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Phytochemical Analysis and Antifungal Activity of Leaves Extract of *Vachellia seyal* on *Phytophthora infestants* Isolated from rotten potatoes

[1]Nura Surajo*., [2]Bem, A.A. and [2]Musa, D.D.

[1]Government Science Secondary School, Dutsin-Ma, Katsina, Nigeria

[2]Department of Plant Science and Biotechnology, Federal University, Dutsin-Ma, Katsina, Nigeria

*Correspondence author: nurasurajo112@gmail.com, +234 803 541 9809

Abstract

Studies looking at the presence of potent antimicrobial compounds in plants have been sparked by the use of plants and their extracts as treatments for various illnesses. The study aimed to analyze the antifungal and phytochemical properties of a methanolic extract of Vachellia seyal leaves on P. infestant isolated from rotten potato tubers. Following drying, the leaves were extracted using methanol as the solvent using cold maceration and percolation. To check for the existence of bioactive components, the extract underwent GC-MS analysis and qualitative phytochemical screening. The antifungal inhibitory activity of the leaf extract was assessed using agar well diffusion techniques. The results of the phytochemical screening indicated the presence of secondary metabolites, including terpenoids, tannins, phenols, flavonoids, alkaloids, saponins, and steroids, in the leaves. Thirty-five (35) compounds were detected from the GC-MS study; based on their peak, six (6) were determined to be the most prevalent. These compounds include 9-Octadecenoic acid (Z)-, methyl ester (23.35%), Squalene (11.84%), 9,15-Octadecadienoic acid, methyl ester, (Z,Z)- (10.63%), Pentadecanoic acid, 14-methyl-, methyl ester (7.95%), (E)-9-Octadecenoic acid ethyl ester (7.19%) and Butylated Hydroxytoluene (1.85%). With a growth inhibition zone of 37mm, the results showed that the leaf extract of V. seyal was effective against P. infestant. The response of V. seyal may have been caused by the presence of phenolic compounds and acetic acid from the leaf extract. Additionally, methanolic crude extract was more effective than ethyl acetate extracts in controlling the activities of certain fungal pathogens. According to the study, V. seyal has antifungal activity and needs to be considered as a possible antifungal agent while looking for novel treatments for fungal infections.

Keywords: Vachellia, Phytochemical/GC-MS Analysis, Antifungal.

INTRODUCTION

The Leguminosae Fabaceae family, which Adanson and de Jussieu previously identified and characterized, has been further divided into three sub-families: Papilionoideae, Mimosoideae, and Caesalpinioideae (Group, 2009). The Fabaceae family, which has about 19500 species and over 765 genera, is the third most significant plant family (Lewis *et al.*, 2013). All tropical and warm temperate regions of the planet are home to species in this family. With over 1350 species recognized, the genus *Vachellia* (formerly the *Acacia* genus) is considered the second biggest genus in the family *Fabaceae* and a part of the *Mimosoideae* subfamily. Australia has the highest concentrations of Acacia spp. (955 species), while there are also significant populations in America (about 185 species), Africa (144 species), and Asia (89 species)(Brockwell, 2005). Members of this family are recognized as a significant source of compounds used to manage numerous illnesses. Medicinal plants are composed of Phytochemicals, which are specific organic components that have specific physiological effects on the human body, and these bioactive substances include tannins, alkaloids, terpenoids, steroids, and flavonoids (Edoega *et al.*, 2005). The Fabaceae family generates a greater quantity of nitrogen-containing secondary metabolites than other plant families, including quinolizidine, pyrrolizidine, indolizidine, piperidine, and pyrrolidine and other several different

