

Evaluation of the Antifungal Activity of the Potent Fraction of Hexane Extract Obtained from the bark of *Acacia Nilotica*

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Abstract: Crude hexane extract and its different fractions (Fraction-1 to Fraction-11) isolated from the stem-bark of *Acacia nilotica* were tested for the antifungal activity against important phytopathogens *Alternaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani* at 15% concentrations were assessed by modified food poison method. Crude hexane extract of stem-bark of *Acacia nilotica* showed high percent mycelial inhibition against *Rhizoctonia solani* followed by *Alternaria brassicae* and *Fusarium oxysporum ciceris* and FRACTION-2 of hexane extract of stem-bark of *Acacia nilotica* was most effective in terms of percent mycelial growth inhibition in case of *Alternaria brassicae* and *Rhizoctonia solani* than all other fractions. The FRACTION-1 of hexane extract of stem-bark of *Acacia nilotica* was most effective in terms of percent mycelial growth inhibition in case of *Fusarium oxysporum ciceris* than all other fractions. This antifungal activity may be due to presence of phytoconstituents such as carbohydrates, sterols, triterpenoids and anthraquinone. The results were compared with standard antifungal acetic acid as a (+ ve) control. By using Kruskal Wallis test, Fraction-2 has high antifungal activity against the plant pathogens *Alternaria brassicae*, *Fusarium oxysporum ciceris*, *Rhizoctonia solani*.

Keywords: Antifungal activity, *Acacia nilotica*, Food poison method, phytochemical screening, *Alternaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani*.

1. Introduction

Fungal phytopathogens that are the cause of many plant diseases and much loss of crop yields, especially in subtropical and tropical regions (Brimner and Boland, 2003). The requirement is to find effective source to check pathogenicity. Chemical fungicides are extensively used in contemporary agriculture. However, these products may cause problems such as environmental pollution and affect human health. Further problems include the development of resistance in the pathogens and to a decrease in the diversity of non-target organisms. To reduce the worsening problems in fungicide usage, new methods for plant protection, which are less dependent on chemicals and are more friendly to environment should be discovered and developed.

Acacia nilotica (Linn.) belonging to the Leguminosae family and sub-family Mimosaceae was taken into consideration found to be rich in antimicrobial activity as an alternative to chemical fungicides. In Australia it is regarded as one of the worst weeds because of its invasiveness, potential for spread, and economic and environmental impacts. The reason for the usage of this weed was its potential to resist pests and pathogens in its environment. The development of resistance in weeds to the common pesticides and the increasing restrictions on the use of toxic material in the environment has given an impetus to search for novel plant protectants that interfere with the pathogenicity factors. Herbal fungicides are gaining growing interest because of their eco-friendly attributes and their cost effectiveness (Dwivedi and Singh, 1998; Karnwal and Singh, 2006).

The use of chemical pesticides is a very popular practice to control various plant diseases management as compare to natural one which are prepared from plants or plant parts. But, consumer now demands less use of synthetic fungicides due to the non-biodegradability, pollutive nature and residual toxicities of chemical pesticides. Several studies have revealed the plant extracts as source of natural pesticides that make excellent efforts for new pesticide development (Arokiyaraj *et al.*, 2008; Gangadevi *et al.*, 2008; Brindha *et al.*, 2009).

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. Natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers were trying to identify the effective natural products to replace the synthetic pesticides (Kim *et al.*, 2005).

2. Materials and Methods

2.1 Collection of Drugs-The powdered bark of *Acacia nilotica*

2 Kg of powdered stem-bark of *Acacia nilotica* was collected from the SHREE *Baidyanath*, Ayurved Bhawan Pvt. Ltd., Allahabad.

2.2 Place of Work

The present work entitled “**Evaluation of the antifungal activity of the potent fraction of hexane extract obtained from the bark of *Acacia nilotica***” was carried out in the Department of Chemistry, School of Basic Sciences and Department of Plant Pathology, SHIATS, Allahabad.

2.3 Extraction of the powdered bark

The plant sample were weighed and extracted in soxhlet extractor with hexane at 57°C. The extraction was monitored continuously at each stage and the completion of extraction was confirmed by colour. The solvent was taken from the thimble and evaporated to check the absence of residue. The extract was filtered through Whatmann no.1 filter paper and evaporated to dryness at 40°C with the aid of distillation and percentage yield was calculated.

Yield of the extract obtained was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of dry powder}} \times 100$$

2.4 Phytochemical Screening of the Hexane Extract

Chemical investigation of the extract for major active ingredients was conducted by standard qualitative method given by **Harborne (1973)** and **Trease and Evans (2002)**.

