

COMPARATIVE EFFICACY OF *ALOE VERA* (LINN) AND *ALOE SCHWEINFURTHII* (BAKER) POWDERED LEAF EXTRACTS IN THE CONTROL OF SOME PLANT FUNGI PATHOGENS

ABSTRACT

Evaluation of the relative efficacy of powdered leaf extracts of *Aloe vera* (Linn) and *Aloe schweinfurthii* (Baker) in the control of some plant pathogens was undertaken in this work. Antimicrobial activities of the extracts obtained using cold water, hot water and ethanol were tested against four fungal spp., namely *Alternaria solani*, *Colletotrichum lindemuthianum*, *Sclerotium rolfsii* and *Trichophyton rubrum*. The phytochemical screening of the leaf extracts of the two aloe species revealed the presence of bioactive compounds such as alkaloids, tannins, saponins, flavonoids, cardiac glycosides, phytates and oxalates. The extracts were observed to exhibit varying inhibitory effects on the selected fungi. Ethanolic extract of *A. vera* at 50mg/ml and 100mg/ml had the greatest impact on *A. solani* and *C. lindemuthianum* respectively. Similarly, cold water extract of *A. schweinfurthii* at 100mg/ml was the most effective against *S. rolfsii* and *T. rubrum*. However, hot water extract of *A. vera* was least effective against *C. lindemuthianum*. Also, the efficacy of cold water extract of *A. schweinfurthii* at 50mg/ml was very low against *T. rubrum* and *A. solani*. The hot water extract of *A. schweinfurthii* at 20mg/ml also showed the least effect against *S. rolfsii*. Consequently, extracts from both Aloe species can be recommended in the management of the four fungal pathogens evaluated in this study. It is hoped that in no distant future, botanical fungicides would be developed from the two Aloe species.

Key words: *Alternaria solani*, *Colletotrichum lindemuthianum*, Phytochemical screening, Inhibitory effects

INTRODUCTION

Plants have been sources of preventive as well as therapeutic medicine among indigenous peoples of various communities for ages [1]. In the past, most of the dwellers of the third world countries rely solely on indigenous plant based medicines as remedies for various health challenges [2]. In fact, it was reported that in South Africa, over 60% of the population consults

the traditional healers [3]. World over, the business interest in these plant species for their proposed medicinal attributes, is on the increase despite the fact that little or no scientific information is available to back the claim of potency attributed to most of them.

The determination of the composition of these plants has been the focus of researchers in recent past [4]. Their efforts have been channeled towards the extraction and classification of biologically active components and subsequent development of drugs as well as herbal products as supplements [5]. Analysis of different species of medicinal plants for biologically active components known to have pharmacological properties had been conducted and most of the studied plants have shown antimicrobial property [6].

Aloe vera (Linn), for example is known to have many bioactive and antipathogenic components and extracts from the Aloe specie can be used in the management of various ailments [7]. The specie is succulent and it has no naturally occurring populations in Nigeria, hence, can only be found in cultivation [8]. When examined, the DNA of *A. vera* shows that it is similar to that of *A. perryi*, a specie endemic to Yemen [9]. Preparations made from *A. vera* are commercially advertised for skin conditions such as sunburns, cold sores and frostbite. *Aloe vera* gel is also used commercially as an ingredient in yogurts, beverages, and some desserts [4].

Aloe schweinfurthii is one of the indigenous Aloe species in West Africa [10]. It is a tropical plant that is customarily used for the treatment of different ailments. These include chronic skin ulcers, coughs, dysmenorrhea, food poisoning, intestinal worms, difficult delivery, dysentery, general stomach aches, and lumbar pain. Other traditional uses for *A. schweinfurthii* include antiseptic usage, purgative, decoagulant, larvicide, vermifuge, stomachic tonic and as a stimulant [11].

The use of plant extracts in the control of pests and diseases as well as in preservation of food is as old as mankind [12]. The ancient Egypt had developed the use of extracts from plants in the preservation of food, as antimicrobial agent and in embalming the dead [13]. For example, the Egyptians were using garlic extract in food preservation. There is continuous rise of interest in the search for antimicrobial agents from plants [14].