nitrogenous chemicals (Wink, 2013).Numerous secondary metabolites, including amines, alkaloids, cyanogenic glycosides, cyclitols, fatty acids, seed oils, amino acids, essential oils, diterpenes, phytosterol, triterpenes, saponins, and hydrolyzable tannins, have been linked to the *Vahellia* genus. The most evident and best-known are polysaccharides (gums) and complex phenolic substances (condensed tannins) (Bodeker *et al.*, 1997). *Vachellia seyal* (Delile.): also called *Gon-ponsego* (Mooré); Gommier, Mimosa épineux (French) is phanerophyte, a thorny tree 6 to 17 m high with smooth and green bark (Thiombiano *et al.*, 2012). The twigs are greenish, and the leaves are alternating and bipinnate, from 3 to 10 cm long, with 3-7 pairs of pinnules. Narrow pods represent the fruits and contain 6 to 10 brown seeds when ripening. Flowering and fruiting usually occur in the second half of the dry season, before foliage. It is a species that is Sahelo-Saharan and Sudano-Sahelian. It's found on low slopes and low ground and generally near rivers. This species has spread from Senegal to Cameroon, Egypt, and Somalia (Arbonnier, 2009).*V. seyal* is a highly nitrogen-fixing and moderately salt-tolerant species and is characterized by a high content of proteins, phenols, and flavonoids (Fatou *et al.*, 2015). The biological activity of *V. seyal* can be attributed to the high content of flavonoid and polyphenolic components contained in it (Sadiq *et al.*, 2015). The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the bindweed or morning glory family, Convolvulaceae. Its large, starchy, sweet-tasting tuberous roots are used as a vegetable. The young shoots and leaves are sometimes eaten as greens. Cultivars of the sweet potato have been bred to bear tubers with flesh and skin of various colors. *Phytophthora infestans* (Anton de Bary) is an oomycete or water mold, a fungus-like microorganism that causes the serious potato and tomato disease known as late blight or potato blight. Early blight, caused by *Alternariasolani*, is also often called "potato blight". Late blight was a major culprit in the 1840s European, the 1845–1852 Irish, and the 1846 Highland potato famines. The organism can also infect some other members of the Solanaceae (Nowicki *et al.*, 2013). Moist, cool environments favor the pathogen: sporulation is optimal at 12–18 °C (54–64 °F) in water-saturated or nearly saturated environments, and zoospore production is favored at temperatures below 15 °C (59 °F). Lesion growth rates are typically optimal at a slightly warmer temperature range of 20 to 24 °C (68 to 75 °F) (Haverkort, *et al.*, 2009). The pathogen is the one that causes late blight disease in potatoes and is thought to be the most destructive oomycete pathogen, which caused the Irish famine in the 1840s (Chowdappa *et al.*, 2015). The illness first manifests as irregular, pale green lesions around the tips of the leaves. These lesions quickly swell into huge, brown-to-black necrotic spots on the upper surface of the leaves, and white hyphae growth is seen on the lower surface of diseased leaves. The infection causes a brown lesion surrounding the infected stem, which finally causes the stem to collapse. In extreme circumstances, the illness can destroy the crop completely in days, turning it into a black-spotted crop. The disease also affects tubers, causing them to become inflexible, dry, and hard, leading to their rotting in storage and the field. Prior to 2006, potato cultivation in the northern Indian states was thought to be frequently threatened by late blight (Chowdappa *et al.*, 2011). *P. infestans* sporangia are spread by wind or water and enable the movement of the pathogen between different host plants. The zoospores released from sporangia are biflagellate and chemotactic, allowing further movement of *P. infestans* on water films found on leaves or soils. Both sporangia and zoospores are short-lived, in contrast to oospores, which can persist in a viable form for many years. People can observe *P. infestans* produce dark green, then brown, blackspots on the surface of potato leaves and stems, often near the tips or edges, where water or dew collects. Under ideal conditions, *P. infestans* completes its life cycle on potato or tomato foliage in about five days (Norwicki, 2013). Sporangia develop on the leaves, spreading through the crop when temperatures are above 10 °C (50 °F) and humidity is over 75–80% for 2 days or more. Rain can wash spores into the soil, infecting young tubers, and the spores can also travel long distances in the wind. The early stages of blight are easily missed. Symptoms include the appearance of dark

blotches on leaf tips and plant stems. White mold will appear under the leaves in humid conditions, and the whole plant may quickly collapse. Following appropriate laboratory procedures, plant pathogenic oomycetes can be quickly and effectively isolated from infected plant tissues using antibiotic-enriched selective media. Antibacterial drugs such as penicillin, ampicillin, and rifampicin, which stop the growth of bacteria and fungi, are frequently present in the selective media used to isolate oomycetes pathogens. The assessment of genetic variability across isolates and the simple identification and confirmation of species may benefit from the molecular characterization of *P. infestans* using the internal transcribed spacer regions (ITS) of rDNA.