Isolation of potent fractions of hexane extract of stem-bark of *Acacia nilotica*

2.5 Isolation of different fractions by column chromatography

Isolation of potent active fraction was done by column chromatography using different concentration of hexane (10-100%), ethyl acetate (10-100%) and methanol (100%) with the increasing order of polarity. Various fractions was collected separately and matched by TLC to check homogeneity; fraction of similar R_F was combined and tested for antifungal activity against plant pathogens.

2.6. Evaluation of antifungal activities of hexane extract and its fraction obtained from stem-bark of *Acacia nilotica*

Isolation of different fungal strains

The different fungal strain *Fusarium oxysprum ciceris* and *Altarnaria brassicae* were isolated from Chick-pea and cabbage plant (infected plant collect from Plant Pathology Central Research Farm) by surface sterilization methods and *Rhizoctonia solani* strain was collected from the microbial culture collection bank of department of Microbiology and Fermentation Technology, JSBB, SHIATS.

2.7 Surface sterilization methods

Portion of the infected plant which was found in the cabbage and chick-pea plant firstly washed by flow of water cut with healthy portion into small pieces into sterile petri dishes with the help of sterile scissors which was flamed over and dipped into methylated spirit (**Thomas, 1979**). The cut

pieces were surface sterilized with 0.01% mercuric chloride for 30 seconds and rinsed in five changes of sterile distilled water and blotted dry with sterile Whatman No. 1 filter papers. Cut pieces were plated aseptically in Petri dishes containing solidified potato dextrose agar (PDA). Solidified plates were incubated at room temperature (28-30°C). After 3 days, a growth of whitish colony in chick-pea and blackish colony growth in cabbage was observed, from this colony growth, a portion from the periphery having single hyphal tip were separated and transferred to other petri-plates having medium to get pure culture. The fungal isolate that were obtained in potato dextrose agar plates were identified by lactophenol cotton blue staining.

2.8 Identification of Isolated Fungi

Microscopic examination was carried out after examining the colony characteristics (**Frazier, 1978**) while the morphological and cultural characteristics were observed under the microscope.

2.9 Methods for testing Antifungal activities

Antifungal activity was done by using Food poison technique elucidated by **Mohanty et al., (2012)** in which firstly 0.15625 g of the hexane extract dissolved in 62.5 ml of DMSO (dimethyl sulphoxide) and 15 ml of this extract and fractions respectively was added to 85 ml of PDA media. The plates were left for 15 mins to solidify. Then 4 mm diameter of fungal colony punched with borer was placed onto plates containing media with extract in aseptic condition. Plates were incubated for 3-5 days at 28°C. The medium without any treatment served as control. Test fungi were inoculated and percent inhibition of mycelial growth was determined. After three days colony diameter was measured in centimetre. For each treatment three replicates were maintained. The fungi toxicity of the extracts and different fractions in terms of percentage inhibition of mycelial growth was calculated by using formula:

$$\% I = \frac{C - T}{C} \times 100$$

Where; % I = percentage inhibition, C = colony diameter in control, T = colony diameter in treatment

2.10 Statistical Analysis

The data obtained from the experiment for the antifungal activities were subjected to statistical analysis using “Analysis of Variance” Technique (Two way classification) (**Fisher and Yates, 1968**). For testing the hypothesis, the following ANOVA table was used (**Chandel, 2002**). The Kruskal-Wallis test and Dunn’s multiple comparison tests were done by using the Software **Graph Pad Instat version 3.10 32 bit for Windows**.

3. Results and Discussion

3.1.1: Macroscopical evaluation of hexane extract of bark of *A. nilotica*

The macroscopical study of the stem-bark of *Acacia nilotica* for the hexane extract was done and shown in table 4.1 below:

Table 4.1: Physical characteristics of hexane extract of stem-bark of *Acacia nilotica*

S.No.	Features	Observations
1	Colour	Brown
2	Odour	Characteristic pungency
3	Taste	Bitter
4	State	Semi-solid

3.1.2: Physicochemical analysis of hexane extract of stem-bark of *A. nilotica*

The percentage yield of hexane extract of stem-bark of *A. nilotica* is 1.718 shown in **Table 4.2** below:

Table 4.2: Percentage yield of hexane extract of stem-bark of *Acacia nilotica*

Plant parts	Solvent	Wt. of plant materials (g)	Wt. of extract materials (g)	Percentage Yield (%)	Colour of extract
Stem bark of <i>Acacia nilotica</i>	Hexane	56	0.962	1.718	Brown

3.1.3: Phytochemical screening of the hexane extract of bark of *A. nilotica*

The analytical results of the qualitative phytochemical analysis of the medicinal plants are given in the **Table 4.3** below:

Table 4.3: Phytochemical screening of the hexane extract of stem-bark of *Acacia nilotica*

