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Evaluation of Acetone, Methanol, and Hexane Extracts from Three Medicinal Plants for the Control of *Fusarium* Wilt of Potato *In Vitro*

Perpetual M. Katsriku* and **Julius Onyango Ochuodho**

Department of Seed and Horticultural Sciences, School of Agriculture and Biotechnology, University of Eldoret, P.O Box 1125, Eldoret, Kenya

Abstract

Fusarium wilt, caused by Fusarium oxysporum, is a destructive vascular disease of Irish potatoes (Solanum tuberosum L.), leading to substantial yield losses. Management is challenging due to the pathogen's persistence in soil and the lack of resistant cultivars, while synthetic fungicides pose environmental and health risks. This study evaluated the in vitro antifungal activity of leaf, root, and stem bark extracts from three medicinal plants: Euclea divinorum, Prunus africana, and Carissa edulis against F. oxysporum. Extracts were prepared using solvents of varying polarity: acetone, methanol, and hexane. Antifungal activity was assessed using the poisoned food technique, measuring mycelial growth inhibition. Results demonstrated that solvent polarity and plant organ significantly influenced efficacy. Acetone and methanol extracts exhibited the strongest activity. Complete mycelial growth inhibition (0 mm growth) was achieved with P. africana stem bark (acetone and methanol), C. edulis root (acetone and methanol), and E. divinorum leaf (acetone only). In contrast, hexane extracts showed only moderate activity, with the highest inhibition (57%) from C. edulis leaf. These findings highlight the importance of solvent selection and organ-specific phytochemical profiles in optimizing antifungal activity. The most effective combinations provide a scientific basis for developing botanical fungicides as sustainable alternatives for managing Fusarium wilt in potato production. Further research is recommended to identify active compounds and validate efficacy under in planta conditions.

Keywords: *Fusarium wilt, Biocontrol, Plant extracts, Medicinal Plants.*

Introduction

Fusarium wilt is a major vascular disease affecting Irish potatoes (*Solanum tuberosum* L.) worldwide. *Fusarium oxysporum* (*F. oxysporum*), a soil-dwelling pathogen, is the primary causative agent. The pathogen infiltrates the root system and commandeers the xylem vessels, resulting in wilting, chlorosis, stunting, and a significant reduction in tuber yield. Controlling this disease is challenging due to the prolonged viability of the pathogen's chlamydospores in soil and the limited availability of resistant potato varieties [20]. The existing dependence on synthetic fungicides prompts apprehensions regarding environmental toxicity, disease resistance, and human health, hence necessitating the exploration of sustainable, eco-friendly alternatives [6]. Plant-derived extracts may serve as a viable source of antimicrobial agents for the formulation of bio-fungicides. This approach adheres to the principles of integrated pest management (IPM) and the increasing demand for safer agricultural chemicals [10].

Euclea divinorum Hiern (Ebenaceae), *Prunus africana* (Hook.f.) Kalkman (Rosaceae), and *Carissa edulis* (Forssk.) Vahl (Apocynaceae) are ethnobotanically important plants in East Africa, historically employed for the treatment of diverse health conditions. This signifies that secondary metabolism is exceptionally resilient. *E. divinorum* is acknowledged for its antibacterial and antioxidant attributes, ascribed to naphthoquinones and triterpenoids [11]. Comprehensive studies have been undertaken on the bark of *P. africana*, which encompasses various bioactive compounds, including phytosterols. Nonetheless, limited research has been undertaken regarding its leaves [19].

Nyagumbo propose that *C. edulis* possesses antibacterial activities and anti-inflammatory advantages [15]. Despite the acknowledged advantages of these plants, there has been insufficient research on their efficacy against phytopathogenic fungi, particularly *Fusarium oxysporum* in potatoes.

The extraction solvent is essential as it modifies the polarity and spectrum of the isolated molecules, hence affecting their bioactivity [13]. Acetone, methanol, and hexane exhibit varying polarities, enabling the extraction of a range of antimicrobial compounds, from non-polar terpenoids in hexane to medium-polarity phenolics in acetone and high-polarity glycosides in methanol. This study evaluated the effectiveness of acetone, methanol, and hexane extracts from the leaves, roots, and stems of *Euclea divinorum*, *Prunus africana*, and *Carissa edulis* against *Fusarium oxysporum*, the pathogen that causes wilt in Irish potatoes.