Despite Fabaceae family members are a significant source of physiologically active chemicals. Only a small percentage of *Vachellia* and *Senegalia* species' secondary metabolites have been thoroughly investigated. Nevertheless, not many species have been carefully checked for these compounds. (Seigler, 2003). Since 2008, South Indian and other African potato harvests, including Nigeria, have had major potato late blight disease outbreaks, which have completely destroyed the crop. (Jain *et al.*,2018). In light of the aforementioned, researchers are increasingly looking to medicinal plants in search of novel strategies to generate potent medications against microbial diseases because the overuse of antibiotics has led to the emergence of multidrug-resistant germs. (Perez *et al.*, 1990). The study aimed to conduct phytochemical analysis and antifungal screening of the methanolic leaves extract of *Vachelliaseyal*against pathogenic fungi *P. infestant* extracted from potato tubers.

MATERIALS AND METHODS

Collection, Identification, and Preparation of Plant Extract

Fresh floral components of *Vachellia seyal*, including leaves, flowers, seeds, and inflorescence, were removed from the plant's natural habitat and brought to the Department of Plant Science and Biotechnology Herbarium Federal University of Dutsin, Ma for identification. The plant was then assigned the voucher number (FUDMA/PSB/00116). Additionally, a voucher specimen was left there. The gathered leaves were cleaned of dust using distilled water, allowed to air dry for three days while being turned frequently to inhibit the formation of fungi, and then ground into a powder using a machine grinder in preparation for additional examination. The ground powder was quickly moved to an airtight container and chilled until needed later, as stated by (Aja *et al.*, 2010).

Extraction of leaves of the study species

The process outlined by Shinkafi (2013) was used to extract the plant material (leaves). Cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with methanol. The extract of the leaves was prepared by soaking 100g of the powdered leaves of the plant species in 400ml methanol in a stoppered container for a defined period (4 days) with frequent agitation. A rotary evaporator was used to filter and concentrate the resultant mixture. The extracts were refrigerated until needed for additional examination.

Qualitative Phytochemical Screening

The crude leaf extracts (methanol) of *V. seyal* underwent a preliminary phytochemical screening using conventional protocols as outlined by Trease and Evans (1989), Sofowora (1993), and Ushie *et al.* (2016).

GC-MS (Gas chromatography-mass spectrometry)analysis

Phytochemical assay was carried out using Gas chromatography and mass spectrophotometry. Volatile compound analysis was performed with a gas chromatography system (Aglient 6890 GC) with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP-5 MS (30 m × 0.32 mm × 0.25 µm film thickness). Helium was used as the carrier gas at a flow of about 1.0 ml/min pulsed split less. The solvent delay was 3 minutes, and the injection size was 1.0 µl. The mass spectrometric detector was operated in an electron impact ionization mode with an ionizing energy of 70 eV. Scanning from m/z 50 to 500 and the ion source temperature was 230°C. The electron multiplier (EM) voltage was maintained

at 1250v above auto-tune. The instrument was manually turned using perfluorotributyl amine (PFTBA). Oven temperature program at 45ºC (2 min), 150˚C (5 min) at a rate of 2˚C min-1, then at 150ºC (2 min), 280˚C (5 min) at a rate of 8˚C min-1; split 30:1 during 1.50min, carrier gas He: 1 ml min-1, constant flow; sample volume 1 µl. Identification was based on a comparison of their mass spectra with a database of National Institute Standard and Technology (NIST) (Romeh *et al.*, 2013).

