 6.0 mm), *Melia azedarach* (Crude) (5.3 and 5.6 mm), *Origanum vulgare* (Crude) (7.5 and 7.0 mm), *Urtica dioica* (Crude) (4.5 and 4.8 mm), *Cassia angustifolia* (Crude) (12.05 and 11.65 mm) were highly effective against *Bacillus cereus*, Table 1. They germinate within the organic matter or if kept within an animal host. In some arthropod intestines there is a filamentous type of growth with many refractile inclusions in multiple cell type. This pattern has been described as arthromitus (rooted), and it is proposed that this is the common digestive phase throughout insects which are domiciled on the terrestrial substratum. The bacilli appear here as long rods, desinucleate the flagella, attached to the epidermis of the intestinal wall of its arthropod host ensuing spores. The saprophytic life cycle of B cereus begins for the spores in the soil, germinates to form a vegetative bacillus that has the ability to sporulate and start the life cycle again. Both cells and spores are released into the ground when the host dies or when the animal defecates. Vegetative cells are able to produce spores and exist in the soil until its consumption by another host organism [15, 16]. In addition to that like Listeria, Bacillus cereus, is found in the soil and as it grows from one cell to many cell it quadruples its motility enabling it to move round the soil. This ability is referred to as motility phenotype. For this reason, Bacillus cereus can translocate. This morphogenic phase appears to be akin to the swarming that has been explained on agar media in relation to sophor grown B. cereus. B. cereus infections range from mild self-limited diarrheal illness to severe emetic poisoning and antibiotics should be based on susceptibility patterns of the identified strain [17]. It has been proved that in the current research that seventy percent of Bacillus cereus isolate possess resistance to Penicillin and Ceph spins, due to production of beta-lactamase [18]. It may be necessary to use empirical therapy when there is expectation of a B. cereus infection to be present in certain contexts. This will be the case when the AST profile is being developed. However, since it was demonstrated hereby that B. cereus was resistant to erythromycin, tetracycline and carbapenem, the selection of an empirical therapy may not be as straightforward as in a case where the result was clearly in favour of the gram-positive cocci [19]. Certain strains of Bacillus cereus are associated with conditions which do not involve the gastrointestinal system. They also boast about pollution of the environment and foods [20].

CONCLUSION

Therefore, in the current study several easily accessible, home remedies are discovered which could be used as adjunct, or could contain a natural medicine. These are easily accessible drugs and in most of the families and these medications are commonly found. Twenty-eight chemically different compounds with bioactivity in the bacterial species Bacillus cereus were detected through GC-MS analysis. The antimicrobial activity was assessed, hence the discovery. Some of the metabolites of Bacillus cereus exhibited inhibition rates of (7.9±0.04) against; Proteus mirabilis. Evaluation discovered that antimicrobic function was present in presence. Its metabolites were most effective (11.03±0.05) against phytopathogen Cladosporium herbarum after 72 hours of incubation. Crude Cassia angustifolia was especially effective on Bacillus cereus with two zones of inhibition of 12.05mm and 11.65mm.

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