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# *Antifungal Potential Of Four Aloe Species*

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**Abstract – Increase in the use of biologically based fungicides for controlling plant and human pathogens in the recent years have led to this research to determine the efficacy of solvent type, to determine the best species of aloe for breeding new variety with more potent biocides for controlling these fungal pathogens under reducing the use of synthetic fungicides which have long term adverse effect on the environment, plant and the end users. The biocides of the leaves and the roots of four species of aloe (*Aloe keayi, Aloe macrocarpa* var. *major, Aloe schwenfurthii* and *Aloe vera*) were extracted with four different solvents (i.e. acetone, ethanol, methanol and hot water) while their fungicidal properties were investigated against three fungal pathogens of crops *(Sclerotium rolfsii, Trichoderma rubrum* and *Colletotrichum lindemuthiamum)*. The result shows that *A. macrocarpa* var. *major* and *A. schwenfurthii* are the most suitable parental genotypes for the breeding of an aloe with more potent biocides.**

**Keywords – Fungicides, Fungi Pathogens, Aloe, Biocides, Parental Genotypes And Breeding.**

## I. INTRODUCTION

Plants remain one of the most common source of antimicrobial agents (Bibithan *et al.,* 2002). The components with phenolic structures, like carvacrol, eugenol, and thymol have were highly active against the pathogen. These groups of compounds show antimicrobial effect and serves as plant defence mechanisms against pathogenic microorganisms (Das *et al.,* 2010).

Even though aloes are now grown around the world for their beautiful forms, flowers, and medicinal properties. They are native to sub-Saharan Africa, the Saudi Arabian Peninsula, and to many islands of the western Indian Ocean, including Madagascar (Botanical Notes, 2009).

Beneficial effect on plant: Aloe Vera can suppress negative plant pathogens, pest and birds. Aloe vera has been used as a major raw material for organic aloe vera fertilizer which contains natural ingredients that promote cell replication (plant growth) with polysaccharides for high absorption of nutrients, Phytochemicals of Aloin, Salicylic Acid and Saponins. These aid with foliar feeding, and balancing the plants health, and are also an excellent fungi food for soil (*Aloe vera* Fertilizer).

Beneficial effects on human include increase in immunity, treatment of stomach ailments, gastro-intestinal problems, skin diseases, constipations, radiations injury, inflammatory effect, healing wounds and burns, ulcer and diabetes (Johnson *et al*., 2012).

## II. MATERIALS AND METHODS

### 2.1. Materials

#### 2.1.1. Sample Collection

The four *Aloe* species used for this study were collected from Alaba layout, off Futa Road and Okuta Elerinla Estate both at Akure South Local Government Area of Ondo State. The three pure fungi samples used were collected from Crop, Soil and

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Pest Management Laboratory, Federal University of Technology, Akure. The names of the four aloe species include *Aloe keayi* Renolds, *Aloe macrocarpa* var. *major* A. Berger, *Aloe schwenfurthii* Bak., *Aloe vera* Linn. while those of the fungi pathogens were *Sclerotium rolfsii, Trichoderma rubrum* and *Colletotrichum lindemuthiamum*. The tools and equipments used were those of Crop, Soil and Pest Management Laboratory, Federal University of Technology, Akure, Nigeria.

### 2.2. Methods

The culturing and the antimicrobial test was done at the Crop, Soil and Pest Laboratory, FUTA. The experimental layout is Randomised Complete Block Design (RCBD).

#### 2.2.1. Preparation of Extracts for Antimicrobial Assay

Leaves and roots of each species were blended using an electrical blender. About 250g of each sample was soaked in 500ml of each of the following extraction media: hot water, acetone, methanol and ethanol for 72hours. The extracts were sieved through double sheet filter paper and concentrated in a rotary evaporator. 0.5mg/ml of the extract formed was prepared using Sterile Distill water. The extracts were sterilized using a membrane filter.

