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Table: 5, Figs : 4

Fungicidal potential of *Amaranthus viridis* L.

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ABSTRACT

Weeds reduce the crops yield and quality of produce. We identified biomolecules and quantified the phenolics and flavonoids contents in the *Amaranthus viridis* L. weed extracts. The antifungal activity of its extracts was determined against *Fusarium* (*F. oxysporum* f.sp *ciceri*, *F. oxysporum* f. sp. *chlamydosporum, F. oxysporum* f.sp. *pallidoroseum* and *F. oxysporum* f. sp. *vasinfectum)* species. We found that *A. viridis* bioactive components bioactivity can be used to controls pathogens. Thus, we identified various bioactive components of this weed and studied their activity, and role in activating defence in plants

INTRODUCTION

The family *Amaranthaceae* includes about 65 genera and 900 species, is mostly distributed in tropical and temperate regions. About 18 genera and over 50 species ARE reported from India. The most widely known are : *Amaranthus blitum* L., *A. dubius* Mart., *A. cruentus* L, *A. graecizans* L, *A. hypochondriacus* L., *A. spinosus* L., *A. thunberch* Moq., *A. tricolor* L., and *A. viridis* L. (1). *A. viridis. Amaranthus viridis* probably originated from America and is found throughout India in waste places. It is a cosmopolitan weed in the tropical and subtropical regions of the world, also penetrating into temperate regions (like Europe, North America, Asia, and Australia). In tropical Africa, it is also a widespread and common weed (28).

Figure 1. *Amaranthus viridis*

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Phenolic compounds are plant secondary metabolites that constitute most widespread groups of substances in plants. These have an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, including functional derivatives (esters, methyl esters, glycosides, and others) (29). These are related to defence responses in plants and also play an important role in other processes (accelerate pollination, flower colours and defence against herbivores, bacteria and fungi (4). Flavonoids protect plants against various biotic and abiotic stresses and play an essential role in the interaction between the plant and their environment. These are responsible for flower colours, protects the plants from pathogenic bacteria, fungi, microbes and insects (19,24). Antifungal activity is based on inhibition of spore development and mycelium hyphae elongation (20,21).

Weeds can have a positive or negative effect on the growth of other plants and organisms through release of chemicals. This is known as allelopathy. According to the 'International Allelopathy Society', any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of any biological system is termed as 'Allelopathy' (15). Chemicals with allelopathic potential are present in all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, seeds, and pollens (5,18). Allelochemicals possess a variety of biological activities and can be used in agriculture to enhance plant growth and productivity due to their herbicidal (15,26) antifungal (14,16), antibacterial (10) and other pesticidal activities (9,30).

This plant, possesses analgesic and antipyretic properties and is used to treat pain and fever in the traditional system of medicine (8). Leaves of *A. viridis* can be used for treating eczema, psoriasis and rashes (25). This herb is used in traditional medicines as diuretic and emollient (3). *Fusarium oxysporum* fungus when treated with $AuCl^{4-}$ ions help in the formation of gold nanoparticles (27). The objective of the present study was to check the antifungal activity of leaf extract of *A. viridis*.

MATERIALS AND METHODS

The study was done at CHARUSAT campus (Latitude: 22.59 ºN, Longitude:72.80 ºE with annual rainfall of 100-400 mm. The maximum temp is 40 ±5 ºC, minimum temp : 20 ±5 ºC, and mean height above the sea level: 39 m.

Preparation of Plant Extracts: Disease-free and fresh *A. viridis* plants were collected from CHARUSAT, Changa campus. The leaves were separated and cleaned under running tap water. The fresh leaves were used to prepare 4 % fresh leaf extract and leachate. The 4 % fresh leaf extract was prepared by crushing 4 g fresh leaves in 1.00 L distilled water. For leachate preparation, 250 g fresh *Amaranthus* leaves were soaked in 500 ml distilled water for 24 h. Similarly, 10 g leaves were oven-dried at 55-60 ºC for 24 h, out of which 1 g dried leaves were mixed in 10 mL distilled water.