Numerous scientific studies have been conducted on *A. vera*, the result of which has shown the plant to have medicinal attributes, hence its relevance to cosmetic, pharmaceutical, nutraceutical and food industries [15]. Some of these companies are left with no choice but to import the components of this plant with attendant financial implications. Consequently, efforts are made by stakeholders to partially replace active components imported from other countries with those of indigenous Aloe species, so as to bring about increased utilization of indigenous plants. In the present work, effort was made to compare one of the Aloe indigenous to Nigeria with *A. vera* in terms of its antifungal properties.

MATERIALS AND METHODS

Aloe vera and *A. schweinfurthii* were collected from stateline area not too far from Federal University of Technology, Akure (FUTA) campus and Ore environs, both in Ondo-State. The help of a botanist was enlisted in the identification process and the plants were potted in the screen house of the Department of Crop, Soil and Pest Management, FUTA.

Collection and preparation of fungal isolates

The four fungal pathogens evaluated in this work, namely; *Alternaria solani*, *Colletotrichum lindemuthianum*, *Sclerotium rolfsii* and *Trichophyton rubrum* were obtained from the culture collections of the pathology unit of the Department of Crop, Soil and Pest Management Federal University of Technology, Akure. Inoculation of each fungus was done separately on freshly prepared Potato Dextrose Agar (PDA) following standard procedure. The pure culture of each fungus was evaluated for susceptibility to the phytochemical components of the Aloe species.

Phytochemical screening

Screening was carried out for the presence of the following phytochemicals: oxalate, alkaloids, saponins, flavonoids, cyanide, phytates and tannins. The standard procedure as described by Sofowara and Harborne was adopted to identify and quantify the bioactive constituents [17, 16].

Preparation of extracts

Eight leaves each of the two species were collected, washed in running tap water and chopped. The chopped pieces were oven dried under a controlled temperature of 65°C for 72 hours. These oven dried leaves of the two Aloe species were crushed separately and ground into powder using a sterile mortar and pestle.

Extracts from the two samples were obtained using cold water (at 25°C), hot water (at 65°C) and ethanol. For cold water extraction, 100ml of sterile distilled water at room temperature were dispensed into conical flasks containing 10g of powdered samples. This was allowed to stand for 24 hrs with intermittent agitation. The mixtures were filtered separately using muslin filter cloth. They were thereafter kept in an incubator (under a controlled temperature of 65°C for 12 hours) so as to obtain a solid extracts. With the aid of scalpel, these solid extracts were scrapped and divided into three portions of 0.2g, 0.5g and 1.0g. Each portion was dissolved in 10ml sterile distilled water to make three different concentrations of 20mg/ml, 50mg/ml and 100mg/ml. The same procedure was repeated for hot water extraction at 65°C. For ethanol extraction, 10g of powdered samples of each species were separately soaked in 100ml of ethanol inside 400ml conical flasks. The mixture was allowed to stand for about 72 hours after which it was filtered using muslin filter cloth.

These filtrates were dried inside an incubator under a controlled temperature of 65°C to obtain a solid extracts. With the aid of scalpel, these solid extracts were scrapped and weighed into 0.2g, 0.5g and 1.0g portions. Each portion was also dissolved in 10ml distilled water to make different concentrations.

Evaluation of extracts against fungal pathogens

Two (2) ml of each extract was aseptically transferred into the petri dishes. 20ml aliquots of molten potato dextrose agar (PDA) at 50°C were poured into the petri dishes containing each extract concentrate and swirled gently such that the extracts and the PDA mixed properly to form a homogeneous mixture. The PDA/extract mixtures were allowed to set at room temperature. A sterile 5mm diameter cork borer was used to punch out agar discs from one week old cultures of the four fungi separately and inoculation was done at the centre of each petri dish containing

PDA/extract mixture. Each extract concentration represented a treatment and each treatment was replicated thrice for the dry leaf samples as well as the three extraction methods.

Two control experiments were set up. The first control involved the use of a standard fungicide i.e. Carbendazim (at 1.0mg per 2ml of sterile distilled water as recommended on Carbendazim 500 Fungicide WP label), while the second control was made up of fungal pathogens on PDA with no extract incorporated.

