

Figure 4.4: Antifungal activity of hexane extract and its different fractions of stem-bark of *Acacia nilotica* against *Rhizoctonia solani*

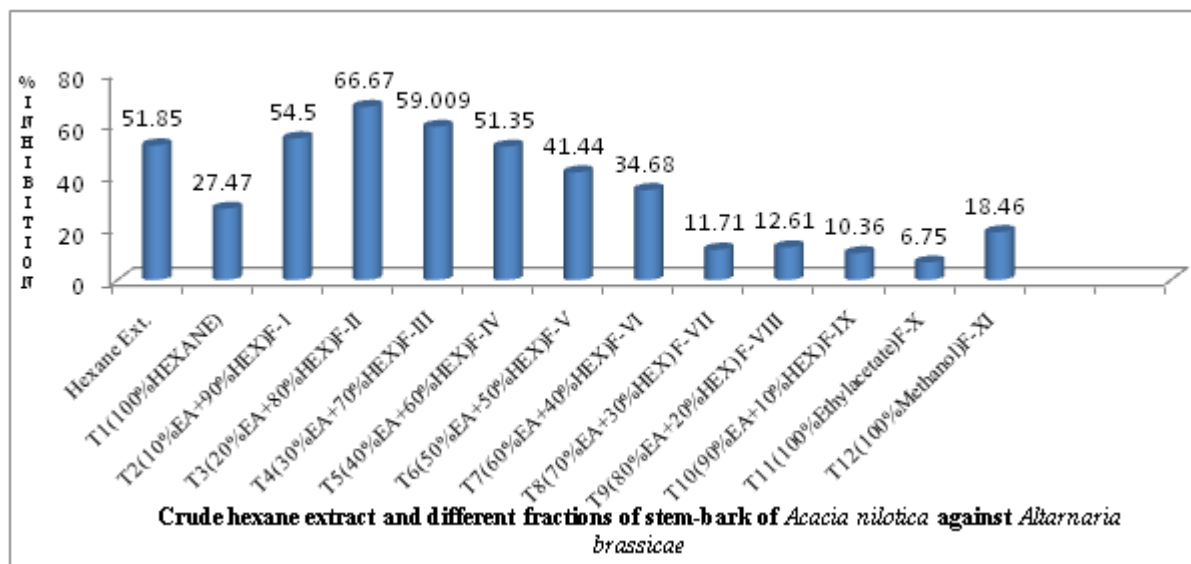


Figure 4.5: Antifungal activity of hexane extract and its different fractions of stem- bark of *Acacia nilotica* against *Alternaria brassicae*

3.1.5 Comparative study of % mycelial growth inhibition of crude hexane extract and its different fractions of stem-bark of *Acacia nilotica* against *Alternaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani*

From the graph which is shown in Table 4.6 and Fig. 4.6 it is clear that the crude hexane extract of stem-bark of *Acacia nilotica* showed high percent mycelial growth inhibition in case of *Rhizoctonia solani* (58.0459%) than *Alternaria brassicae* (51.85%) and *Fusarium oxysporum ciceris* (46.009%). Among all the hexane extract and different

fraction of stem-bark of *Acacia nilotica*, **Fraction-2** was more effective in terms of percent mycelial growth inhibition in case of *Alternaria brassicae* (66.67%) than *Fusarium oxysporum ciceris* (**Fraction-1**) (65.59%) and *Rhizoctonia solani* (**Fraction-2**) (62.17%). These differences in the susceptibility of the test organisms to the different fractions might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structures.

Table 4.6: Percentage (%) of mycelial growth inhibition of hexane extract and different fractions of stem-bark of *Acacia nilotica* against *Alternaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani*

Fungal strain	Hex. Extract	100% Hex.	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10	F-11
<i>Alternaria brassicae</i>	51.85	27.47	54.50	66.67	59.09	51.35	41.44	34.68	11.71	12.61	10.36	6.75	18.46
<i>F. oxy. Ciceris</i>	46.09	20.43	65.59	60.75	47.31	31.18	27.95	17.74	15.05	18.27	12.90	9.13	17.20
<i>Rhizoctonia solani</i>	58.04	26.59	51.31	62.17	49.06	43.07	39.32	41.19	34.45	39.70	40.07	14.23	32.95