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Perpetual M. Katsriku | Katsrikuperpetual@gmail.com

The results will enhance the scientific validation of these ethnobotanical resources and pinpoint potential lead extracts for the formulation of botanical fungicides.

Fusarium wilt, caused primarily by the soil-borne pathogen *Fusarium oxysporum (F. oxysporum)*, is a devastating vascular disease affecting Irish potato (*Solanum tuberosum* L.) cultivation worldwide [12]. The pathogen invades the root system, colonises the xylem vessels, and leads to wilting, chlorosis, stunting, and significant tuber yield losses. Managing this disease is challenging due to the longevity of the pathogen's chlamydospores in soil and the limited availability of resistant potato cultivars [20].

Current dependence on synthetic fungicides raises concerns regarding environmental toxicity, pathogen resistance, and human health, driving the search for sustainable, eco-friendly alternatives [6]. Plant-derived botanicals represent a promising reservoir of antimicrobial compounds, offering a potential basis for bio-fungicide development. This approach aligns with integrated pest management (IPM) principles and the growing demand for safer agricultural inputs [10].

Euclea divinorum Hiern (Ebenaceae), *Prunus africana* (Hook.f.) Kalkman (Rosaceae), and *Carissa edulis* (Forssk.) Vahl (Apocynaceae) are ethnobotanically significant species in East Africa, traditionally used to treat various ailments, indicative of their rich secondary metabolism. *E. divinorum*, for instance, is renowned for its antimicrobial and antioxidant properties, attributed to naphthoquinones and triterpenoids [11]. *P. africana* bark is rich in bioactive compounds like phytosterols and is widely studied; however, its leaves are less explored [19]. *C. edulis* is reported to possess antimicrobial and anti-inflammatory activities [15]. While these plants have documented uses, their efficacy against phytopathogenic fungi, specifically *Fusarium oxysporum* affecting potatoes, remains underexplored.

The choice of extraction solvent is critical, as it influences the polarity and spectrum of compounds recovered, thereby affecting bioactivity [13]. Acetone, methanol, and hexane offer a range of polarities, facilitating the extraction of diverse antimicrobial compounds, from non-polar terpenoids in hexane to medium-polarity phenolics in acetone and high-polarity glycosides in methanol.

Therefore, this study aimed to evaluate the *in vitro* antifungal activity of leaf, root, and stem bark extracts of *Euclea divinorum*, *Prunus africana*, and *Carissa edulis*, prepared using acetone, methanol, and hexane, against *Fusarium oxysporum* causing wilt in Irish potato. The findings will contribute to the scientific validation of these ethnobotanical resources and identify potential lead extracts for developing botanical fungicides.

Methodology

2.1. Isolation and identification of *Fusarium oxysporum*

Irish Potato plants exhibiting indications of Fusarium wilt were transported to the laboratory in sterile paper bags. The Irish potato plants were individually washed with tap water to eliminate dirt, cut into 1 cm fragments, surface sterilised with 0.1% sodium hypochlorite for three minutes, and rinsed three times alternately with sterile distilled water. The specimens were subsequently infected at five equidistant locations on PDA within a 90 mm Petri dish and cultured for 7 days at room temperature (28 ± 2 °C). Mycelia were sub-cultured onto new PDA, and additional sub-culturing was performed until pure cultures of *F. oxysporum* were achieved.

Slides of 7-day-old mycelia from pure cultures of *F. oxysporum* were analysed using a compound microscope (Leica DME, Leica Microsystems, Shanghai, China). The identification as *F. oxysporum* was validated by contrasting their physical and cultural traits with the photographs and descriptions provided by [2].

2.2. Collection of Plant Samples

Leaves and roots of *Euclea divinorum* Hiern (Ebenaceae) and *Carissa edulis* (Forssk.) Vahl (Apocynaceae) and the stem barks and leaves of *Prunus africana* (Hook.f.) Kalkman (Rosaceae) were intentionally harvested from Elgeyo Marakwet County in 2025. The specimen was subsequently transported to the Department of Botany Herbarium for verification and allocation of a voucher specimen number.