Isolation of *Phytophthora infestans* from Infected Potato tubers

After washing the contaminated potato tuber sample to remove debris, a fresh razor blade was used to aseptically chop the potato into tiny pieces (2–5 mm2). In order to prevent bacterial contamination, the infected potato samples were inoculated on Sabouraud dextrose agar supplemented with 200 mg of chloramphenicol per milliliter. After that, the infected plate was incubated for five (5) days at 25 ˚C.

Microscopic observation of Sporangia

In order to evaluate the morphological features of the recovered sporangia, seven-day-old P. infestans cultures were used. The sporangia were aseptically transferred into Lactophenol cotton blue mountant on a clean, grease-free glass slide using a straight wire and was covered with a glass coverslip. The slide was viewed under the microscope using an x10 objective lens to focus and x 40 objective lens to confirm the spore of *P. infestans*. The morphology of the spores was confirmed using a fungi atlas (Levetin, 2004).

Antifungal Screening

The antifungal properties of leaf extracts of the study species were tested against *Phytophthorainfestant*using agar well diffusion method on Sabouraud Agar (SA) plates.) *P. infestant* isolate spore suspensions were aseptically added to sterile Petri plates. After adding 20 milliliters of the cooled, molten Sabouraud's agar to each plate, the plates were spun clockwise and counterclockwise to ensure that the fungal spore suspensions were evenly mixed. Using a sterile cork-borer, wells of 6 mm diameter were created on the agar medium's surface following solidification. The extracts were put into each well in a volume of 0.1 ml. The ketoconazole control was utilized at a 10 mg/ml concentration. For 48 hours, the plates were incubated at 28 ˚C. Using a transparent ruler, the inhibition zones around the agar wells were measured millimeters after incubation (Mercy *et al.*, 2014).

Figure 1: Infected Potato tubers

RESULTS

Phytochemical screening

Steroids, flavonoids, alkaloids, tannins, terpenoids, phenols, and saponins were the secondary metabolites tested and found all present in the methanolic leaves extracts of *V. seyal*, according to preliminary phytochemical analysis (Table 1).

GC-MS chromatogram of the *V. seyal* leaves extracts reveals thirty-five (35) peaks, signifying the presence of thirty-five (35) chemicals, as seen in Figure 2. Table 2 displays a variety of compound names, retention times, molecular formulas, molecular weights, peak areas (%), and biological activities. The compounds are detected between the ranges of 13.9499 to 38.6234 minutes. The compounds found have a lower retention time of 13.9499 minutes for butylated hydroxytoluene and a higher retention time of 38.6234 minutes for E-15-Heptadecenal.

Antifungal screening

The antifungal efficacy of methanolic leaf extract of *V. seyal* was carried out against pathogenic *P. infestant*. As shown in (Table 3), the results revealed that *V. seyal* was found to inhibit the growth of the tested *P. infestant* fungal strain with growth inhibition of 37%.

Table 1: Preliminary phytochemical screening of methanolic leaves extracts of *V. seyal*

Secondary metabolites	Steroid	Flavanoid	Alkaloid	Tannin	Terpenoid	Phenol	Saponin
Key	+	+	+	+	+	+	+

