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# Antifungal Properties of Seeds Cassia Farmasiana

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## ABSTRACT

The antifungal activities of extracts in organic solvents from seeds of cassia farmasiana were tested. Based on the polarity, three organic solvents like Petroleum ether, Chloroform and Methanol were used for preparing the extracts (2.0% and 3.0%) from the stem and seeds of cassia farmasiana against three common forest fungi Viz. Colletotrichum gloeosporiodes, Fusarium oxysporum and Ganoderma lucidum. The inhibitory activity of all the extracts against three fungi remained consistently high in the chloroform extract i.e., 36.2%, 31.9% and 36.2% for C. gloeosporiodes, F. oxysporum and G. lucidum respectively; next effective reagent was Petroleum Ether. In general, higher concentration of extract (3.0%) was found to be more effective against all three fungi than lower concentration of 2.% chloroform extract (3.0%) was found to be most suppressive of C. Gloeosporiodes. (65.4 and 68.4%) than other fungi, while Petroleum Ether (3.0%) was found to be reasonlably inhibitory (57.3%) to F. oxysporum. It may be concluded from these observations, that concentration of stem and seed extracts from cassia farmasiana may change the extent of fungal growth Its important to completion of screening.

Keywords : Antifungal cassia farmasiana, Colletrotrichum gloeosporiodes, Fusarium oxysporum and Ganoderma lucidum.

## I. INTRODUCTION

Medicinal Plant, herbs, species & herbal remedies are integral components of alternative system of medicine science since times immemorial cassia farmasiana. Around 2,300 Species of plants possess pesticidal properties.[1] cassia farmasiana belongs to the family leguminosae. It is an annual monsoon weed prevalent in wastelands having antimicrobial properties.[2] The ecofriendly antimicrobial properties of its seed gum has been reported by different workers. [3-5] Keeping the problem of pollution and health peril of conventional agrochemicals in view, a study was conducted to test its seed-extract against the common aerial fungus (*Colletortrichum gloeosporioides),* soil-fungus (*Fusarium oxysporum)* and root-fungus (*Ganoderma lucidum)* found abundant in forest ecosystem seed-extract varied concentrations of cassia farmasiana in different organic solvents were screened to identify the maximum antifungal activity.

**Plant extracts and test fungi:-** The seed of cassia farmasiana was shade dried and powdered; the seed-powder was again shade-dried. 3.5Kg of seed-powder was defatted with Petroleum Ether and then extracted with 5 liter of 95% Methanol, Petroleum Ether or chloroform at 40-60$^0$ C in Soxhlet apparatus for 17 hours. The respective extracts were then suspended in 1 liter $H_2O$.

Chloroform, Petroleum Ether and Methanol extracts of seeds of cassia farmasiana were then tested for their efficacy on the fungi Colletrotrichum gloeosporioides, Fusarium oxysporum and Ganoderma lucidum. The initial screening wsas done at 1.0% concentration of extracts of both stem and seed. At later stage, only chloroform and petroleum ether extracts of seeds were tested at 2.0 and 3.0 percent concentration.

**Detoxification test :-** The principle contiguous in this technique is to toxify the nutrient medium with a fungi toxicant and then allowing a test fungus to grow on such a medium. Potato-dextrose agar medium was prepared in flask and sterilized. The petri plates having 8 cm inner diameter were used. 240 ml sterilized PDA containing 1.2 gm/ml extract were poured aseptically in nine plates. After solidification, agar disc of 7 days old activated culture were placed in the center with the help of sterile cork-borer. Control experiments were undertaken parallelly like PDA+ Fungus without addition of seed-extract, Solvents (Acetone and Methanol)+ Fungus without addition of seed-extract. The diameter of the clolonies were compared with these controls to determine the extent of fungitoxicity of seed-extract from cassia farmasiana.

The percentage calculated using the following formula:

$$I=C-T/C \times 100$$

Where, I= Percent Growth Inhibition; C=Radial Growth in cm of Test Fungus in control Petri Plate; T= Radial Growth in cm of Test Fungus in treated Petri Plate.

**Statistical analysis**

The experiments of antifungal activities were analyzed by two way factorial models. The significance of difference between the treatments was tested at tested at 10% level of significance.