S. No.	Chemical Constituents	Result
1	Alkaloids	
	Dragendroff's test	-
	Mayer's Reagent	-
	Hager's Reagent	-
	Wagner's Reagent	-
2	Flavonoids	-
	Shinodo test (Magnesium Hydrochloride-reduction test)	-
	Alkaline reagent test	-
3	Tannins	-
	4	Amino acid and Protein test
4	Biuret test	-
	Ninhydrin test	-
	5	Carbohydrates test
5	Molisch test	+
	6	Reducing Sugar

	Fehling test	-
	Benedict's test	-
7	Saponin	-
8	Saponin Glycosides	-
9	Sterols and triterpenoids	
	Salkowski's test	+
10	Anthraquinone test	+
11	Glycosides test	-
12	Anthracenes test	-

Antifungal activity of hexane extract and its different fractions isolated from stem-bark of *Acacia nilotica*
Morphological & culture characteristics of the isolated plant pathogens

The infected chick-pea and cabbage plant showing characteristic symptoms of wilt disease and leaf spot disease, a whitish and blackish colony growth was observed which is shown in **Plate 4.1** below. The isolated fungi from disease chick-pea and cabbage plant were observed and identified under the microscope on the basis of its structure and it was confirmed by its conidia. The pure culture, conidia of the infected cabbage plant were shown in below **Plate 4.2**. Thus the isolated fungi from the diseases chick-pea and cabbage plant was *Fusarium oxysporum ciceris* and *Altarnaria brassicae*.



Plate 4.1: *Fusarium oxysporum ciceris* and *Altarnaria brassicae* isolated from Chick-pea and Cabbage plant



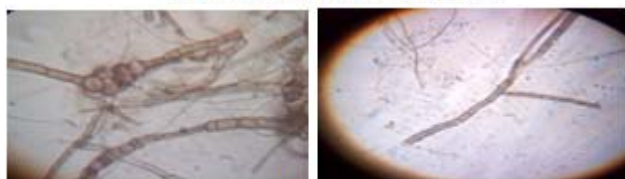
Plate 4.2: Pure culture and conidia of the isolated fungi *Altarnaria brassicae* of the infected cabbage plant

Plate 4.3: Pure culture and conidia of the isolated fungi *Fusarium oxysporum ciceris* of the infected chick pea plant

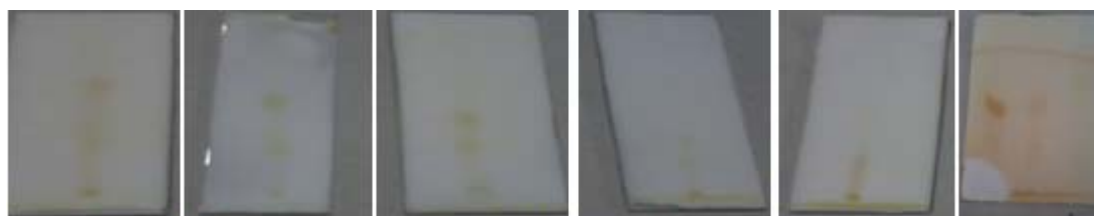
Rhizoctonia solani strain collected from the microbial culture collection bank of department of Microbiology and Fermentation Technology, JSBB, SHIATS was observed and identified under the microscope on the basis of its structure and it was confirmed by its conidia. The pure culture, conidia of the *Rhizoctonia solani* were shown in below **Plate 4.4**.



Pure culture of *Rhizoctonia solani*



Conidia of *Rhizoctonia solani*



10%EA+90%HEX (F-1)

20% EA+80% HEX.(F-2)

30%EA+70%HEX.

Figure 4.1: Different spot appeared in mixture of 10%, 20%, 30% ethyl acetate and 90%, 80%, 70% hexane respectively used as solvent

Antifungal activity of the hexane extract of stem-bark of *Acacia nilotica* against *Fusarium oxysporum ciceris*, *Altarnaria brassicae* and *Rhizoctonia solani*

Crude hexane extract and different fractions of stem- bark of *Acacia nilotica* plants used in this study were preliminary screened against important phytopathogenic fungus *Altarnaria brassicae*, *Fusarium oxyporum ciceris* and *Rhizoctonia solani* at 15% concentration were assessed by

Plate 4.4: Pure culture and conidia of fungi *Rhizoctonia solani*

Isolation of active ingredient from hexane extract of stem-bark of *Acacia nilotica*

The different fractions of hexane extract of stem-bark of *Acacia nilotica* were isolated by eluting with hexane, ethyl acetate and methanol as organic solvent in the order of the increasing polarity. The fractions were concentrated by evaporating the excess of the solvent by distillation and the residues were identified by using TLC plate runs, in different solvents and the different spots that appeared in the TLC plates were shown in **Fig.4.1** below:

food poison method of **Mohanty et al., (2012)**. Crude hexane extract of stem-bark of *Acacia nilotica* showed high percent mycelial inhibition against *Rhizoctonia solani* (58.0459%) followed by *Altarnaria brassicae* (51.85%) and *Fusarium oxysporum ciceris* (46.0099%) which are shown in below **Table 4.4** and **Fig. 4.6**.

