Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

EVALUATION OF MYCOCHEMICAL PROFILE OF *ALTERNARIA JAPONICA* THROUGH GC-MS ANALYSIS

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ABSTRACT

The present study was carried out to find out mycochemical profile of culture filtrates of *Alternaria japonica* through GC-MS analysis. The fungus was incubated for 21 days using malt extract broth as growth medium. Culture filtrate was filtered and concentrated by evaporating water at 45 °C. Filtrates were partitioned using *n*-hexane followed by chloroform. Both the organic solvent fractions were analyzed through GC-MS. In *n*-hexane and chloroform fractions, 14 and 10 compounds were identified, respectively. Major compounds in *n*-hexane fraction were *o*-xylene (36.9%); ethyl benzene (19.3%), hexane, 2-4-dimethyl- (16.8%), 1-2 dimethylbenzene (7.56%), cyclohexane, 1-ethyl-1-methyl (3.36%), heptane, 2,5-dimethyl- (3.36%) and octane, 4-methyl- (2.52%). Likewise, predominant compounds in chloroform fraction were octane-4 methyl (24.62%), carbonic dihydrazine (16.41%), octane, 2,4,6-trimethyl (12.56%), hydrazine, (1,1-dimethylethyl) (8.75%), 16-hexadecanoyl hydrazide (7.66%) and stearic acid hydrazide (7.08%). Literature surveyed showed that many compounds identified in both the fractions possess antimicrobial, pharmacological, antioxidant, antidepressant, anticancer and anti-inflammatory properties.

Keywords: *Alternaria japonica,* antimicrobial, antioxidant, GC-MS analysis, mycochemicals.

INTRODUCTION

During the evolution of microorganisms, phytopathogens appeared as the filamentous fungi with the ability to invade and multiply on the host plants to complete their life cycle (Patriarca *et al*., 2019). Genus *Alternaria* is example of phytopathogenic imperfect fungi which usually survive on decomposed organic compounds and produce toxins that are host specific. It has drastic impacts on nutritionally important food crops belonging to Solanaceae, Brassicaceae and Cucurbitaceae families (Al-Ghafri *et al*., 2019). In addition to plant pathogens, they fabricate strong secondary metabolites in humans with toxic effects that produce certain cancer types, potent allergens with mutagenic potential on immune-comprised patients affecting their immune system (Cabral *et al*., 2019).

Alternaria japonica is a seed inhabiting, cosmopolitan, notoriously destructive post-harvest pathogen of vegetables and fruits with world-wide economic losses. It is widely distributed in plant decayed organic material, soil, air and household dust (Al-Lami *et al*., 2019). It is host specific pathogen producing specific toxins with adverse effects on food, plant tissues, mammalian cells and other microorganisms. Toxins produced by *Alternaria* spp. are secondary metabolites with low molecular weights belongs to a diverse group of compounds that contaminate foodstuffs, feed and agricultural commodities with lethal effects on living organisms (Siciliano *et al*., 2018). The known mycotoxins produced by *Alternaria* spp. are altenuisol, altenuene, alternariol, altenusin, tetramic acid, peptides, quinones, tentoxin, curvularins, altenin, altertoxin and zinniol which cause antimicrobial, phytotoxic, cytotoxic, antifungal, antibacterial and insecticidal toxicities (Dang *et al*., 2015). In general, *A. joaponica* produces mycotoxins in plant products that are responsible for

Submitted: November 14, 2019
Revised: December 03, 2019
Accepted for Publication: December 20, 2019
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devastating diseases with their toxic effects (Gambacorta *et al*., 2019). Therefore, the present study was carried out to evaluate the mycochemical profile of culture filtrates of *A. joaponica* through GC-MS analysis for identification of harmful and beneficial compounds.

MATERIALS AND METHODS

An isolate of *A. joaponica* was obtained from Culture Bank of Pakistan (FCBP) Punjab University, Lahore, Pakistan. The culture was multiplied on malt extract agar (MEA) plates by placing a single spore technique under aseptic conditions and incubated at 28 °C for 10 days. After the culture matures, the agar plugs containing fungal mycelia were transferred to malt extract broth in flasks and incubated for 21 days at 28 °C on a shaker for the production of secondary metabolites secreted by the pathogen. The culture was then filtered and the filtrate after making concentrated was partitioned using *n*-hexane and chloroform (Akhtar and Javaid, 2018). The two organic solvents fractions were analyzed by GC-MS for identification of compounds present in these fractions. Literature survey was carried out to gather information regarding bioactivities of the various identified compounds (Javaid *et al.,* 2019).