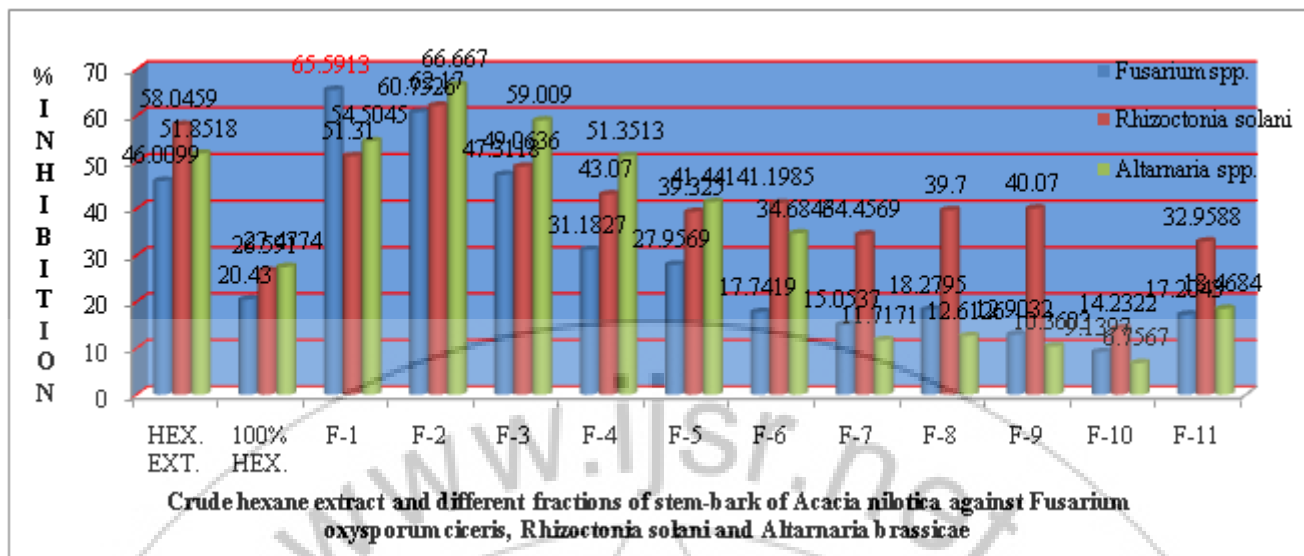


Figure 4.6: % mycelial growth inhibition of crude hexane extract and different fractions of stem-bark of *Acacia nilotica* against *Alternaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani*
Kruskal-Wallis Test (Non-parametric ANOVA)

The P value is **0.0019**, considered very significant. By using Kruskal-Wallis test, the sequence of the order of ranking was given as **Fraction-2 > Fraction-1 > Crude hexane extract > Fraction-3 > Fraction-4 > Fraction-5 > Fraction-6 > Fraction-7 > Fraction-8 > Fraction-9 > Fraction-11 > Fraction-10** shows that **Fraction-2** was shown the highest antifungal activity against the plant pathogen *Alternaria brassicae*, *Fusarium oxysporum ciceris*, *Rhizoctonia solani*.

The arrays of phytochemical compound present in the **Fraction-2** shows potent antifungal activity against *Alternaria brassicae*, *Fusarium oxysporum ciceris*, *Rhizoctonia solani*. This suggests that **Fraction-2** has the synergistic effect of the compounds present in it that contributed to its antifungal properties. Therefore, phytochemical profiling for the individual components of the fraction would be effective in dealing with the phytopathogens.

3.1.6 Dunn's Multiple Comparisons Test

Comparison between % mycelial growth inhibition of crude hexane extract and different fractions against plant pathogens *Alternaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani* by using Dunn's Multiple Comparison Test shows that it was non-significant ($p > 0.05$).