2.3. Preparation of Plant Extracts

The roots, leaves, and stem barks of the collected plants were washed with tap water and distilled water, then shade-dried for two weeks. The materials were converted into fine powders using a laboratory grinding mill. Subsequently, they were enclosed in airtight bags, tagged, and maintained in darkness until extraction. Approximately 50 grams of each plant powder were measured and extracted with 0.5 litres of ethanol by continuously swirling with a magnetic stirrer for three hours. The extract was subjected to filtration using filter paper and subsequently evaporated to dryness under vacuum conditions. The extract was subsequently stored at 2-8 °C until utilised.

2.4. Isolation and Cultivation of Fungal Pathogens

Fusarium oxysporum was identified from infected Irish potato plants exhibiting characteristic wilt symptoms, obtained from a contaminated field at the University of Eldoret. Small segments of vascular tissue from the lesion margins were subjected to surface sterilisation using 1% NaOCl for 2 minutes, subsequently washed with sterile distilled water, and inoculated onto Potato Dextrose Agar (PDA) supplemented with streptomycin sulphate at a concentration of 50 mg/L. Following incubation at 25±2°C for 5-7 days, hyphal tips from proliferating colonies were sub-cultured to get pure cultures. Morphological identification (micro- and macroconidia, chlamydospore development) was conducted, and pathogenicity was validated by Koch's postulates on healthy potato seedlings. A pathogenic culture was preserved on PDA slants at 4°C for the investigation.

2.4. *In Vitro* Antifungal Activity Assessment

The antifungal efficacy of the plant extracts was assessed against the mycelial development of *F. oxysporum* using the poisoned food method established by [8] with some modification. Five (5) millilitres of each concentration of plant extract were introduced into a 9 cm diameter Petri plate, followed by the addition of 20 millilitres of molten PDA media. The mixture was subsequently agitated for homogeneous integration prior to the solidification of the PDA. A 5 mm mycelial disc of pure *F. oxysporum* culture was positioned at the centre of the Petri dish and kept at ambient temperature (28 ± 2 °C) for 5 days. The growth of mycelia was assessed by measuring the colony radius on the fifth day of inoculation using a transparent ruler along two orthogonal lines on the base of the Petri dish, and calculating the average for each Petri dish [18]. Potato Dextrose Agar, supplemented with sterilised distilled water, functioned as the negative control. Each treatment was replicated three times.

The treatments were organised in a Completely Randomised Design. The percentage inhibition of mycelial growth was calculated using the formula established by [9]. Mycelia growth inhibition = $\frac{C-T}{C} \times 100$

where C = Radial growth of fungus in control
T = Radial growth of fungus in treatment

2.5. Statistical Evaluation

Data regarding % inhibition were analysed using analysis of variance (ANOVA) with R statistical software (version 4.3.0). A two-factorial design (plant extract type x solvent) was employed. Treatment means were differentiated via Tukey's Honest Significant Difference (HSD) test at a 5% significance threshold ($p < 0.05$). Results are expressed as the mean.

Results and Discussion

3.1 Antifungal Activity of Plant Extracts on Mycelia Growth

3.1.1 Antifungal Activity of Methanolic Plant Extracts

The *in-vitro* assay results for the methanolic extracts against *Fusarium oxysporum* exhibit significant and diverse antifungal activity among the evaluated botanicals. The complete inhibition of mycelial growth (0 mm) exhibited by *Prunus africana* stem bark and *Carissa edulis* root extracts indicates the highest level of bioactivity, significantly differing from the negative control and other treatments ($p < 0.001$). This total suppression demonstrates that these specific plant-solvent combinations efficiently extracted and delivered a high concentration of fungistatic or fungicidal compounds into the growth medium. The remarkable effectiveness of the methanolic extracts aligns with established phytochemical principles. Methanol is a polar, protic solvent proficient at extracting many antimicrobial secondary metabolites, including phenolics, flavonoids, tannins, and saponins, commonly linked to antifungal activity [16][13]. The notable effectiveness of *P. africana* stem bark corroborates its recognised phytochemistry, rich in bioactive compounds including phytosterols (e.g., β-sitosterol), ferulic acid esters, and pentacyclic triterpenoids, which have antibacterial activity [17][19]. The finding that the stem bark extract outperformed the leaf extract from the same species underscores the importance of choosing specific plant organs, as biosynthetic pathways and chemical accumulation might vary significantly among tissues. The complete inhibition shown with *C. edulis* root extract, as opposed to the partial inhibition demonstrated by its leaf extract (0.75 mm), indicates a higher concentration or a different composition of active compounds in the root tissue. Carissa species are acknowledged for their antibacterial compounds, which encompass cardiac glycosides, lignans, and simple phenolics [15]. The significant activity of root extracts from both *C. edulis* and *E. divinorum* (1.2 mm) may possess ecological significance, as roots directly engage with soil-borne pathogens like *F. oxysporum* and may have evolved improved chemical defences. The mild but significant inhibition by *E. divinorum* leaf and root extracts, along with *P. africana* leaf, confirms their antifungal effectiveness, albeit they were less potent than the primary agents under these assay circumstances. *E. divinorum* is notable for its naphthoquinones, including 7-methyljuglone, which exhibit significant redox-cycling activity capable of disrupting fungal cellular processes [11].