Data Collection

Data was collected on the mycelial growth of the four fungal pathogens. The zone of mycelial growth was measured after 48 hours of inoculation. All measurements taken were recorded. Percentage mycelial growth inhibition was determined using the formula:

$$\% \text{ mycelia growth} = (a-b)/a \times 100$$

Where: a = Zone of mycelia growth on non treated sample
b = Zone of mycelia growth on treated sample

All data were subjected to analysis of variance (ANOVA) and means were separated using the Duncan's multiple range test (DMRT).

RESULTS

This study showed that Aloe leaves have alkaloids, tannins, saponins, flavonoids, cardiac glycosides, phytates and oxalates. The results also showed that there is significant difference in the proportion of phytochemicals in *A. schweinfurthii* and *A. vera*. This can be seen on Figure 1. In terms of proportion, oxalate, saponins, flavonoids and cyanide were observed to be more in *A. schweinfurthii* than in *A. vera*. However, alkaloids, phytates and tannins were observed to be more in *A. vera* than in *A. schweinfurthii*. These differences are significant.

Also, the result on the percentage mycelial growth inhibition of the selected fungi by the leaf extracts of the two Aloe species are presented in Figures 2 to 4. Significant differences exist among the extracts with regards to inhibition of mycelial growth in the fungi species.

For example, the mycelial growth of *Trichophyton rubrum* was seen to be highly inhibited when tested against 20mg/ml S2, whereas the same fungus was observed to be more resistant to the effect of V1. Similarly, 100mg/ml S2 had the highest efficacy against *Sclerotium rolfii* while V1 showed no inhibitory effect against the fungus. In contrast, 50mg/ml and 100mg/ml of EA had the greatest effects against *Alternaria solani* and *Colletotrichum lindemuthianum* respectively. Highest inhibitory effects were observed in both cases. However, S1 and ES had mild to moderately high effects against the fungi.