#### 2.2.2. Antifungi Assay

Poisoned Food Technique (Shukia et al., 2008) was used for the investigation. 5ml of the reconstituted extract were aseptically mixed with 20ml of sterile molten Potato Dextrose Agar (PDA) that have been cooled to $45^0$C before pour plating and allowed to solidify at ambient temperature. The fungi were inoculated at the centre of the PDA was poured into each Petri dish and swirled gently so that the extracts and the PDA plates with the aid of4mm cork borer sterile inoculating needle. 'Ketocolazone', a standard antifungi agent was used as a positive control at 0.5mg/ml. A negative control plate (NTR) without any treatment were also set up. All the plates were incubated at $27^0$C for 3-7 days. Zone of mycelia growth were measured with the aid of Vernier calippers every 24hours [1]. Mycelia growth inhibition were calculated in % using the formular below:

$$\% \text{ Mycelia growth} = \frac{Zone\ of\ mycelia\ growth\ NTR - Zone\ of\ mycelia\ TR}{Zone\ of\ mycelia\ growth\ NTR} \times \frac{100}{1}$$

Where NTR = Average diameter of fungal colony in Negative control sets i.e. plates hwithout any treatment.

TR = Average diameter of fungal colony in treated sets.

Graphical representations were made using SPSS computer software statistic.

## III. Results

Figure 1 shows that *Sclerotium rolfsii* (54.32, 55.15, 58.16, 56.96) and *Colletotrichumnm lindemuthianum* (79.46, 69.89, 58.34, 64.83) exhibited high and non-significantly different mycelia growth inhibition with the leaf extracts of the four species of Aloe. In Figure 2, the mycelia growth inhibition of *S. rolfsii* by root extracts of the four aloe species (55.84, 47.80, 56.36 and 52.91) are not significantly different from each other at mean value $p<0.05$. Also, there is high and significantly similar growth inhibition of *Colletotrichum lindemuthianum* with the root extracts of *A. macrocarpa* var. *major* (68.87), *A. schwenfurthii* (72.25) and *A. vera* (66.94) root extracts. *A. macrocarpa* var. *major* (66.68) root extract has the highest antimicrobial effect on *T. rubrum* when compared to that of *A. vera* (33.90) which has the least effect on the same pathogen.

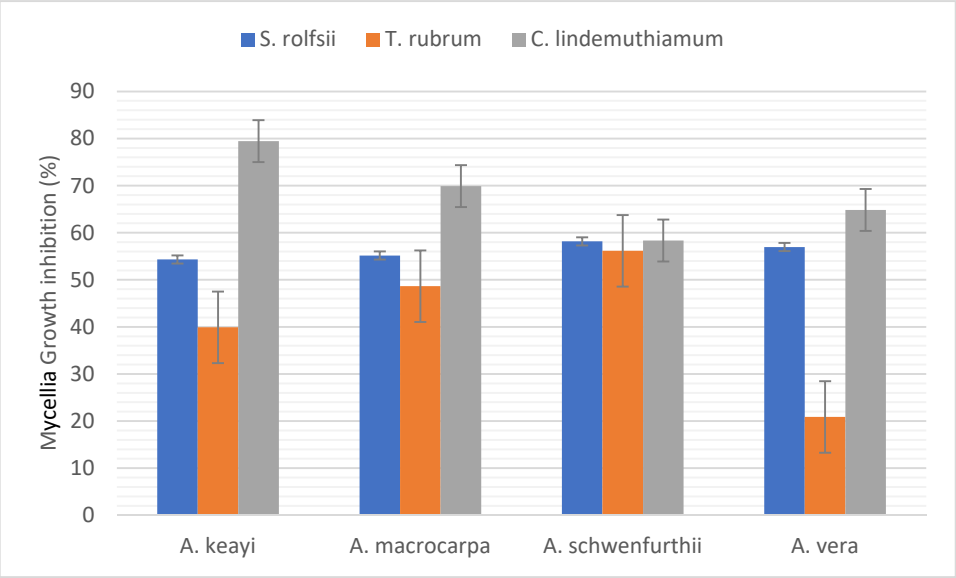


Fig. 1: The Effect of Leaf extracts of Four Aloe species on the Mycellia growth of Three Fungal Pathogens.