GC-MS Analysis: Chemical Analysis of fresh leaf extract by GC-MS (17) was done using Thermo GC- Trace Ultra Ver: 5.0. Pyrolysis auto sampler interfaced with a Perkin Elmer Turbo mass Gold equipped with a fused silica capillary column. The fraction was pyrolyzed at 610 ºC and then introduced to the GC column. Helium was employed as a carrier gas (1 ml/min). Qualitative identification of the different constituents was done by the composition of relative retention time and mass spectra with those of authentic reference compounds by retention indices (RI) and mass spectra. Interpretation of spectrum of GC-MS was done

using the database of the National Institute Standard and Technology (NIST). This was performed at SICART.

Total Phenolics: The total phenolic content was estimated by the Folin Ciocalteu method (17,23). 1 mL leaf extract was mixed with 5 ml distilled water, 1 mL sodium carbonate (20 %), and 1 mL Folin Ciocalteu reagent. The mixture was allowed to stand in water bath at 40 ℃ for 30 min. The absorbance was measured at 765 nm using UV-Visible spectrophotometer (Shimadzu). The standard curve was prepared by using different concentrations of phenol in the range of 0.1- 5 mg/ml.

Total Flavonoids: The flavonoid content was determined by aluminium chloride method (11,15). A volume of 125 µL of the extract was added to 75 µl of 5 % $NaNO_2$ solution. The mixture was allowed to stand for 6 mins, and then 150 µL of aluminium chloride was added and incubated for 5 mins, followed by the addition of 750 µL of NaOH (1M). The final volume was adjusted to 2500 µL with distilled water. After 15 min the mixture turned to pink, and the absorbance was measured at 510 nm using UV visible spectrophotometer (Shimadzu).

Fungicidal Bioassay: Potato dextrose agar (PDA) was prepared (17) by dissolving 6.72 g potato dextrose broth (PDB) and 8.5 g Agar (Bacteriological) in 250 ml distilled water. After autoclaving, PDA was poured into Petri plates. Agar well diffusion method was used, where a drop of culture suspension was placed in centre of the nutrient agar plate and spread all over the plate with a sterile spreader. Three wells were made on the potato dextrose agar medium containing plate with a sterile cork borer and wells were filled with 100 µL of 4 % plant extract. Plates were observed for a zone of inhibition by measuring the colony diameter. Sterile water was used as a control. Further, 100 ml Potato Dextrose Broth (9.6 g PDB was dissolved in 400 ml distilled water) was prepared in a conical flask and it was inoculated with *F. oxysporum*f.*sp ciceri*, *F. oxysporum* f. sp. *chlamydosporum, F. oxysporum* f.sp. *pallidoroseum* and *F. oxysporum* f. sp. *vasinfectum* and tested against 4 % *Amaranthus* fresh leaf extract (aqueous) and *Amaranthus* fresh leaf leachate.

RESULTS AND DISCUSSION

GC-MS Analysis: GC-MS analysis was done to find the types of compounds present in the extract. Artemisyl acetate, a phytochemical (flavonoid, molecular weight -196, Retention time – 19.98) (Figure 1 and 2, Table 1 and 2) was confirmed using PubChem. Artemisyl acetate is a flavonoid and contains antimicrobial properties. Fresh leaf extracts from *Amaranthus* contains Artemisyl acetate, a flavonoid which has anti-microbial properties. Artemisyl acetate is also fungistatic against *Tiarosporella phaseolina* (1000 µL/L), *Fusarium moniliforme* (750 µL/L), and *F. solani* (750 µL/L) (22).The antimicrobial activity of *A. viridis* leaves and seed extracts were comparable with the standard drugs, streptomycin and mecanozol (11).

Table 1. Contents of total phenolics and flavonoids in *Amaranthus* preparations.