RESULTS

The extraction of *A. japonica* was aimed to analyze the mycoprofile of this pathogenic fungus through GC-MS by using two different solvents *n*-hexane and chloroform. GC-MS chromatograms of the two solvent fractions are given in Figure 1 and 2. The chromatogram of *n*-hexane indicates the occurrence of 14 major and minor compounds (Table 1). The most abundant compounds were *o*-xylene (36.9%) followed by ethyl benzene (19.3%) and hexane, 2-4-dimethyl- (16.8%) as indicated by their peak area percentages. The compounds such as 1-2 dimethylbenzene (7.56), cyclohexane, 1-ethyl-1-methyl (3.36%), heptane, 2,5-dimethyl-(3.36%), octane, 4-methyl-(2.52%) and 3-butyn-1-ol (2.52%) were recorded as moderately abundant. On the other hand, borane carbonyl (1.68%), methoxyacetonitrile (1.68%), 1,2,5-oxadiazole (1.68%), propanedioic acid, oxo, diethyl ester (0.84%), acetaldehyde (0.84%) and butanal, 3-hydroxy-(0.84%) were recorded as least abundant compounds (Table 1).

Table 1. Compounds identified through GC-MS in *n*-hexane fraction of culture filtrate of *Alternaria japonica*.

Sr. No.	Names of compounds	Formula	Weight	Retention time (min)	Peak area (%)
1	Ethyl benzene	C_8H_{10}	106	5.275	19.3
2	*o*-Xylene	C_8H_{10}	106	5.567	36.9
3	Cyclohexane, 1-ethyl-1-methyl	C_9H_{18}	70	6.267	3.36
4	1-2 Dimethylbenzene	C_8H_{10}	106	6.425	7.56
5	Hexane, 2-4-dimethyl-	C_8H_{18}	128	6.717	16.8
6	Borane carbonyl	CH_3BO	42	8.092	1.68
7	Octane, 4-methyl-	C_9H_{20}	128	8.267	2.52
8	3-Butyn-1-ol	C_4H_6O	42	9.717	2.52
9	Heptane, 2,5-dimethyl-	C_9H_{20}	128	12.033	3.36
10	Methoxyacetonitrile	C_8H_{10}	42	13.383	1.68
11	1,2,5-Oxadiazole	$C_2H_2N_2O$	42	19.092	1.68
12	Propanedioic acid, oxo, diethyl ester	$C_7H_{10}O_5$	74	45.292	0.84
13	Acetaldehyde	C_2H_4O	44	48.517	0.84
14	Butanal, 3-hydroxy-	$C_4H_8O_2$	270	50.492	0.84

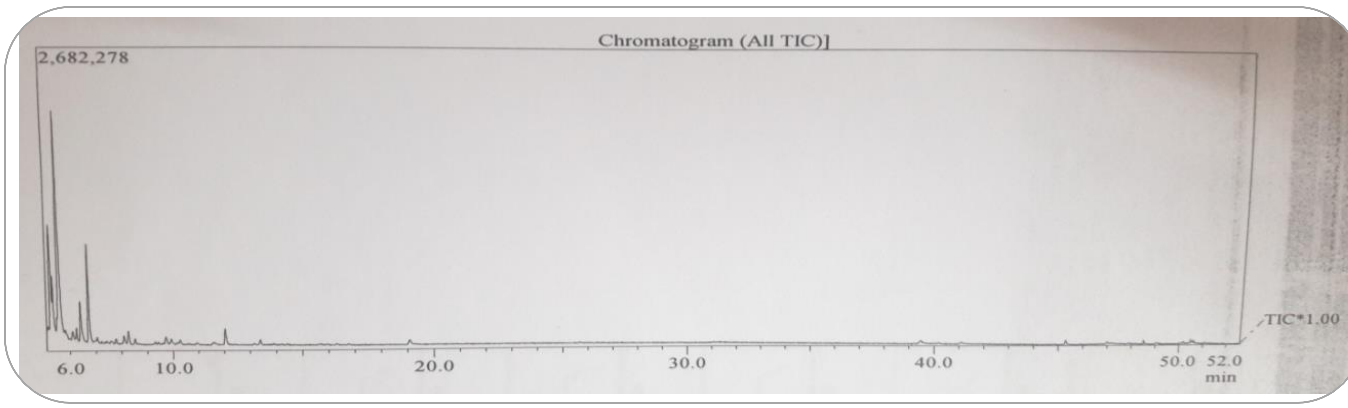

Figure 1. GC-MS analysis of *n*-hexane fraction of culture filtrate of *Alternaria japonica.*

GC-MS analysis of chloroform fraction exhibited the presence of 10 major and minor compounds The most abundant compounds were octane-4 methyl (24.62%), carbonic dihydrazine (16.41%) and octane, 2,4,6-trimethyl (12.56%). The moderately abundant compounds were hydrazine, (1,1-dimethylethyl) (8.75%) followed by 16-hexadecanoyl hydrazide (7.66%), stearic acid hydrazide (7.08%), octadecane (6.02%), hydrazine carboxamide (5.97%), propanoic acid (5.47%) and acetic acid (4.85%) (Table 2).

Table 2. Compounds identified in chloroform fraction of culture filtrate of *Alternaria japonica* through GC-MS.