KEY: + = Present

Table 2: GC-MS result of methanolic leaf extract of *V. seyal*

PK	**RT (min)**	**Name of compound**	**MF**	**MW (g/mol)**	**Qual**	**Peak area%**	**Activity**
1.	13.9499	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.4	98	1.8455	Antioxidant
2.	15.7173	2-Tridecanol	$C_{13}H_{28}O$	200.4	72	0.8404	Antioxidant
3.	15.8652	1-Heptadecanamine	$C_{17}H_{37}N$	255.5	59	0.5331	Antimicrobial
4.	19.4792	Decanoic acid, 3-methyl-	$C_{11}H_{22}O$	186.3	50	1.3035	Antioxidant/inflammatory
5.	19.5773	Butanoic acid, 3-hydroxy-, butyl ester	$C_8H_{16}O_3$	160.3	37	1.2783	Antimicrobial
6.	19.6236	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194.2	47	0.9371	Antimicrobial
7.	19.6599	Decanoic acid, 3-methyl-	$C_{11}H_{22}O$	186.3	47	0.8848	Antimicrobial
8.	19.7378	.beta.-d-Mannofuranoside, methyl	$C_7H_{14}O_6$	194.2	50	1.4096	Antimicrobial
9.	19.7615	Dimethyl-cyano-phosphine	C_3H_6NP	87.06	47	0.8689	Antimicrobial
10.	19.7973	4-O-Methylmannose	$C_7H_{14}O_6$	194.2	40	0.72	Antifungal
11.	20.2242	5-Eicosene, (E)-	$C_{20}H_{40}$	280.5	46	1.7389	Antifungal
12.	23.094	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O$	270.5	98	7.949	Antifungal
13.	23.9343	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334.5	80	3.3728	Antimicrobial
14.	24.3745	1-Docosene	$C_{22}H_{44}$	308.6	91	1.1684	Antioxidant

To be continued next page

Table 2 Continued

PK	RT (min)	Name of compound	MF	MW (g/mol)	Qual	Peak area%	Activity
15.	24.4516	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O$	384.5.5	93	4.9242	Antimicrobial
16.	26.3795	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	$C_{19}H_{32}O$	292.5	99	10.6339	Antimicrobial
17.	26.4958	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O$	296.5	83	23.3483	Antioxidant
18.	26.7793	Phytol	$C_{20}H_{40}O$	276.5	76	3.7507	Antioxidant
19.	27.0049	Methyl stearate	$C_{19}H_{38}O_2$	298.5	30	1.5717	Antimicrobial
20.	27.6246	Methyl tetratriacontyl ether	$C_{35}H_{72}O$	508.9	98	0.5242	Antimicrobial
21.	27.7291	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.5	53	7.1933	Antimicrobial
22.	27.85	2- Chloropropionic acid, octadecyl ester	$C_{21}H_{41}C_{lO}$	361.0	62	1.2353	Antioxidant
23.	28.1525	17-Pentatriacontene	$C_{35}H_{70}$	490.9	93	0.5285	No activity
24.	28.2268	Octadecanoic acid, 17-methyl-, methyl ester	$C_{20}H_{40}O$	312.5	46	3.194	Antimicrobial
25.	29.4614	3,7-Nonadien-2-one, 4,8-dimethyl-	$C_{11}H_{20}O$	168.3	93	0.2035	Antioxidant
26.	30.6068	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	697.0	64	0.7372	Antimicrobial
27.	31.4284	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.5	93	2.2209	Antioxidant
28.	31.4546	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.5	38	1.8591	Antidiabetic
29.	34.2435	Oxalic acid, allyl octadecyl ester	$C_{23}H_{42}O_4$	382.6	58	0.6793	Antioxidant
30.	37.8459	Octadecyl propyl	$C_{21}H_{44}O3s$	376.6	45	0.6086	Antimicrobial
31.	37.9671	Carbonic acid, but-3-en-1-yl tetradecyl ester	$C_{18}H_{34}O_3$	298.5	99	0.0173	No activity

To be continued next page

Table 2 Continued

PK	RT (min)	Name of compound	MF	MW (g/mol)	Qual	Peak area%	Activity
32.	38.094	Squalene	$C_{30}H_{50}$	410.7	64	11.8389	Antifungal
33.	38.368	Heptadecanoic acid, heptadecyl ester	$C_{18}H_{36}O$	284.5	83	0.3055	Antioxidant
34.	38.4054	Pentadecafluorooctanoic acid, octadecyl ester	$C_{26}H_{37}F_{15}O_2$	666.5	78	0.0523	Antimicrobial
35.	38.6234	E-15-Heptadecenal	$C_{17}H_{32}O$	252.4	83	-0.2772	Antimicrobial