***Amaranthus* Preparation**	**Total Phenolics (mg/ml)**	**Total Flavonoids (mg/ml)**
Fresh leaf extract (4 %)	2.2±0.3	1.3±0.3
Fresh leaf leachate (1:2w/v)	1.0±0.1	1.8±0.4
Dried leaf paste (1:10 w/v)	1.3±0.2	1.3±0.3

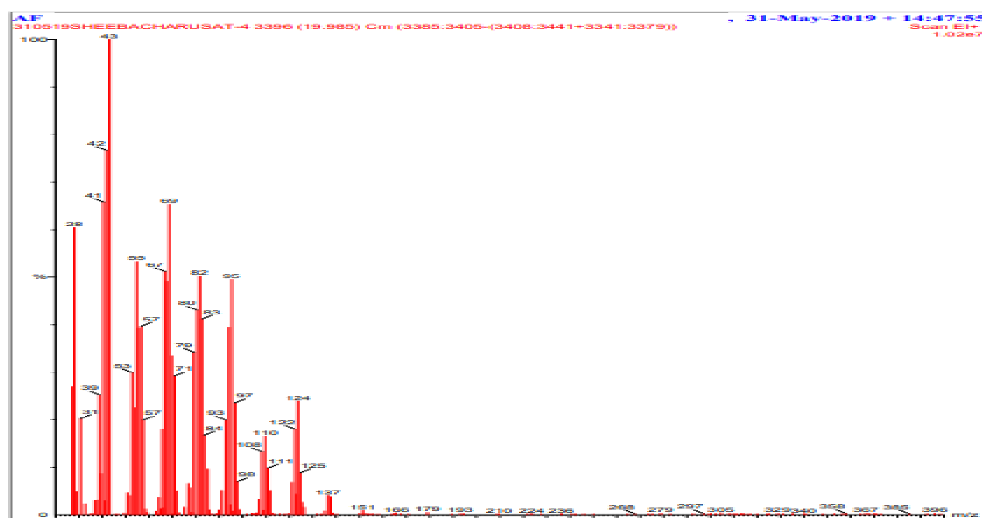

Figure 2. Peaks of Mass spectroscopy for the compound with Retention time- 19.98 in *A. viridis* fresh leaf extract

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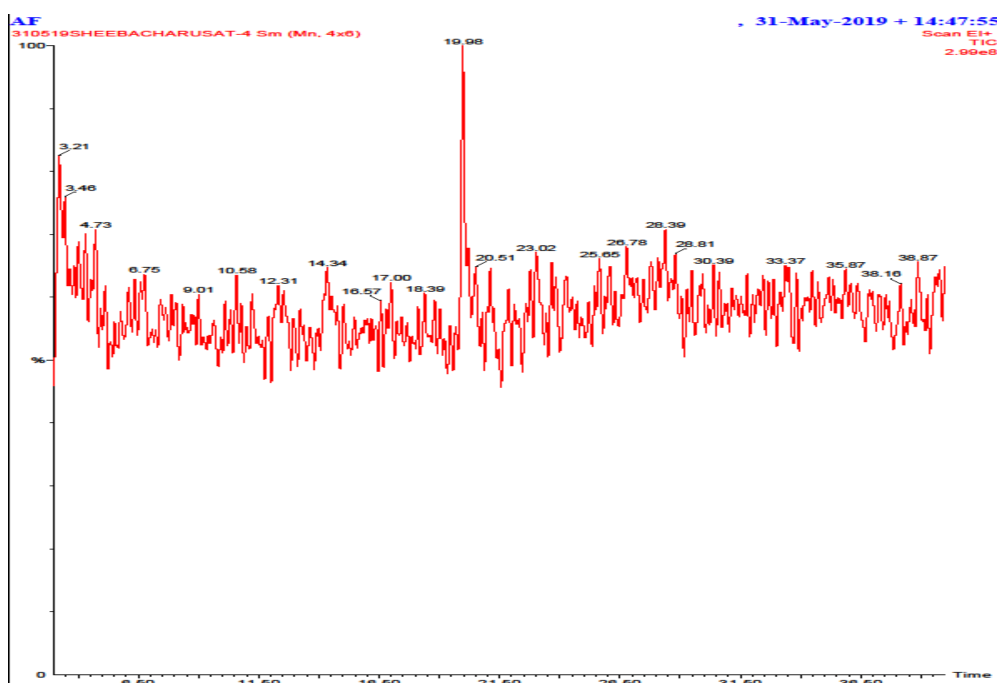