Table 4.4: Antifungal activity of the hexane extract of stem-bark of *Acacia nilotica* against *Fusarium oxysporum ciceris*, *Altarnaria brassicae* and *Rhizoctonia solani*

Hexane extract	Control	<i>F. oxy. Ciceris</i>	Control	<i>R. solani</i>	Control	<i>A. brassicae</i>
R1 (cm)	4.7	2.4	5.8	2.5	6.3	3
R2 (cm)	4.7	2.8	5.8	2.4	6.3	3.3
R3 (cm)	4.7	2.4	5.8	2.4	6.3	2.8
MEAN	4.7	2.5333	5.8	2.43333	6.3	3.0333
STD		0.230940		0.057735		0.25166
MEAN±STD (n=3)		2.533±0.2309		2.43±0.05		3.033±0.251
% mycelial growth inhibition		46.0099		58.0459		51.8518

Antifungal activity of the different fractions of the hexane extract obtained from stem-bark of *Acacia nilotica* against *Fusarium oxysporum ciceris*, *Altarnaria brassicae* and *Rhizoctonia solani*

By using column chromatography, different fractions (**Fraction-1 to Fraction-11**) of hexane extract obtained

from stem-bark of *Acacia nilotica* were isolated and tested for antifungal activity against important phytopathogens *Altarnaria brassicae*, *Fusarium oxyporum ciceris* and *Rhizoctonia solani*.

The statistical analysis of the data revealed that at 5% level of significance, in comparison to control, each crude hexane

extract and its different fraction significantly inhibited the growth of tested pathogens. It was observed that at 15% concentration of stem-bark of hexane extract of different fractions the **FRACTION-2** of the hexane extract of stem-bark of *Acacia nilotica* was most effective in terms of percent mycelial growth inhibition in case of *Altarnaria brassicae* (66.67%) and *Rhizoctonia solani* (62.17%) than all other fractions. The effect of different fractions at 15% concentrations on antifungal activity against *Altarnaria brassicae* and *Rhizoctonia solani* was found to be statistically significant ($F_{cal} 163.3432 > F_{tab} 2.18$ due to fractions) and ($F_{cal} 116.8963 > F_{tab} 2.18$ due to fractions) respectively at 5% level of significance (**Appendix II Anova Table 3, 2**). The **FRACTION-1** of hexane extract of

stem-bark of *Acacia nilotica* was most effective in terms of percent mycelial growth inhibition in case of *Fusarium oxysporum ciceris* (65.59%) than all other fractions. The effect of different fractions at 15% concentrations on antifungal activity against *Fusarium oxysporum ciceris* was found to be statistically significant ($F_{cal} 87.04 > F_{tab} 2.18$ due to fractions) at 5% level of significance (**Appendix II Anova Table 1**). Acetic acid (positive control) for *Altarnaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani* showed the highest antifungal activity in comparison to all other fractions which was shown in the **Table (4.5)**.

Table 4.5: Antifungal activity of the different fractions of the hexane extract obtained from stem-bark of *Acacia nilotica* against *Fusarium oxysporum ciceris*, *Altarnaria brassicae* and *Rhizoctonia solani*