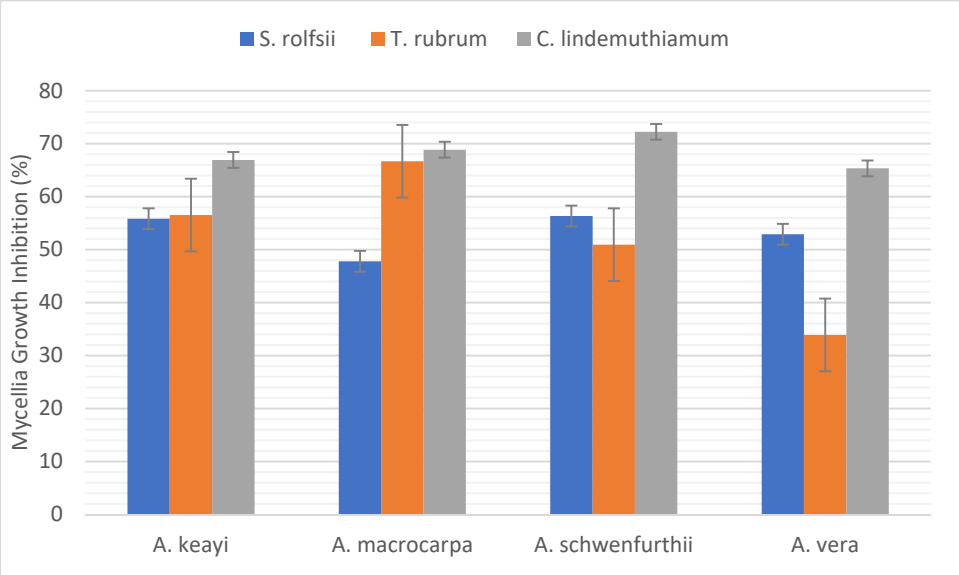


Fig. 2: The Effect of Root extracts of Four Aloe species on the Mycellia growth of Three Fungal Pathogens.

Where A. macrocarpa means *A. macrocarpa* var. *Major* and A. schwenfurthii means *A. Schwenfurthii.*

As shown in Figure 3, *C. lindemuthiamum* (71.24, 64.68, 75.14 and 61.46) exhibit maximum and equal growth suppression with all the extracts. Also, there is no significant different among the growth inhibition caused by acetone (47.51) and ethanol (47.84) leaf extract on *T. rubrum* at mean value $p>0.05$. In figure 4, the highest inhibition was observed in *T. rubrum* with acetone root extract (73.54) while the least was recorded with hot water extract (32.36). Low and significantly similar mycelia growth inhibition was observed in *C. lindemuthiamum* with hot water (59.92) and methanol (62.17) root extracts while the highest inhibition was shown by the same pathogen with ethanol root extract (83.13).

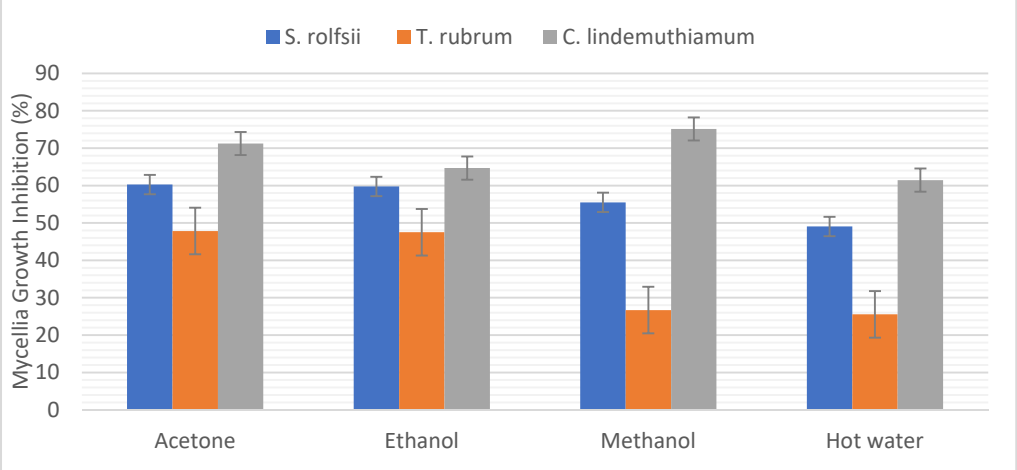


Fig. 3: The Effect of Solvent Type on the Efficacy of the leaves of Aloe species in the Inhibition of Three Fungi pathogens