Figure 3. Gas chromatogram of *A.* ***viridis*** fresh leaf extract

Phenolics and Flavonoids: This study evaluated the presence of secondary metabolites like phenolics and flavonoids in *Amaranthus* 4 % fresh leaf extract, *Amaranthus* leachate and in *Amaranthus* dried leaf paste. Allelochemicals can stimulate or inhibit plant growth depending on their concentration (2). Allelopathy has been recognized in weed-crop interaction and its beneficial effects are being applied to get higher yield of crops (12). Allelopathy is a form of plant interference that can significantly influence the ecosystem and agroecosystem dynamics (31). The presence of Total phenolics and flavonoids in different *Amaranthus* preparations was estimated (Table 1,2) and was found highest in *Amaranthus* 4 % fresh leaf extract, whereas flavonoids were maximum in *Amaranthus* fresh leaf leachate.

Table 2. List of compounds in Mass spectroscopy of *A. viridis* fresh leaf extract, Retention time-19.98 m

Hit	REV	for	Compound Name	M W	Formula	CAS
1	823	637	Undecane,1,2-Dibromo-2-Methyl	326	$C_{12}H_{24}Br_2$	55334-43-5
2	817	629	Z,Z-4,16-Octadecadien-1-Olacetate	308	$C_{20}H_{36}O_2$	900130-95-7
3	796	625	Cis-9,10-Epoxyoctadecen-1-Ol	284	$C_{18}H_{36}O_2$	13980-12-6
4	793	611	E-2-Trtradecen-1-01	212	$C_{14}H_{28}O$	900130-83-7
5	789	630	E-2-Octadecadecen1-Ol	268	$C_{18}H_{36}O$	900131-10-2
6	788	583	Undec-10-Ynoicacid, Dodecylester	336	$C_{22}H_{40}O_2$	900406-16-4
7	787	590	9-Octaecenal(2)	266	$C_{18}H_{34}O$	2423-10-1
8	785	581	Undec-10-Ynoicacid, Dodecylester	350	$C_{23}H_{42}O_2$	900406-16-5
9	781	601	2-Methyl-Z,Z-3,3-Octadecadienol	280	$C_{19}H_{36}O_2$	900130-90-5
10	780	540	Cyclopentane,1-1`-Hexadecylidenebis	362	$C_{26}H_{50}$	55401-16-8
11	779	610	Cis-9-Hexadecenal	238	$C_{16}H_{30}O$	56219-04-6
12	778	559	Z-(13,14-Epoxy) Tetra Dec-11-En-1-Olacetate	268	$C_{16}H_{28}O_3$	900131-33-2
13	777	509	4-Methyl-Z-4-Hexadecebn-1-Ol	254	$C_{17}H_{34}O$	900130-89-0
14	776	501	Hexacosanal	380	$C_{26}H_{52}O$	26627-85-0
15	775	596	E,E-2,13-Octadecadien-1-Ol	266	$C_{18}H_{34}O$	900131-09-8
16	775	587	Myrcenylacetat	196	$C_{12}H_{20}O_2$	900149-84-5
17	774	562	Z-2-Octadecen-1-1-Ol	268	$C_{18}H_{36}O$	900131-11-0
18	774	601	Z-3,17-Octadecadien-1-Olacetate	308	$C_{20}H_{36}O_2$	900130-95-6
19	773	561	Tridecanal	198	$C_{13}H_{26}O$	10486-19-8
20	773	525	Undec-10-Ynoic Acid, Tetradecyl Ester	378	$C_{25}H_{36}O_2$	900406-16-7