RT= retention time, **MF**= molecular formula, **MW=** molecular weight

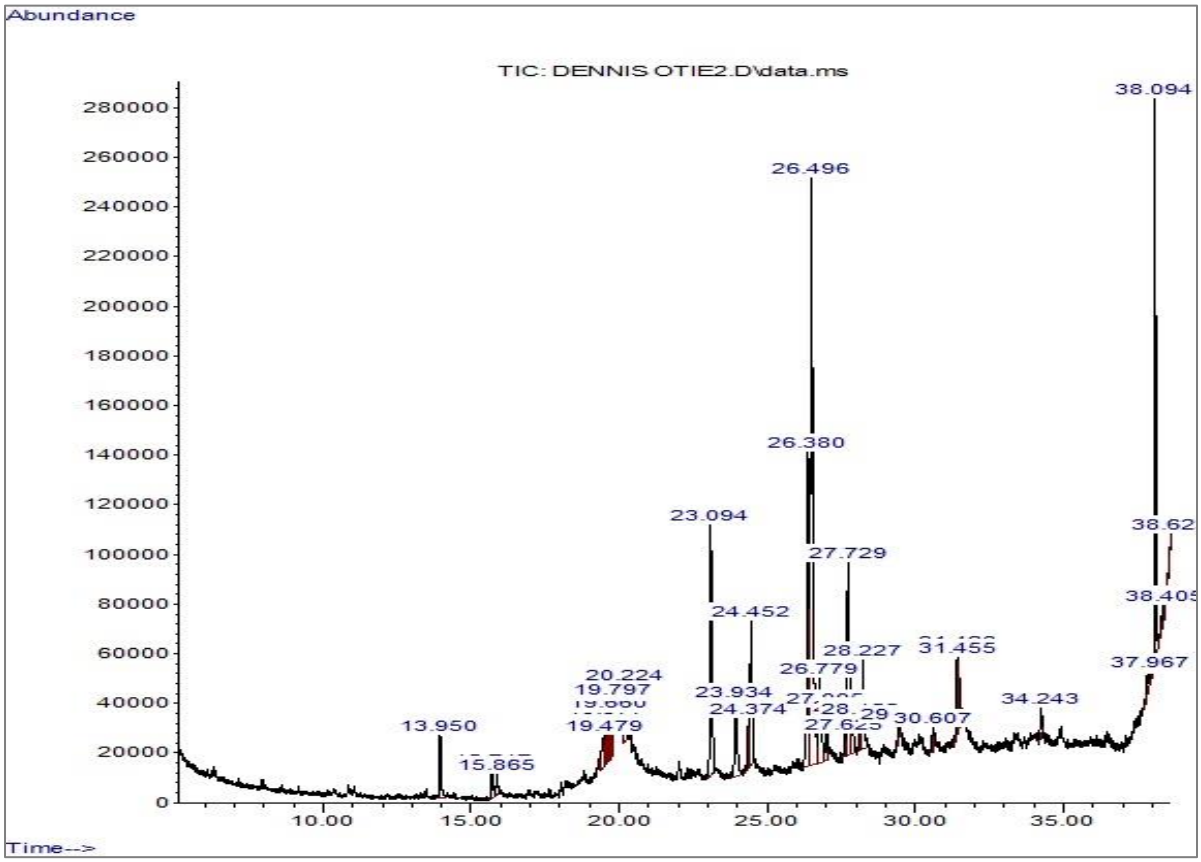

Figure 2. GC-MS Chromatogram of *V. seyal* leaves extract

Table 3: Antifungal screening of methanolic leaves extract of *V. seyal*on *P. infestant*

Species	Part used	*Phytophthora infestant*	Inhibition zone
V. seyal	Leaves	+	37mm