The extraction of considerable amounts of these or other active compounds using methanol, leading to a notable reduction in growth, confirms its effectiveness as an extraction solvent for this species. These findings are highly promising for the management of plant diseases. The complete inhibition observed with the primary extracts suggests the existence of compounds that can halt pathogen multiplication at the application site. A concentration of 10 mg/mL is utilised as a benchmark for future formulation studies. The findings support the ethnobotanical use of these plants and necessitate further investigation of their active compounds for developing bio-fungicides aimed at Fusarium wilt, aligning with the global effort to reduce reliance on synthetic chemicals in agriculture [6].

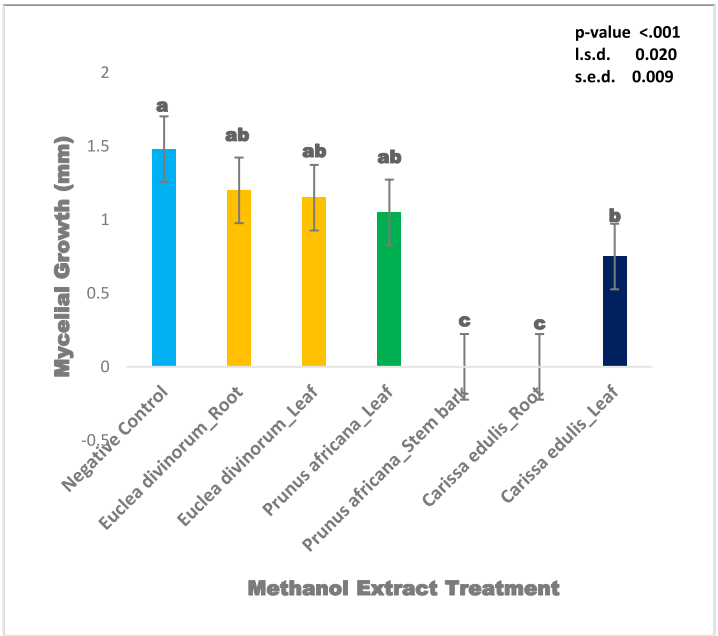

Figure 1: Mycelial Growth in methanol extract treatment

3.1.2 Antifungal Activity of Acetone Plant Extracts

Acetone is a great solvent for removing bioactive compounds from plants, as shown by the acetone extract bioassay. The acetone extract bioassay exhibits a strong and unique antifungal profile. Three extracts (the stem bark of *Prunus africana*, the leaf of *Euclea divinorum*, and the root of *Carissa edulis*) showed total mycelial suppression (0 mm), whilst the other extracts showed notable but partial inhibition, indicating a distinct bimodal distribution of activity. This pattern illustrates how well acetone works to get rid of particular antibacterial substances, which vary depending on the plant species and organ in question.

The substance is quite active, as seen by the modest LSD (0.020) and SED (0.009) results. Tight confidence intervals show that the inhibition observed with these three extracts is statistically significant ($p < 0.001$). Acetone may even be a better solvent than methanol for removing antifungals from these species. Medium-polarity compounds that often have strong antibacterial properties, such as different flavonoids, tannins, quinones, and mid-polarity terpenoids, are effectively extracted by acetone. This is because it cannot extract very non-polar waxes, such as hexane or methanol, or excessively polar sugars [5][3].