Fungicidal Assay: The aqueous 4 % *Amaranthus* fresh leaf extract was also tested against *Fusarium oxysporum* f.sp. *ciceri*, *F. oxysporum* f.sp. *chlamydosporum*, *F. oxysporum* f.sp. *pallidoroseum* and *F. oxysporum* f. sp. *vasinfectum* and it effectively inhibited the fungal growth. The zone of inhibition was highest in *F. oxysporum* f. sp. *vasinfectum* (Table 3). Antifungal activity of *Amaranthus* 4 % fresh leaf extracts and *Amaranthus* fresh leaf leachate (quantitative activity) on various *Fusarium* species was also analyzed. *Amaranthus* leaf extracts and leachate were analyzed against *Fusarium* species and were found effective against *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f.sp. *chlamydosporum*, *F. oxysporum* f.sp. *pallidoroseum* and *F. oxysporum* f. sp. *vasinfectum.* Fungal growth in potato dextrose broth was 7.22 g/100 mL (FOV) and in a flask with potato dextrose broth having extract was 0.13 g/100 mL, whereas, fungal growth in potato dextrose broth was 0.87 g/100 mL (FOP) and in a flask with potato dextrose broth having leachate was 0.16 g/100 mL (Figure 3 and Table 4,5). Control of *Fusarium* species may be due to the presence of various alleochemicals like artemisyl acetate, flavonoid, phenolics etc in these plants.

Table 3. Antifungal activity of *Amaranthus* fresh leaves extract

***Fusarium* spp.**	**Inhibition Zone (mm)**
F. oxysporum f.sp. *ciceri*	0.2±0.1
F. oxysporum f.sp. *chlamydosporum*	0.4±0.1
F. oxysporum f.sp. *pallidoroseum*	0.4±0.03
F. oxysporum f.sp. *vasinfectum*	0.8±0.1

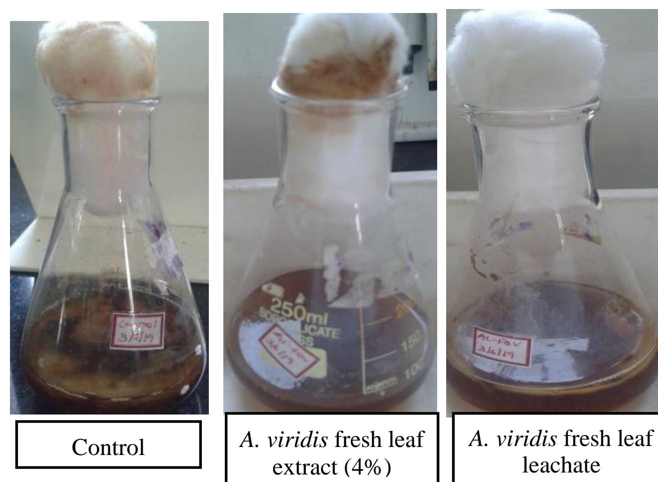

Figure 5. Antifungal activities of *A. viridis* fresh leaf extracts (aqueous) and *A.viridis* leachate (aqueous) on *F. oxysporum* f. sp.*vasinfectum* (FOV).

Table 4. Antifungal activity of *A. viridis* 4 % fresh leaf extracts on various *Fusarium* species

	Mycelial weight (g/100 mL)		
Type of pathogen	Control	Fresh extract (4 %)	Inhibition (%) over control
Extract			
F. oxysporum f.sp. *ciceri*	4.21	1.89	55.1
F. oxysporum f.sp. *vasinfectum*	7.22	0.13	98.2
F. oxysporum f.sp. *pallidoroseum*	0.87	0.17	80.5
F. oxysporum f.sp. *chlamydosporum*	4.66	1.29	72.3

Table 5. Antifungal activity of *A. viridis* leaf leachate on various *Fusarium* species

Fusarium spp	Mycelial weight (g/100ml)		
	Control	Leachate	Inhibition (%) over control
Leachate			
F. oxysporum f.sp. *ciceri*	4.21	1.15	72.7
F. oxysporum f.sp. *vasinfectum*	7.22	2.02	72
F. oxysporum f.sp. *pallidoroseum*	0.87	0.16	81
F.oxysporum f.sp. *chlamydosporum*	4.66	1.17	74.9

CONCLUSIONS

The readings were taken after 28 days every month, and continued for 18 months (from June 2017 to June 2019). Pathogen (*Fusarium* sp) inhibition was observed when 4 % *Amaranthus* leaf extract and *Amaranthus* leaf leachate and pathogen were incubated together. Further, after incubation the leaf extract and leachate decreased fungal growth. This explored the antifungal potential of the leaf extracts and leachate. The Antifungal activity with aqueous leaf extract and leachate had great effects. Hence, the weeds can have various applications including as fungicide and their more effect son plants needs to be studied**.**