Hex. extract of bark of <i>A. nilotica</i> of different fractions	<i>Fusarium oxysporum ciceris</i>		<i>Altarnaria brassicae</i>		<i>Rhizoctonia solani</i>	
	Mean % mycelial Diametre (cm) growth	% mycelial growth inhibition	Mean % mycelial Diametre (cm) growth	% mycelial growth inhibition	Mean % mycelial Diametre (cm) growth	% mycelia growth inhibition
T1 (100%HEX.)	4.93333±0.15	20.43010	5.3667±0.11	27.47747	6.533±0.1527	26.591
T2 (10%EA+90%HEX.) F-I	2.133±0.0577	65.59139	3.3667±0.15	54.50450	4.333±0.0577	51.310
T3 (20%EA+80%HEX.) F-II	2.433±0.1527	60.75268	2.467±0.057	66.6667	3.3667±0.251	62.17
T4 (30%EA+70%HEX.) F-III	3.2667±0.208	47.31182	3.033±0.208	59.00909	4.5333±0.057	49.0636
T5 (40%EA+60%HEX.) F-IV	4.2667±0.115	31.18279	3.6±0.2	51.3513	5.0667±0.152	43.07
T6 (50%EA+50%HEX.) F-V	4.4667±0.208	27.95698	4.333±0.152	41.4414	5.4±0.264575	39.325
T7 (60%EA+40%HEX.) F-VI	5.1±0.173205	17.74193	4.833±0.251	34.6846	5.2333±0.115	41.1985
T8 (70%EA+30%HEX.) F-VII	5.26667±0.15	15.05376	6.5333±0.15	11.7117	5.8333±0.057	34.4569
T9 (80%EA+20%HEX.) F-VIII	5.0667±0.378	18.27956	6.4667±0.20	12.6126	5.3667±0.208	39.700
T10 (90%EA+10%HEX.) F-IX	5.4±0.5	12.90322	6.6333±0.64	10.3603	5.3333±0.152	40.07
T11(100%Ethyl acetate) F-X	5.6333±0.251	9.13978	6.9±0.11547	6.7567	7.6333±0.450	14.2322
T12(100%Methanol) F-XI	5.13333±0.20	17.20430	6.0333±0.32	18.4684	5.9667±0.115	32.9588
Acetic acid (+ve control)	0	100	0	100	0	100

Antifungal activity of hexane extract and different fractions of stem-bark of *Acacia nilotica* against *Fusarium oxysporum ciceris*, *Altarnaria brassicae* and *Rhizoctonia solani* showed

the different mycelial growth inhibition in **Fig. 4.2, 4.3, 4.4 and 4.5**.

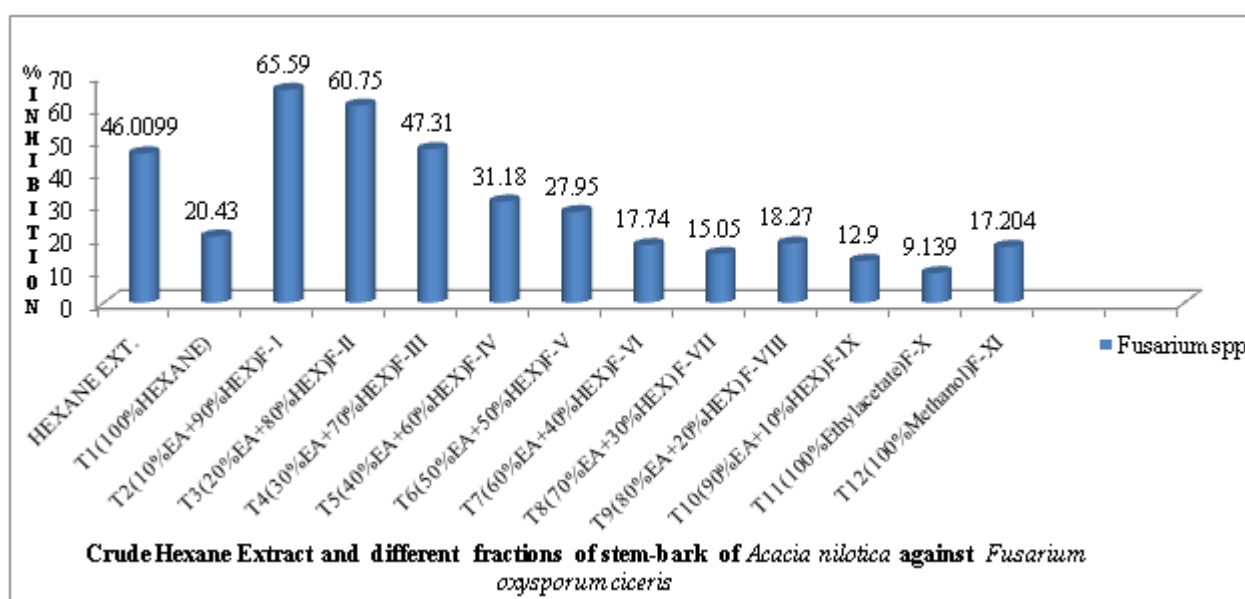


Figure 4.3: Antifungal activity of hexane extract and different fractions of stem-bark of *Acacia nilotica* against *Fusarium oxysporum ciceris*