Organ-specific factors control the effectiveness of phytochemicals, which differ between species. While the leaf extract (0 mm) completely inhibited *Euclea divinorum*, the root extract (1.17 mm) only partially affected it.

This departure from the trend seen in other solvents clearly shows that *E. divinorum* leaves collect acetone-soluble antifungal compounds that are not present in the roots. This supports earlier findings that Euclea species biosynthesise bioactive flavonoids and naphthoquinones, with notable differences in derivative profiles and concentration across aerial and subterranean tissues [11].

This corroborates previous research suggesting that Euclea species biosynthesise bioactive naphthoquinones and flavonoids, with significant variation in concentration and derivative profiles between aerial and subterranean tissues [11]. The acetone-soluble fraction of the leaf appears to be particularly effective against *F. oxysporum*.

Similarly, the leaf extract (1.0 mm) of *Carissa edulis* did not completely inhibit acetone, although the root extract did. This suggests that the roots contain a reservoir of potent, medium-polarity antifungal compounds that are efficiently extracted by acetone, thereby confirming a significant variation in bioactive chemistry among the organs.

The presence of lignan, phenolic acid, and coumarin is distinctive of Carissa species was asserted [1]. They possess solubility qualities suitable for acetone.

The primary antifungal constituents have a broad spectrum of polarity, evidenced by the constant and total inhibition demonstrated by *Prunus africana* stem bark extract in acetone and methanol solvents. Both protic (methanol) and aprotic (acetone) polar solvents are capable of dissolving them. Although the aglycone nucleus remains soluble in acetone, the carbohydrate moiety enhances the solubility of glycosylated phenolics and terpenoids in methanol. The bark of *P. africana* provides a dependable source of antifungal chemicals effective in various solvents [7].

Chemicals extracted using acetone are expected to engage in molecular interactions with several fungal targets. A considerable quantity of medium-polarity chemicals, including quinones and flavonoids, can induce oxidative stress in fungal cells, compromise membrane functionality, and hinder the functioning of fungal enzymes [4]. The 100% inhibition of growth at the evaluated concentration indicates a fungicidal mechanism of action.

These results facilitate the advancement of biofungicides. Acetone is commonly utilised as a solvent in agricultural and downstream processes due to its higher volatility and reduced hazards compared to methanol. The initial phase of bioassay-guided fractionation, compound characterisation, and formulation development for disease management involves identifying specific plant combinations and their components (e.g., *E. divinorum* leaf, *C. edulis* root, and *P. africana* bark) that produce acetone extracts capable of completely inhibiting Fusarium wilt.

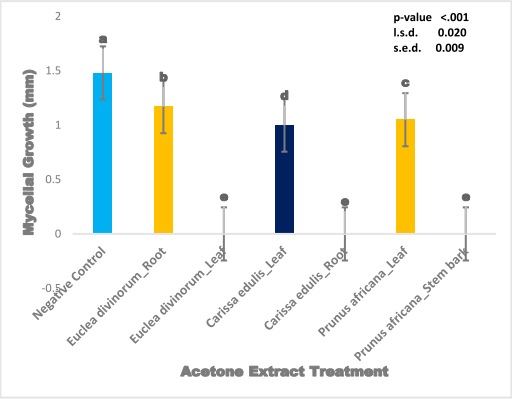

Figure 2: Mycelial Growth in Acetone extract treatment

3.1.3 Antifungal Activity of Hexane Plant Extracts

The bioassay results for hexane extracts demonstrate markedly different antifungal profiles compared to methanolic extracts. This indicates that the bioactive compounds in these plants are distinctly characterised by their polarity. Statistically substantial antifungal activity was seen ($p = 0.006$); however, the overall inhibitory effect was significantly reduced across all regimens. None of the extracts entirely inhibited the growth of mycelium.

The most effective treatment (*Carissa edulis* leaf, 0.63 mm) reduced growth by about 57% compared to the negative control (1.48 mm). This is very different from the total suppression seen with methanol extracts of *P. africana* stem bark and *C. edulis* root.

The moderate to poor activity of hexane extracts can be directly linked to the polarity of the solvent.