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ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

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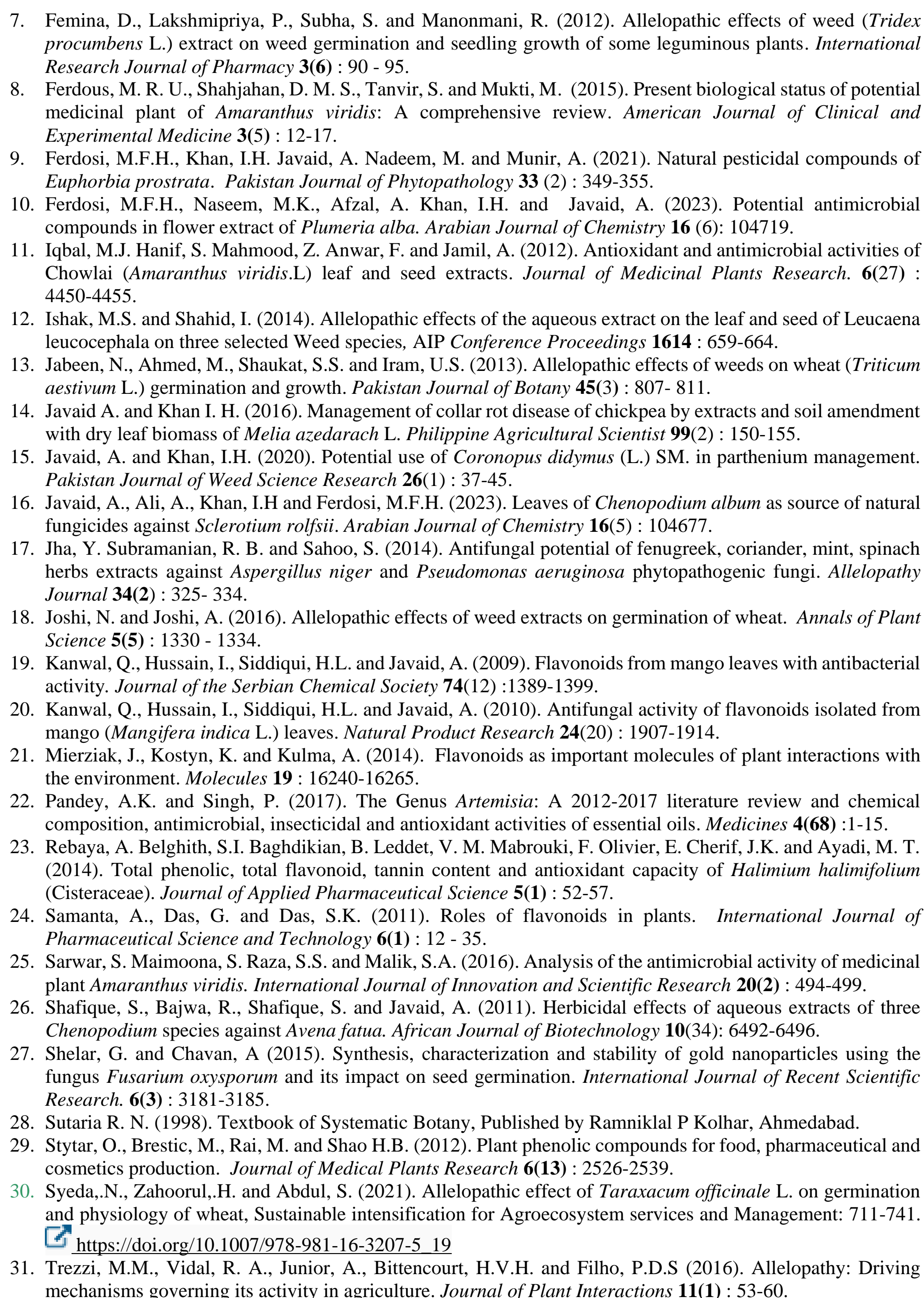

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