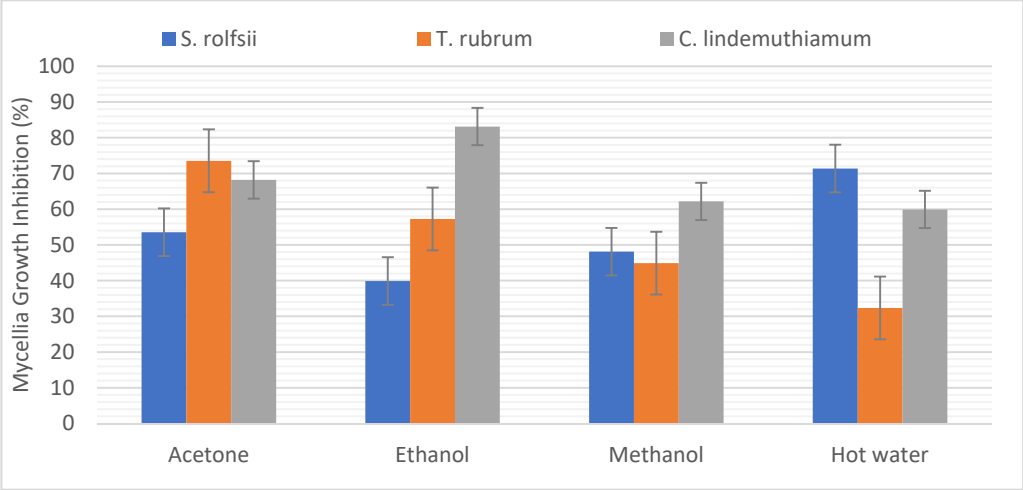


Fig. 4: The Effect of Solvent Type on the Efficacy of the roots of Aloe species in the Inhibition of Three Fungi pathogens.

Where S. rolfsii means *Sclerotium rolfsii*, T. rubrum means *Trichoderma ru*rubru

C. lindemuthiamum means *Colletotrichum lindemuthiam*

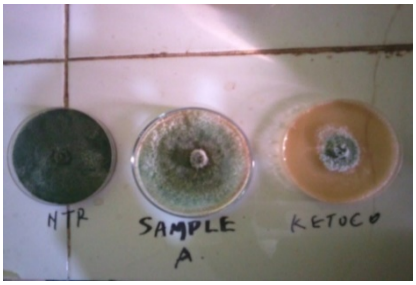


**Plate1**

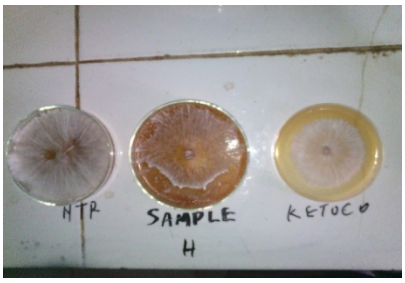


**Plate2**

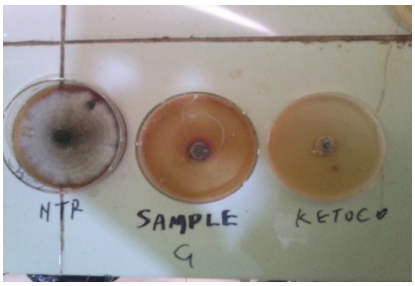


**Plate3**

**Plate 1: Activity of *Colletotrichum lindemuthiamum* on Hot water *Aloe vera* root extracts in comparism with standard and untreated plate at 72hours. Plate 2: Activity of *Sclerotium rolfsii* on Ethanol *Aloe macrocarpa* root extract in comparism with standard and untreated plate at 72hours. Plate 3: Activity of *Trichoderma rubrum* on Acetone *Aloe schwenfurthii* leaf extracts in comparism with standard and untreated plates at 72hours.**