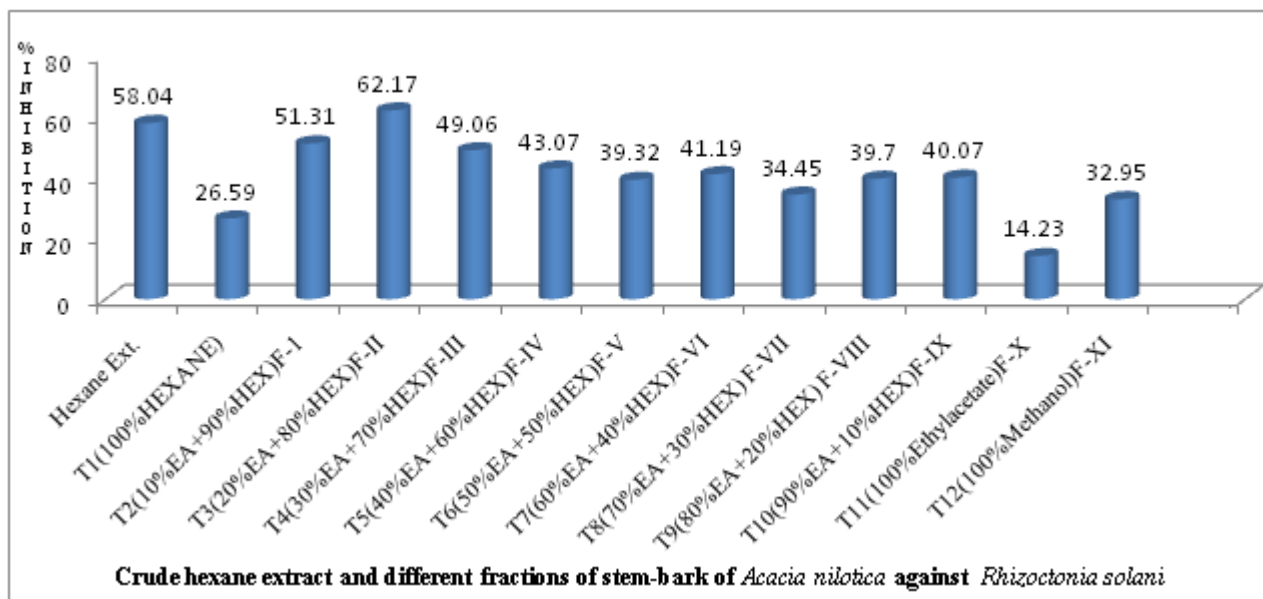


Figure 4.4: Antifungal activity of hexane extract and its different fractions of stem-bark of *Acacia nilotica* against *Rhizoctonia solani*

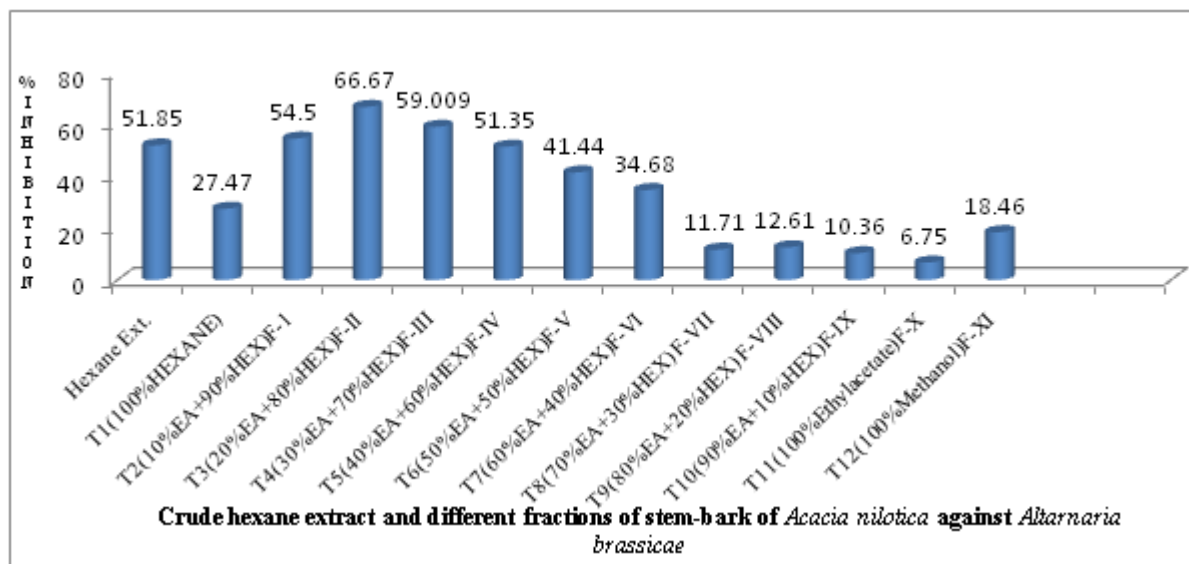


Figure 4.5: Antifungal activity of hexane extract and its different fractions of stem- bark of *Acacia nilotica* against *Alternaria brassicae*