Hexane is a non-polar, aprotic solvent effective for extracting lipophilic compounds. These comprise essential oils, fatty acids, sterols, waxes, and non-polar terpenoids [3]. The significant efficacy of *C. edulis* leaf extract (0.63 mm), recognised as the most effective hexane extract, suggests the possible buildup of antibacterial lipophilic compounds within this organ. Carissa species possess non-polar components, such as monoterpenes and sesquiterpenes, in their essential oils, which can compromise fungal membrane integrity [15][1].

The superior performance of the leaf compared to the root in hexane extraction further substantiates that non-polar metabolites are segregated by organ.

It is noteworthy that the extract of *Prunus africana* stem bark has superior efficacy in methanol compared to hexane (1.33 mm). The notable disparity indicates that the primary antifungal compounds in *P. africana* bark are likely medium to high-polarity substances, such as phenolic glycosides or more polar triterpenoid derivatives, which exhibit poor solubility in hexane [7][19]. The reduced effectiveness of *Euclea divinorum* extracts in hexane suggests that its distinctive bioactive naphthoquinones (e.g., 7-methyljuglone), despite being somewhat lipophilic, may be more efficiently extracted or present in more active forms in polar solvents like acetone or methanol [11].

The statistical study reveals significant disparities. The LSD (0.377) and SED (0.173) results significantly exceed those of the methanol assay, indicating greater variability in the reactivity to hexane extracts.

This could be because non-polar substances were extracted in different ways or because the extracts didn't dissolve well in the water-based PDA medium, which could make the bioavailability in the experiment unpredictable. The "ab," "bc," and "c" groupings reveal that the *C. edulis* leaf was statistically different from the control. However, the differences between some of the other treatments were not as clear.

These results are very important for phytochemical screening.

The minimal effectiveness of hexane extracts indicates that non-polar compounds are not the primary source of potent, broad-spectrum antifungals in these species against *F. oxysporum*. Nevertheless, they may collaborate with other components of a full-spectrum crude extract or assist plants in self-defence through mechanisms such as creating impermeable surfaces to water. This shift focuses from hexane as the primary extraction solvent for these species in bio-fungicide development, unless particular lipophilic fractions are aimed at for specialist formulations.

The comparative solvent efficacy underscores a fundamental principle in natural product research: bioactivity is intrinsically linked to extraction methods. The results endorse a solvent-guided discovery strategy, highlighting the utilisation of methanol or other polar solvents for preliminary screening against this pathogen, while hexane extracts may be allocated for the investigation of specific non-polar defence mechanisms or for integration into combination therapy.

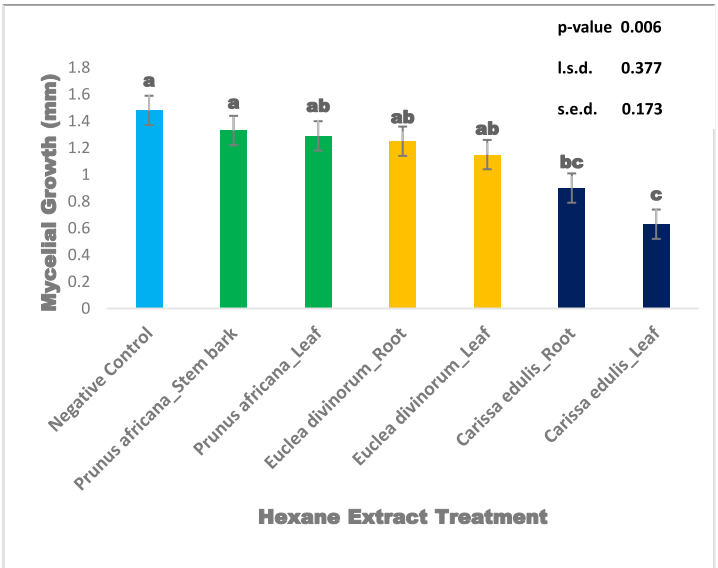

Figure 3: Mycelial Growth in Hexane extract treatment

3.1.4 Integrated Antifungal Efficacy Across Plant Species and Solvents

The extensive analysis of several plant extract treatments produces a complete bioactivity map, emphasising notable interactions between plant species, organ type, and solvent polarity in the suppression of *Fusarium oxysporum* development. The hierarchical clustering of efficacy, as demonstrated by the statistical groupings (a-e), indicates that 100% inhibition (Group 'e') is achievable but contingent upon specific, optimised combinations rather than being an intrinsic property of the plants.