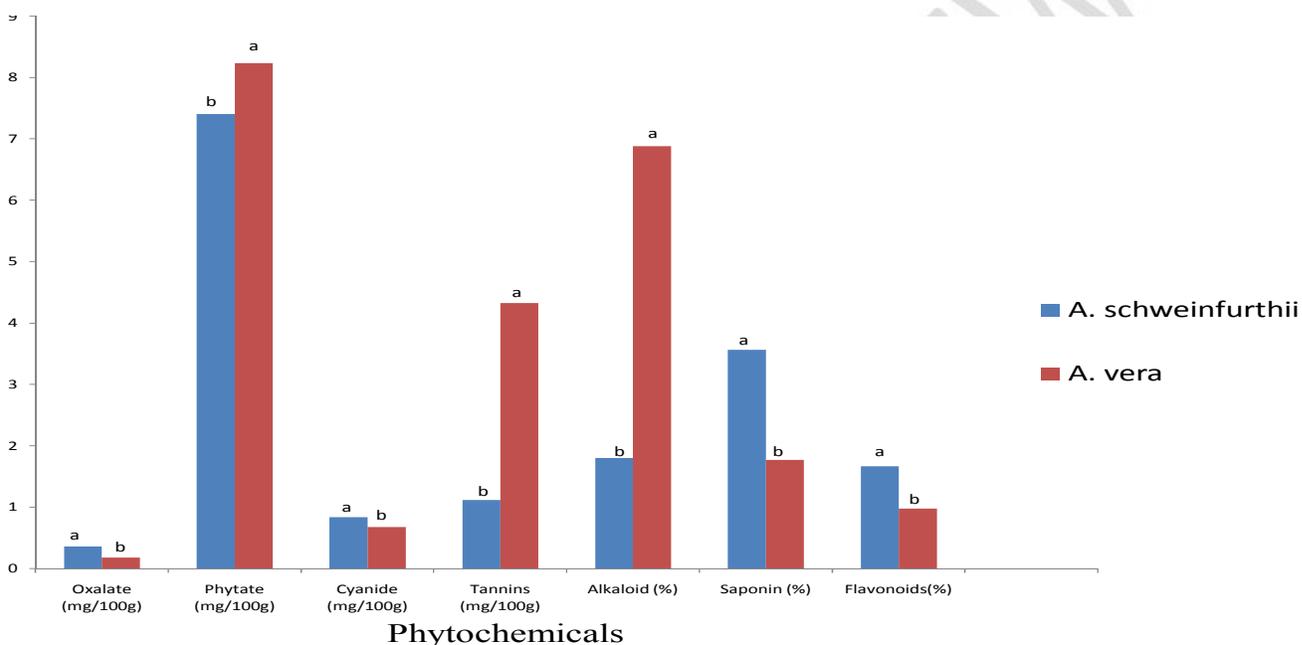


Fig. 1: Quantitative analysis of phytochemical constituents of the two aloe species

NOTE: Mean values followed by different letters are significantly different from one another ($p < 0.05$).

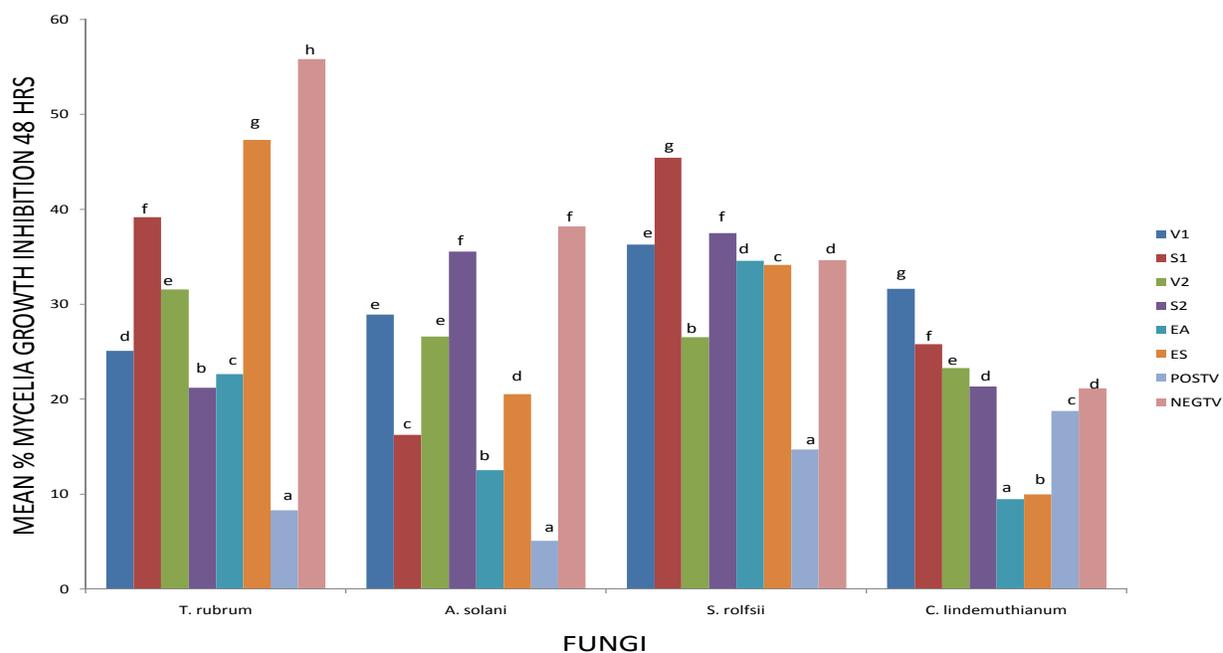


Fig. 2: Effect of the extracts at 20mg/ml against the fungi after 48 hours.