DISCUSSION

The methanolic leaf extract of *Vachelliaseyal*species underwent qualitative preliminary phytochemical screening, demonstrating secondary metabolites like tannins, flavonoids, alkaloids, phenols, saponins, terpenoids, and steroids. This result corresponds with the findings of Bojaxa et al.'s (2010) investigation into the phytochemical screening of *Fabaceae* family plants, which revealed the presence of terpenoids, tannins,

flavonoids, alkaloids, phenols, saponins, and steroids. According to Saleh *et al.* (2015), some secondary metabolites have previously been proven to exhibit antimicrobial action. Moreover, the phytochemicals that have been recorded correspond with the findings of a study carried out by Bodeker *et al.* (1997), which stated that the formerly genus *Acacia* recorded the presence of secondary metabolites, including amines, alkaloids, glycosides, fatty acids, seed oils, essential oils, saponins, and hydrolyzable tannins. Additionally, the phenolic molecule found in the phytochemical screening aligns with the results of Fatou *et al.* (2015), who found that *V. seyal* is a species that is highly nitrogen-fixing, moderately salt-tolerant, and has a high protein, phenol, and flavonoid content. Furthermore, the results corroborated earlier research indicating that the genus *Acacia* is diverse and contains a range of bioactive substances, including tannins, phenolic compounds, alkaloids, flavonoids, and terpenoids (Singh *et al.*, 2009), which are responsible for diverse pharmacological and biological characteristics as reported by (Seigler, 2003).

Numerous peaks were identified by the GC-MS analysis of the methanolic leaf extracts of various plant species, each peak belonging to a different phytochemical component. Records included chemical names, molecular formulas, retention periods, molecular weight peak area percentages, and biological activity information. The methanolic leaves extract of *V. seyal* included thirty-five (35) compounds, of which six (6) were determined to be the most prevalent compounds based on their peak, including 9-Octadecenoic acid (Z)-, methyl ester (23.35%), Squalene (11.84%), 9,15-Octadecadienoic acid, methyl ester, (Z,Z)- (10.63%), Pentadecanoic acid, 14-methyl-, methyl ester (7.95%), (E)-9-Octadecenoic acid ethyl ester (7.19%) and Butylated Hydroxytoluene (1.85%). The compounds discovered from the GC-MS analysis were compared with substances in the V. seyal extract phytochemical study library. The components primarily correspond to the classes of alkaloids, flavonoids, phenols, and tannins, as reported by (Kamaraj *et al.*, 2011). According to earlier studies, these chemicals may have antibacterial, antidiabetic, antioxidant, antifungal, and anticancer properties (Ikram *et al.*, 2016).

Different bioactive compounds detected from the leaf extract of *V. seyal* confirmed its antifungal activity, and this is in agreement with the previous findings of Kubmarawa *et al.* (2007), who revealed that plant parts containing fatty acid compounds possessed antimicrobial activity. Also, a study by Shinkafi (2013, 2014) discovered that plants with tannin, flavonoids, phenols, steroids, and saponins had antimicrobial action. Additionally, the study's antifungal screening results align with the findings of Rathod and Pawar (2012), who reported that plant extracts of the *Vachellia* species (previously known as the genus *Acacia*) had an inhibitory effect on *P. infestant's* linear growth. Furthermore, the research plant's leaves extract's inhibitory activity corresponds with the results of Mahesh and Satish (2008), who found that methanolic extracts of the bark and leaves of *Vachellia* species, including *V. seyal*, had noteworthy antifungal activity against root extract. Similar to this, Hubert *et al.*'s (2013) study found that plant components that produce secondary metabolites like phenolic/flavonoids and terpenes/monoterpenes are known to stop the proliferation of pathogens. Moreover, using methanol as a solvent supported the study findings of Prabhahar *et al.* (2012) that methanolic extract demonstrated greater fungal inhibitory activity against *P. infestant*.

CONCLUSION

Ultimately, the investigation determined and verified that different bioactive compounds detected from the leaf extract of *V. seyal* confirmed its antifungal activity against *P. infestant*. To offer a thorough evaluation of the extract's antifungal efficacy, a clinical trial should be conducted to ascertain the safety and efficacy of the herbal formulation.

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