4. Conclusions

The less yield of hexane extract indicates that the solvent system plays a significant role in the solubility of the bioactive ingredients which influence the different activities. Hexane is non-polar in nature and it extracted the non-polar materials but yielded in least quantity, which is one of the evidence that these plants contain very small amount of non-polar compounds (Abbas *et al.*, 2014). The preliminary phytochemical screening carried out on hexane extracts of stem-bark of *Acacia nilotica* revealed the presence of phytoconstituents such as carbohydrates, sterols, triterpenoids, and anthraquinone. Alkaloids, flavonoids, amino acid, protein, tannins, reducing sugars, saponins,

glycosides and anthracenes were absent in hexane extract of stem-bark of *Acacia nilotica*, despite the fact that it is present in the ethanolic extract of *Acacia nilotica* (Banso, 2009) except flavonoids

As the plant derived substances have recently become of great interest owing to their versatile applications. They constitute a rich source of bioactive chemical and the use of these natural plant derived products as fungicides for the control of diseases in plant can be attributed to their low cost, locally available, non-toxic, biodegradable (Alam *et al.*, 2002) and their low negative impacts on the environment, especially for the farmers who cannot afford expensive synthetic pesticides. Many plant extracts used as pesticides were fast acting, quickly inhibiting pest feeding with quick knock down effect and therefore save crop from additional crop damage rather quickly. Natural products from many plants were known to control plant pathogens (Khan *et al.*, 1979).

The results obtained from the present investigation revealed that all of the tested hexane extracts and its different fractions at 15% concentration inhibited the growth of pathogens. This antifungal activities of hexane extracts and its different fractions may be attributed to the presence of variety of active ingredient such as sterols, triterpenoids and anthraquinone may have the capacity to rupture the cytoplasmic membrane of the fungal cells and damage the intracellular compounds or they may interact with lipid bilayers or inhibit the protein and nucleic acid synthesis of the fungal cell.

Triterpenoids are known to weaken the membranous tissue, which results in dissolving cell wall of microorganism (Hernandez *et al.*, 2000). The rate of mycelial growth inhibition was increased by increasing the concentration which deeply penetrated the fungal cell wall/membrane and might have killed them completely.

Various plant extracts e.g., *Cicer arietinum* (Bajwa *et al.*, 2006), *Parthenium hysterophorus* (Bajwa *et al.*, 2004) and

Magnolia grandiflora (Ahmed and Abdelgaleil, 2005) etc. have also been examined for their antifungal activity with the objective of exploring environmentally safe alternatives of plant disease control.

Sehajpal *et al.*, (2009) reported the effect of several plant extracts against *Rhizoctonia solani* causing sheath blight of rice. Chatterjee and Das (2014) reported that the *Acacia nilotica* (L.) Willd. exhibited the antifungal effect against *Rhizoctonia solani*. Rathod and Pawar (2012) reported that the several species of fungi belonging to 12 genera were isolated from seeds of soybean. Among these fungi *Aspergillus flavus*, *Fusarium oxysporum*, and *Alternaria alternata* were found to be dominant which could be controlled by using leaf extract of medicinal plants like *Acacia nilotica* (L.).

Many fungicides have been tested for effectiveness in controlling *A. brassicae* and conclusions were often conflicting. Ansari *et al.*, 1990 evaluated eighteen fungicides as to their control of *A. brassicae* in artificial cultures, infected seeds and as a foliar spray on infected plants of *B. campestris* var. yellow sarson (a highly susceptible rape cultivar).

Accordingly our extract was also found effective against the fungi. Further more if we separate these spots again by using column chromatography from the Fraction-2 of hexane extract of stem-bark of *Acacia nilotica* probably these pure isolated compounds get through the fungal cell wall/membrane and suppress their growth or if these compounds deeply penetrated, might kill them completely. Thus it might be used as antifungal agent for curing different fungal diseases which reduce toxicity and adverse effect and would be good agreement in controlling the fungal diseases such as *Fusarium oxysporum ciceris*, *Alternaria brassicae* and *Rhizoctonia solani*.

The results obtained from this work conclude that the plant is promising for development of phytomedicine for antifungal properties and also indicates the potential usefulness of *Acacia nilotica* in the treatment of various pathogenic diseases. Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, scientifically supported by the results obtained in this work.

The further studies might be needed to determine the chemical identity of the bioactive compound which was responsible for the observed antifungal activity. The finding of the active compound present in the hexane extract fractions could be an important step towards the possibilities of using natural plant products as pesticides in the plant disease control.

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