- Notes:
- V1 Hot water extract from powdered *Aloe vera*
 - S1 Hot water extract from powdered *Aloe schweinfurthii*
 - V2 Cold water extract from powdered *Aloe vera*
 - S2 Cold water extract from powdered *Aloe schweinfurthii*
 - EA Ethanolic extract on powdered *Aloe vera*
 - ES Ethanolic extract on powdered *Aloe schweinfurthii*
 - POSTV Standard
 - NEGTV Control

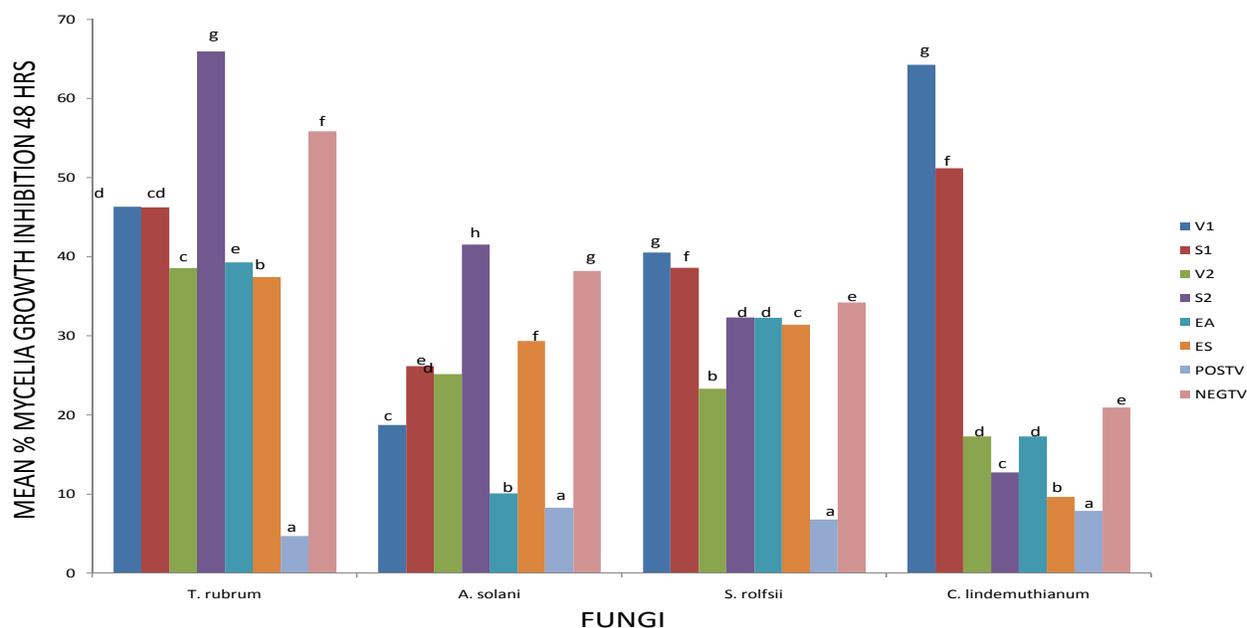


Fig. 3: Effect of the extracts at 50mg/ml against the fungi after 48 hours.

- Notes:
- V1 Hot water extract from powdered *Aloe vera*
 - S1 Hot water extract from powdered *Aloe schweinfurthii*
 - V2 Cold water extract from powdered *Aloe vera*
 - S2 Cold water extract from powdered *Aloe schweinfurthii*
 - EA Ethanolic extract on powdered *Aloe vera*
 - ES Ethanolic extract on powdered *Aloe schweinfurthii*
 - POSTV Standard
 - NEGTV Control