***1. Identification of Exceptional Bioactive Combinations*:** Three distinct combinations were recognised as completely inhibitory (0 mm growth), forming an elite group of treatments: The effectiveness of *Prunus africana* stem bark, extracted using acetone and methanol, demonstrates the existence of a variety of antifungal compounds with broad solubility across multiple polarity categories. This signifies the presence of both glycosylated (methanol-soluble) and aglycone (acetone-soluble) forms of active chemicals, potentially providing diverse mechanisms of action against the pathogen [7]. The root of Carissa edulis yields completely inhibitory extracts in both acetone and methanol. The organ-specific efficacy highlights the root as a concentrated source of antifungal chemicals for this species, presumably including lignans, phenolic acids, or other metabolites effective against soil-borne fungi [1]. The leaf of *Euclea divinorum* achieved complete inhibition exclusively via acetone extraction, in contrast to the previous two examples. This demonstrates a specific phytochemical synergy wherein the medium-polarity compounds in the leaf, likely some naphthoquinone derivatives or flavonoids, are effectively solubilised by acetone and display unique efficacy [11].

2. Solvent Polarity Influences Bioactivity Profile: The results provide a specific solvent efficacy gradient for antifungal discovery aimed at F. oxysporum:

- *Acetone and Methanol (High Efficacy)*: These polar solvents consistently yielded the most potent extracts, achieving complete suppression in all instances. Their success is attributed to their effectiveness in isolating phenolic acids, flavonoids, tannins, and quinones, which are recognised antimicrobials that disrupt fungal membranes, inhibit enzymatic function, and induce oxidative stress [16][4][3].
- *Hexane (Low to Moderate Efficacy)*: As a non-polar solvent, hexane had the lowest overall activity. The best hexane extract from *C. edulis* leaves (0.63 mm) reduced growth by around 57%. This suggests that highly lipophilic compounds (e.g., essential oils, waxes) in these species are not the primary fungicidal agents against this vascular wilt pathogen, although they may contribute to other facets of plant defence [5].

3. Organ-Specificity is a Principal Factor:

The data unequivocally demonstrate that the plant organ is as crucial as the species itself. For example, the stem bark of *Prunus africana* regularly surpassed its leaves in efficacy across all solvents. The root of *Carissa edulis* shows far greater potency than its leaves. The leaf of *Euclea divinorum* exceeded its root, especially when extracted with acetone.

This organ-specificity suggests differential allocation in chemical defence, potentially shaped by ecological pressures and the physiological roles of each tissue [14].

4. Implications for Bio-Fungicide Development and Subsequent Investigation:

These findings provide a strategy framework for developing specialist botanical fungicides. The chosen elite combinations, including *P. africana* stem bark extracted with acetone, represent the most promising prospects for commercialisation due to their consistent and powerful efficacy. Subsequent investigations must emphasise:

a. Bioassay-Guided Fractionation of the superior acetone and methanol extracts to identify and define the unique antifungal compounds.

b. Synergy investigations to determine if combinations of active extracts (e.g., *P. africana* bark + *C. edulis* root) exhibit additive or synergistic effects, potentially reducing the required effective dosage.

c. In-Planta Validation to ascertain efficacy and assess phytotoxicity in live potato plants under controlled and field conditions.

d. Analysis of the Mechanism of Action to determine if these extracts possess fungicidal or fungistatic characteristics and to identify their cellular targets in *F. oxysporum*.

This comprehensive study demonstrates that successful antifungal discovery from botanicals requires a meticulous approach, optimising the selection of plant species, plant organs, and extraction solvent. The findings strongly support the ethnobotanical use of these plants and transform traditional knowledge into validated, scientifically-based pathways for sustainable agriculture.