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

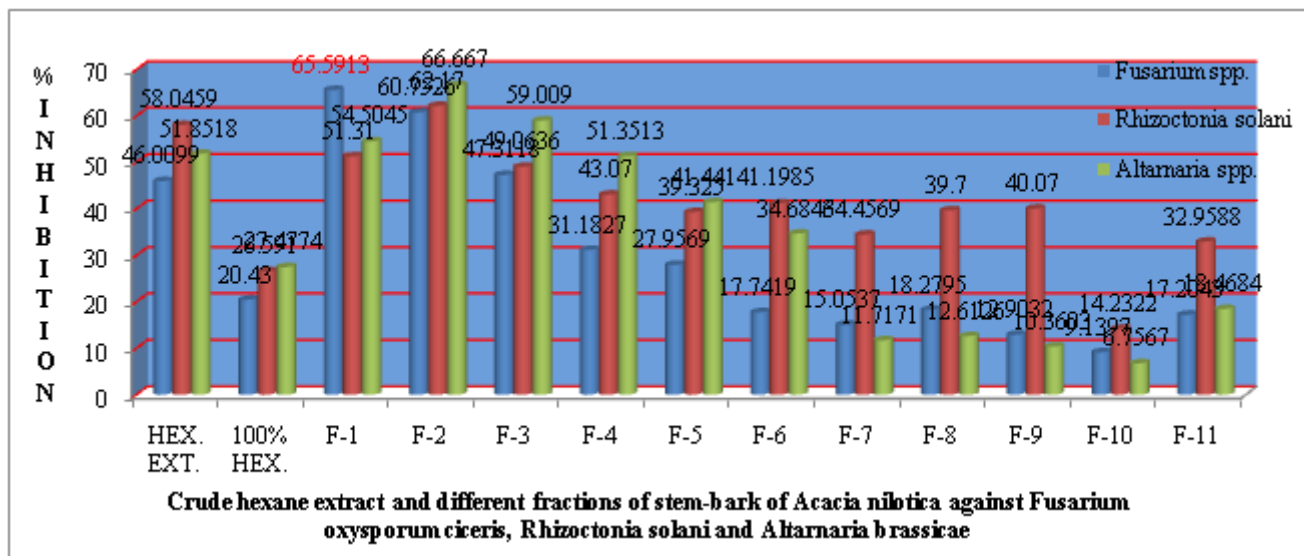


Figure 4.6: % mycelial growth inhibition of crude hexane extract and different fractions of stem-bark of *Acacia nilotica* against *Alternaria brassicae*, *Fusarium oxysporum ciceris* and *Rhizoctonia solani*
Kruskal-Wallis Test (Non-parametric ANOVA)

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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References

- [1] Fisher, R.A. and Yates, S. (1968). Analysis of variance, Handbook of biostatistics.
- [2] Harborne, J.B. (1973). Phytochemical Methods. Chapman and Hall Ltd., London, UK: 49-188.
- [3] Frazier, W. C. (1978) Food Microbiology , USA, Tata Mcgraw Hill Co. inc. :20-40
- [4] Khan, I. A., Subhan, A. and Ahmad, A. (1979). Inhibition of spore germination of *Halminthosporium turcicum*, the incitant of sorghum leaf blight by chemicals and plant extracts. *Indian Journal of Plant protection* 7:77-81
- [5] Thomas, G.A. (1979). Medical Microbiology London; Macmillan Press: 403
- [6] Ansari, N. A., Kahn, M. W. and Muheet, A. (1990). Evaluation of some fungicides for seed treatment and foliar application in management of damping-off of seedlings and blight of rapeseed caused by *Alternaria brassicae*. *Mycopathologia* 110:163-167
- [7] Dwivedi, S.K. and Singh, K.P. (1998). Fungitoxicity of some higher plant products against *Macrophomina phaseolina* (Tassi) Goid. *Journal of Flavour and Fragrance* 13: 397-399
- [8] El-Tahir, A., Satti, G.M. and Khalid S.A. (1999). Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Acacia nilotica*. *Phytotherapy Research* 13: 474-478.
- [9] Hernandez N.E., Tereschuk M.L., Abdala L.R. (2000). Antimicrobial activity of flavonoids in medicinal plants from Tafi del Valle (Tucumán, Argentina) *Journal of Ethnopharmacology* 73 (1-2): 317-322
- [10] Alam, S., Akhter, M., Begum, F., Banu, M.S., Islam, M. R. and Chawdhary, A.N. (2002). Antifungal activities (*in vitro*) of some plant extracts and smoke on four fungal pathogens of different hosts. *Pakistan Journal of Biological Sciences* 5 (3): 307-309
- [11] Chandel, S.R.S. (2002). Handbook of biostatistics: 255
- [12] Trease, G.E. and Evans, W.C. (2002). Pharmacognosy 15th Ed. Saunders Publishers, London: 42-44,221-229,246-249,304-306,331-332,391-393
- [13] Brimmer, T.A. and Boland, G.J. (2003). A review of the non-target effects of fungi used to biologically control plant diseases. *International Journal of Agriculture, Ecosystems and Environment* 100(1): 3-16
- [14] Bajwa, R. Shafique, S., Anjum, T. and Shafique, S. (2004). Antifungal activity of allelopathic plant extracts IV: Growth response of *Alternaria alternata*, *Fusarium moniliforme* and *Drechslera hawaiiensis* to aqueous extract of *Parthenium hysterophorus* L. *International Journal of Agriculture Biology* 6: 511-516
- [15] Ahmed, S.M. and Abdelgaleil, S.A.M. (2005). Antifungal activity of extracts and sesquiterpene lactones from *Magnolia grandiflora* L. (Magnoliaceae). *International Journal of Agriculture Biology* 4: 638-642
- [16] Kim, D. I., Park, J. D., Kim, S. G., Kuk, H., Jang, M. J. and Kim, S. S. (2005). Screening of some crude plant extracts for their acaricidal and insecticidal efficacies. *Journal of Asian Pacific Entomology* 8: 93-100