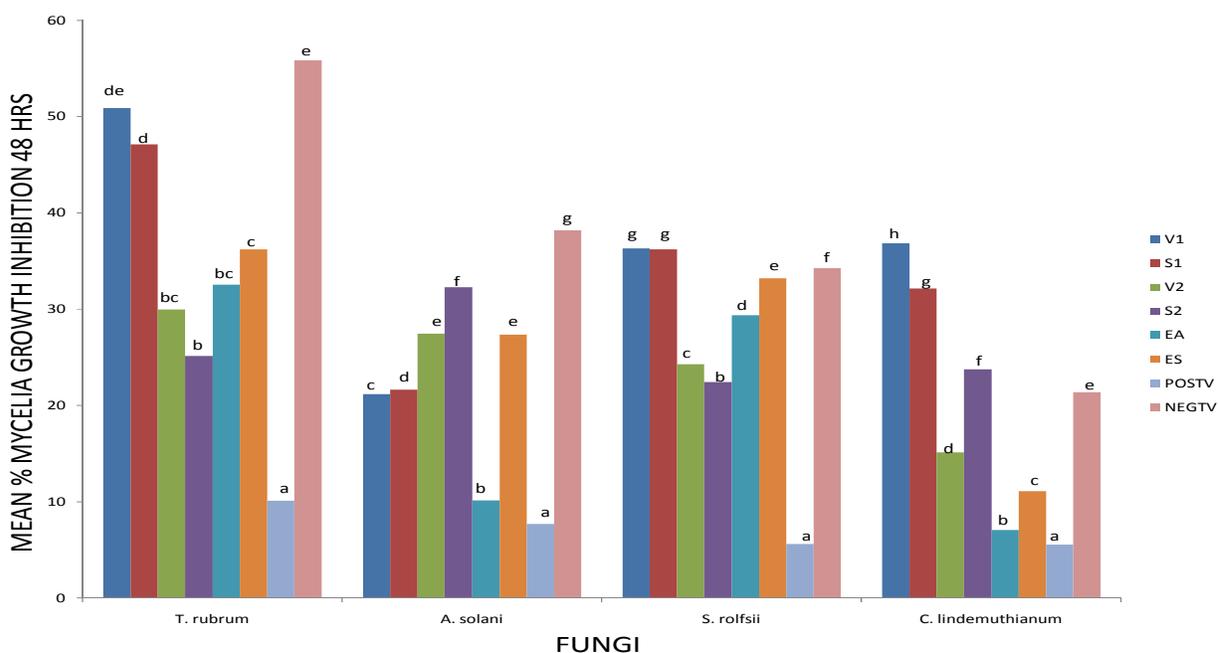


Fig. 4: Effect of the extracts at 100mg/ml against the fungi after 48 hours.

- Notes:
- V1 Hot water extract from powdered *Aloe vera*
 - S1 Hot water extract from powdered *Aloe schweinfurthii*
 - V2 Cold water extract from powdered *Aloe vera*
 - S2 Cold water extract from powdered *Aloe schweinfurthii*
 - EA Ethanolic extract on powdered *Aloe vera*
 - ES Ethanolic extract on powdered *Aloe schweinfurthii*
 - POSTV Standard
 - NEGTV Control

DISCUSSION

This work showed that powdered leaf extracts of the two aloe species have varying degree of inhibition of mycelial growth against the selected fungi pathogens. *Alternaria solani* has been

reported to cause dark concentric rings on the fruits, stems and leaves of tomatoes. Early blight of tomatoes is also known to be caused by the same fungus [18; 19]. All the extracts except S2 had significant inhibition on the mycelial growth of the fungus. The inhibitory effect of the ethanolic extracts from *A. vera* was more when compared to other extracts. One can therefore say that ethanol was more efficient in the extraction of phytochemicals which are effective against the fungus. This is in line with the claim that the amount of an active ingredient in an extract is a principal function of solubility of the active ingredient, which in turn is influenced by the type of solvent used in the extraction [20]. This is the underlying factor responsible for the variation of efficacy in assayed extracts [21; 20]. EA at 50mg/ml exhibited the greatest effect against the fungus. This is in consonance with the discovery made when the extract of *Aloe vera* was tested against pathogenic species of genus *Alternaria* [22]. The inherent ability to induce toxic effect on mycelial growth and proliferation of these fungi was said to be responsible for the efficacy of *A. vera* extract [23]. The presence of alkaloid which was found to be more in *A. vera* extract could also be responsible for this. One of the major properties of alkaloids is their toxicity against the cells of foreign organisms [24]. Extracts of *A. schweinfurthii* also inhibited the growth of the fungus except S2 which was of no effect against the pathogen. The aqueous extracts that demonstrated the least activity against *Alternaria* spp. could be explained by the fact that when plant materials are ground in water, some phenolases and hydrolases are released and could have modulating effects on the activity of the compounds in the extracts. It could also be due to incomplete extraction of the active principles. This finding is in tune with a study carried out in Uruguay, where it was observed that a high proportion of the test plant extracts in water (80% of them) did not inhibit the fungus *Alternaria* sp [25].