## IV. Discussion

High mycelia growth inhibition exhibited by *Sclerotium rolfsii* and *Colletotrichum lindemuthianum* towards the leaf extracts of the four aloe species is an indication of presence of high antimicrobial elements in the leaf extracts hence their ability to control the said fungi pathogens. In consistent with this present work, most of the previous works on *Aloe* leaf extract had showed its effectiveness in the control of fungi and bacteria pathogens of economic and agricultural importance among which are control of fungi that cause tinea, inhibition of growth of *Streptococcus progenes* and *Shigella flexneri* in vitro with *A. barbadensis* inner gel [8], maximum growth inhibition of *Streptococcus pyogenes and Pseudomonas aeroginosa* by *A. vera* leaf extract [4]. In contrast, *Aloe vera* extracts failed to show antibiotic properties against *Xanthomonas* species and evidence for control beneath human skin remains to be established [8].

Maximum growth inhibition of *Sclerotium rolfsii* with root extracts of the four aloe species, that of *Colletotrichum lindemuthianum* with root extracts of three aloe species and that of *Trichoderma rubrum* with *A. macrocarpa* var. *major* root extract indicate their high antimicrobial strength. Minimum growth inhibition of *Trichoderma rubrum* with *A. vera* root extract showed its low antimicrobial strength making it unsuitable for the control of the fungi pathogen. The above findings is similar to that of previous work which demonstrated that *C. albicans* was less susceptible to the extracts as compared to the cutaneous mycosis causing fungal agent (*T. mentagrophytes*) among the pathogenic fungal used. This might be due to the presence of different bioactive entities that potentially inhibit the growth of cutaneous mycosis, like that of the multidrug-resistant bacteria. The difference in extracts efficacy on the growth of *T. mentagrophytes* and *C. albicans* indicates the presence of antifungal constituents in the crude extracts of each plant [16]. Another work also reported that though all the bacteria tested in an investigation were sensitive to all the plant extracts, their effectiveness varied in different extracts. The difference in the antimicrobial efficacy of the plant extracts is suggested to be depended on the variation in their phytochemical content. Less effectiveness of some of the plant extracts against the microbes may be due to the absence or insufficient concentrations of the antibacterial constituents [14]. Other studies had also documented that medicinal plants contain coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, and polyacetylenes which have the potential as a bactericidal, bacteriostatic, or fungicidal effect against selected human pathogens [12, 8]. Some other authors proposed that this inhibitory activity of secondary metabolites emanates from the sequential inhibition of the biochemical pathway, inhibition of protein synthesis, and disintegration of the outer membrane [15, 9].

Growth inhibition of *C. lindemuthiamum* by the four different solvents used for the extraction of the leaf of four different aloe species is an indication of high presence of required inhibitory constituents extracted by the said solvents. Also, insignificant difference among the effect of acetone and ethanol leaf extract toward *T. rubrum* indicate similar amount of required inhibitory constituents extracted by the solvents. *T. rubrum* and *C. lindemuthiamum* display appreciable inhibitory property toward acetone and ethanol root extracts respectively while hot water root extract is not effective in the control of these said pathogens. Also, methanol and hot water root extracts have similar inhibitory effect on *C. lindemuthiamum*. The above findings is in agreement with [4] whom had previously reported that the ineffectiveness of some of the extracts on some of the bacteria and all the fungi he tested may likely be due to the fact that the solvents used could not extract the active components of the plant required for their control [2].

## V. Conclusion

Though other aloe species controlled the fungi pathogens under study, w4but the best antimicrobial effect towards the three fungi pathogens was exhibited by root extract of *A. macrocarpa* var. *major,* leaf extracts of *A. macrocarpa* var. *major* and *A. schwenfurthii* making these two aloe species good parental genotype for the production of a new variety of aloe with extracts that are more effective in the control of fungal diseases. Also, acetone and ethanol gave the best result with the said aloe species in the extraction of required phytochemicals for controlling the fungi pathogens under study making them the best solvents for extraction purposes.

## VI. Acknowledgements

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