- [17] **Bajwa, R., Anjum, T., Shafique, S. and Shafique, S. (2006).** Evaluation of antifungal activity of *Cicer arietinum* L. *Pakistan Journal of Botany* **38**: 175-184
- [18] **Karnwal, P. and Singh, P. (2006).** Antifungal activity of *Cassia fistula* leaf extract against *Candida albicans*. *Journal of Microbiology* **46(2)**: 169-170
- [19] **Arokiyaraj, S., Martin, S., Perinbam, K., Marie, A., Pand, B. V. (2008).** Free radical scavenging activity and HPTLC finger print of *Pterocarpus santalinus* L. *in vitro* study. *Indian Journal of Science and Technology* **1(7)**: 1-7
- [20] **Gangadevi, V., Yogeswari, S., Kamalraj, S., Rani, G and Muthumary, J. (2008).** The antibacterial activity of *Acalypha indica* L. *Indian Journal of Science and Technology* **1(6)**.
- [21] **Mahesh, B. and Satish, S. (2008).** Antimicrobial activity of some important medicinal plant against plant and human pathogen. *World Journal of Agriculture Sciences* **4 (S)**: 839-843
- [22] **Mann, A., Banso, A. and Clifford, L. C. (2008).** An antifungal property of crude plant extract from *Anoqissus leiocarpus* and *Terminalia avicennioides*. *Tanzania Journal of Health Research.* **10 (1)**: 34-38
- [23] **Banso, A. (2009).** Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *Journal of Medicinal Plants Research* **3(2)**: 82-85
- [24] **Brindha, V., Saravanan, A and Manimekalai, R. (2009).** Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarinin extracted from *Terminalia arjuna*. *Indian Journal of Science and Technology* **2(2)**: 22-26
- [25] **Sehajpal, A., Arora, S. and Kaur, P. (2009).** Evaluation of plant extracts against *Rhizoctonia solani* causing sheath blight of rice. *Journal of Plant Protection Science* **1**: 25-30
- [26] **Singh, B.N., Singh, B.R., Sarma, B.K. and Singh, H.B. (2009b).** Potential chemoprevention of N-nitrosodiethylamine-induced hepatocarcinogenesis by polyphenolics from *Acacia nilotica* bark. *Chemico-Biological Interaction* **181**: 20-28
- [27] **Mohanty, R.C., Ray, P. and Rath, S. (2012).** In-vitro antifungal efficacy study of plant leaf extracts against three *dermatophytes* *CIBTech Journal of Microbiology* **1(2-3)**: 27-32
- [28] **Rathod, L.R. and Pawar, P.V. (2012).** Antimicrobial activity of medicinal plant to control seed borne pathogen of Soybean *Journal of Current Botany* **3(2)**: 10-12
- [29] **Chatterjee, P. and Das, N. (2014).** Evaluation of antimicrobial potentiality of 50% aqueous ethanolic leaf extract of *Acacia nilotica* Willd. *Asian Journal of Pharmacy and Clinical Research* **7 (1)**: 95-98
- [30] **Abbas, K., Niaz, U., Hussain, T., Saeed, M.A., Javaid, Z., Idrees, A. and Rasool, S. (2014).** Antimicrobial activity of fruits of *Solanum nigrum* and *Solanum xanthocarpum*. *Acta Poloniae Pharmaceutica - Drug Research* **71(3)**: 415-421

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