Sr. No.	Names of compounds	Formula	Weight	Retention time (min)	Peak area (%)
1	Carbonic dihydrazine	CH_6N_4O	90	5.850	16.41
2	Acetic acid	CH_4CO_2	75	39.467	4.85
3	Propanoic acid	$C_3H_6O_2$	270	40.183	5.47
4	Hydrazine, (1,1-dimethylethyl)	$C_4H_{12}N_2$	88	41.067	8.75
5	Hydrazine carboxamide	CH_6N_4O	75	45.292	5.97
6	16-Hexadecanoyl hydrazide	$C_{16}H_{34}N_2O$	217	46.992	7.66
7	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298	47.875	7.08
8	Octadecane	$C_{18}H_{38}$	254	50.492	6.02
9	Octane-4 methyl	C_9H_{20}	270	51.083	24.62
10	Octane, 2,4,6-trimethyl	$C_{11}H_{24}$	298	51.833	12.56

Table 3. Potential activity of compounds identified in *n*-hexane and chloroform fraction of *A. japonica*.

Sr. No.	Names of compounds	Property	Reference
1	o-Xylene	Organopollutant, carcinogenic, neurotoxic	Zhang *et al*. (2013)
2	Ethyl benzene	Microbial volatile organic compound	Tabarestani *et al*. (2016)
3	Cyclohexane, 1-ethyl-1-methyl	Antimicrobial	Harikrishnan *et al*. (2010)
4	Octane, 4-methyl-	Antimicrobial, use in cosmetics and biomedicine	Ubaid *et al*. (2016) Peng *et al*. (2013)
5	3-Butyn-1-ol	Antiproliferative activity, antimicrobial	Sabitha *et al*. (2014) Alabi *et al*. (2019)
6	1, 2, 5-Oxadiazole	Antiproliferative activity	Gelain *et al.* (2019)
7	Propanedioic acid, oxo, diethyl ester	Pharmacological activity	Firdouse *et al.* (2019)
8	Acetaldehyde	Carcinogenic, irritant	Sivakumar *et al*. (2011)
9	3-hydroxy- hydrazide	Antibacterial, antifungal	Kaymakcioglu *et al*. (2012) Kumar *et al*. (2012)
10	Carbonic dihydrazine	Use in biomedicine, antimicrobial properties	Iqbal *et al*. (2017)
11	16-Hexadecanoyl hydrazide	Antimicrobial, antioxidant and pharmacological properties	Sneha and Sumathy, (2016) Igwe and Obiukwu, (2017)
12	Stearic acid hydrazide	Antidepressant, antioxidant and antimicrobial activities	Jubie *et al*. (2012)
13	Octadecane	Antimicrobial	Girija *et al*. (2014)
14	Hydrazine carboxamide	Antifungal, antibacterial and anticancer	Chandra *et al*. (2015) Gilani *et al*. (2011)
15	Propanedioic acid	Anti-inflammatory activity	Mokale *et al*. (2010)
16	Acetic acid	Antiproliferative activity	Germani *et al*. (2010)

DISCUSSION

Various biological activities the identified compounds in *n*-hexane and chloroform fractions are given in Table 3. Among the identified compounds profile, *o*-xylene was previously investigated as a toxin that found excessively in industrial waste and critically contaminates the environment (Zhang *et al*., 2013). Ethyl benzene is reported by Tabarestani *et al*. (2016) as the microbial volatile organic compound formed during the process of fungal metabolism and is in practice as a diagnostic tool for the determination of certain microbial flora. Octane, 4-methyl- and cyclohexane, 1-ethyl-1-methyl also possesses excellent antimicrobial properties. These compounds are in practice for the production of biomedicine, skin care and pharmaceutical products (Ubaid *et al*., 2016). Similarly, 3-butyn-1-ol and 1,2,5-oxadiazole compounds are known for antiproliferative activities and were tested against cancer cells such as SK-N-SH, HepG2 and HeLa with potent inhibitory potetial against these cell lines (Sabitha *et al*., 2014). Recently, Firdouse *et al.* (2019) reported that propanedioic acid, oxo, diethyl ester and propanedioic acid have pharmacological activities against diseases of rectum, gastrointestinal ulceration, rectal, renal dysfunction and hemorrhoids. Sivakumar *et al*. (2011) worked on a naturally occurring compound acetaldehyde found in fruits, vegetables and plants and reported that it is carcinogenic in humans and if applied for prolonged period then it works as an irritant to respiratory tract, skin, throat and eyes. Kaymakcioglu *et al*. (2012) synthesized the 3-hydroxy- hydrazide compound and evaluated its efficacy against pathogenic fungal species such as *Fusarium*, *Botrytis*, *Colletotrichum* and *Candida albicans* with promising results. Recently, it was noted that carbonic dihydrazine, acetic acid and hydrazine carboxamide compounds are highly efficient against breast cancer cells inhibition as well as against pathogenic fungi and bacteria. These compounds are used in medicine since the previous decades for the treatment of cancer, glaucoma and tumor therapy (Chandra *et al*., 2015; Iqbal *et al*., 2017). Thus in the current study, it was concluded that the *n*-hexane and chloroform fractions of *A. japonica* contains an excellent profile of beneficial as well as harmful compounds.

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Arshad Javaid	:	Design research, manuscript write up.
Iqra H. Khan	:	Conduct research.
Khajista Jabeen	:	Help in conducting research and write up.
Uzma Bashir	:	Help in conducting research and write up.