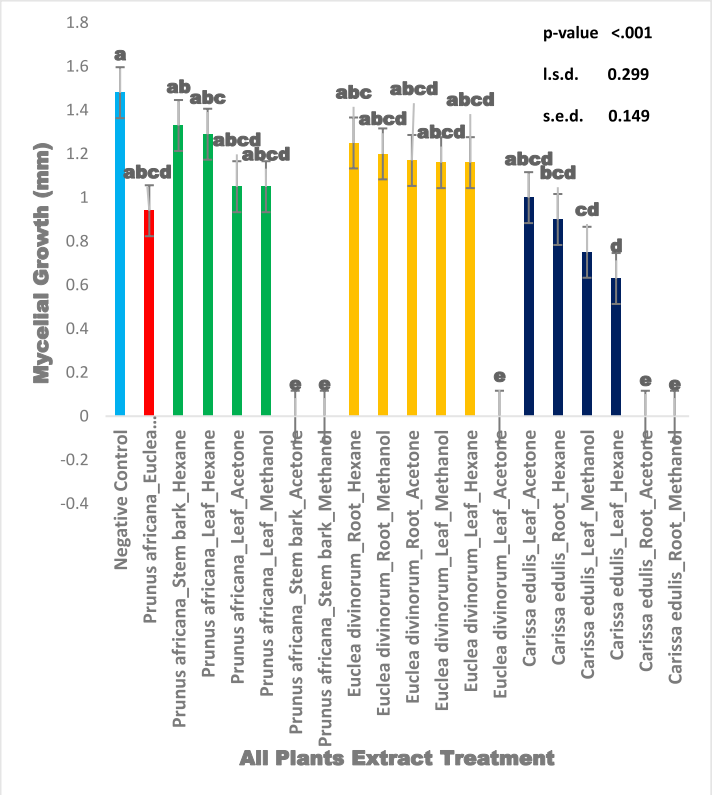

Figure 4: Mycelia Growth among all plant extracts treatment

Conclusion

This investigation presents strong in vitro evidence that extracts from *Euclea divinorum*, *Prunus africana*, and *Carissa edulis* exhibit notable antifungal activity against *Fusarium oxysporum*, the pathogen responsible for potato wilt. The effectiveness was variable but was explicitly determined by a crucial interaction among the plant species, particular plant organs, and solvent polarity. A definitive hierarchy of solvent effectiveness was established, with polar solvents (acetone and methanol) producing markedly superior results in comparison to the non-polar hexane.

Three superior, entirely inhibitory combinations were identified: *Prunus africana* stem bark extracted with acetone or methanol, Carissa edulis root extracted with acetone or methanol, and, uniquely, *Euclea divinorum* leaf extracted exclusively with acetone. These findings highlight the significance of a targeted, phytochemically guided strategy for bioprospecting, wherein the selection of plant tissue and extraction solvent is carefully optimised.

The findings provide robust scientific validation of the ethnobotanical applications of these plants and convert traditional knowledge into concrete insights for sustainable agriculture. By identifying specific, highly effective plant-solvent combinations, this research establishes a solid foundation for the subsequent development, formulation, and evaluation of botanical fungicides as part of an integrated approach to address the economically important issue of Fusarium wilt in potato cultivation.

Recommendation

Based on the in vitro findings, the following strategic recommendations are proposed to facilitate the translation of this research into practical application:

1. Conduct In-Planta Efficacy Trials: Immediate research efforts should shift from in vitro studies to controlled environment (greenhouse) experiments and subsequently to field trials. These trials are required to assess the protective and curative efficacy of the lead extracts against Fusarium wilt in potato plants, while concurrently evaluating any phytotoxic effects on the crop.

2. Initiate Bioassay-Guided Fractionation: To determine the precise active constituents, it is imperative to perform bioassay-guided fractionation on the most potent extracts, such as the *P. africana* stem bark acetone extract. Methods such as column chromatography and preparative HPLC should be utilised to isolate purified compounds, which can subsequently be characterised through spectroscopic techniques (NMR, MS). This will clarify the molecular foundation underlying the observed activity.

3. Clarify the Mechanism of Action: The research should focus on elucidating the underlying mechanistic basis of fungal inhibition. This involves examining whether the extracts exhibit fungicidal or fungistatic activity and determining their cellular targets (e.g., cell membrane integrity, mitochondrial function, or specific enzymatic pathways) through assays such as membrane permeability assessments and metabolic inhibition analyses.

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