Trichophyton rubrum is known to inhabit different environment including animals, humans and soils. It is a dermatophyte fungus that is economically important in hair, skin and nail infections in human [26]. The result of this study agrees with previous study which shows *A. vera* extracts to have consistent inhibitory effects on *T. mentagrophytes* and *T. rubrum* [27]. However, in the current work, the effect of the extract of *A. schweinfurthii* on the pathogen was observed to be more when compared to that of the *A. vera*. The cold water extract of *A. schweinfurthii* followed by ethanolic extract of *A. vera* were seen to have significant inhibitory effect (62.01% and 59.47% respectively) on *T. rubrum* at 20mg/ml concentration. This study revealed that cold water extract from powdered *Aloe schweinfurthii* at 20mg/ml have the greatest inhibitory effect.

The low water temperature unlike hot water could also be said to maintain the nature of the bioactive ingredients. Saponin which was observed to be present in higher proportion in *A. schweinfurthii* could also be responsible for this high activity of S2. This is in line with the report which states saponins to be important in plants as antifeedant and in protecting plants against microbes and fungi [28]. The presence of biologically active compounds in plants makes them to be of medicinal importance especially for antimicrobial activity against pathogenic organisms.

Similarly, the cold water extract from powdered *A. schweinfurthii* at 100mg/ml concentration proved to be very active against *Sclerotium rolfsii*. Flavonoids which is present in higher proportion could be responsible for this. Flavonoids have antimicrobial properties [29]. V2 was also seen to have significant effect against the fungus. The fungus has been known to infect a wide range of agricultural and horticultural crops. In Nigeria, cocoyam is seriously affected by tuber rot caused by *S. rolfsii* [25]. It has also been reported that the attacks from this fungus could cause a drop in production figures of cocoyam by 11% [30]. Although no worldwide compilation of host genera has been published, over 270 host genera have been reported in the United States alone. Susceptible agricultural hosts also include sweet potato (*Ipomoea batatas*), pumpkin (*Cucurbita pepo* L.), corn (*Zea mays*), wheat (*Triticum vulgare*) and peanut (*Arachis hypogea*). Horticultural crops infected by the fungus are included in the genera *Narcissus*, *Iris*, *Lilium*, *Zinnia*, and *Chrysanthemum* [31]. This work revealed S2 at 100mg/ml and S1 at 20mg/ml, to show the greatest and least efficacy against *S. rolfsii*.

Colletotrichum lindemuthianum is the causal agent of anthracnose of *Phaseolus vulgaris*. It produces red to brown, irregular-shaped blotches on pods. Small, thin lesions of the same colour can be seen on stem. The fungus causes irregular seed development [32]. The attack by the fungus may lead to total loss of crop if left unchecked [33]. The highest percentage inhibition of the fungus was by EA (66.79%) at 100mg/ml, followed by the same extract at 20mg/ml and ES (53.91%) at 50mg/ml. The ability of ethanol to extract the active ingredients that are potent against this fungus could have been responsible for this. On the other hand, hot water extract from powdered *A. schweinfurthii* and hot water extract from powdered *A. vera* proved to be less effective against *C. lindemuthianum*. The reason for this is explained by the fact that the structure of the phytochemicals is unstable when exposed to high temperature [34]. Hence, plant extracts, notwithstanding their increasing relevance in therapeutic treatment, have limitation in

being rapidly degraded by high temperature and ultra-violet radiations [35; 36; 21]. The least effective extract against the growth of the fungus out of the extracts of *A. vera* and *A. schweinfurthii* is V1.

CONCLUSION

This research work clearly suggests that *Aloe schweinfurthii* may be a good substitute for *Aloe vera* in the area of plant protection. The result showed that cold water extract from powdered *A. schweinfurthii* at 20mg/ml and 100mg/ml were the most effective against *T. Rubrum* and *S. rolfsii* respectively. On the other hand, the efficacy of ethanolic extract from powdered *A. vera* at 50mg/ml and 100mg/ml was observed to be the greatest against *Alternaria solani* and *Colletotrichum lindemuthianum* respectively. Further work should be done on the identification and quantification of the active constituents of each species of Aloe. The appropriate mix ratio of active constituents that will be most potent against fungal pathogens should also be investigated.

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