## II. Result

It was observed that than that of seed extract (4.6%), stem extract of cassia farmasiana had significantly more antifungal activity (19.4%) (Table 1), irrespective of nature of extract used. Highest activity was found in the Chloroform extract (38.4%) followed by Petroleum Ether (35.4%) Least antifungal activity was quantified in the Methanol extract (20.4%), irrespective of plant part tested. While studying interaction between the plant part and extract (P x T), it was found that significantly more activity was present in chloroform extract of stem (65.2%) followed by Petroleum Ether (48.9%). Methanol extract had least antifungal activity (41.0%) and very low activity in all the seed extract (14.2%) in case of Petroleum Ether as well as for chloroform extract).

**Table I-** Percent of inhibition of growth of Colletotrichum gloeosporiodes with different extracts at 1.0% of stem and seed of Cassia farmasiana.

| Part | Treatments | | | | | | Mean | |
|---|---|---|---|---|---|---|---|---|
| | CK | CKA | CKM | PE | CHL | ME | | |
| Stem | 0.0[a] | 0.0[a] | | 0.0[a] | 48.9[b] | 65.2[bc] | 41.0 | 28.5 |
| Seed | 0.0[a] | 0.0[ab] | 28[c] | 14.2[c] | 14.1[cd] | 0.0[e] | 5.1 | |
| Mean | 0.0 | 0.0 | 1.4 | 35.4 | 38.4 | 20.4 | | |

| Part | Treatments | Interaction (PxT) |
|---|---|---|
| SEM | 0.0 | 0.4 |
| CD (1%) | 0.2 | 0.5 |

*Means with similar superscripts are not significantly different from each other

**Abbreviations:** CK, Check, Without plant extract; ckA, acetone check (acetone, without plant extract); CKM, methanol check (methanol, without plant extract); PE, petroleum ether, with plant extract, CHL, chloroform with plant extract, ME, methanol, with plant extract.

**Inhibitory activity of Cassia farmasiana seed-extract against Fusarium oxysporum**

Irrespective of treatments, the stem extract was significantly more suppressive (29.0%) than seed extract (14.1%) (Table 2) The trend for inhibition of fungal growth was similar to C. gloeosporioides i.e., chloroform extract could inhibit the growth of F. oxysporum to the maximum extent (52.4%) followed by petroleum ether (25.0%) The least inhibitory activity was quantified in Methanol extract (21.8%) whether it was prepared from stem or seed of Cassia farmasiana Interaction (P x T) analysis revealed that stem extract has anti-fusarium activity than seed which was found maximum and significantly more in chloroform extract (63.8%) followed by petroleum ether (44.2%) least activity was recorded in methanol extract of Cassia farmasiana stem (29.0%). In case of seed, maximum inhibition of fungal growth was observed when chloroform extract (54.4%) was used followed by methanol extract (14.1%), which was at par with chemical check of acetone as well as methanol 14.1% each).

**Table 2**- Percent inhibition of growth of Fusarium oxysporum with different extracts at 1.0% of stem and seed of Cassia farmasiana.

| Part | Treatments | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | CK | CKA | CKM | PE | CHL | ME | |
| Stem | 0.0[a] | 29.0[bc] | 14.1[de] | 44.2[e] | 63.8[ef] | 21.5[d] | 30.1 |
| Seed | 0.0[a] | 14.1[bc] | 14.0[cd] | 56[ef] | 54.4[fg] | 14.1[h] | 15.0 |
| Mean | 0.0 | 21.8 | 14.0 | 25.0 | 56.7 | 21.8 | |

| | Part | Treatments | Interaction (PxT) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| SEM | 0.0 | 0.4 | 0.4 |
| CD (1.0%) | 0.4 | 0.7 | 0.8 |

**Inhibitory activity of cassia farmasiana seed-extract against Ganoderma lucidum**

The growth of Ganoderma lucidum was more inhibited (20.3%) when treated with the stem extract than seed extract of cassia farmasiana whether it was prepared in any of the organic solvent (table 3). However, contrary to the previous results, the growth of this fungus was suppressed in the Petroleum Ether extract (57.3%) chloroform extract had significantly more inhibitory activity (36.2%) chloroform extract had significantly more inhibitory activity (36.2%) than Methanol (16.0%) but it had significantly less than Petroleum Ether extract irrespective of plant part used for extraction. The interaction analysis shows that the maximum anti-Ganoderma activity was seen in Petroleum Ether extract of the cassia farmasiana seed (72.4) However in general, stem extract had more activity for example chloroform and methanol extract had inhibited the fungus up to 72.4 and 29.0 percent respectively.

**Table 3**- Percent inhibition of growth of Ganoderma lucidum with different extract at 1.0% of stem and seed of cassia farmasiana.

| Part | Treatments | | | | | | Mean | |
|---|---|---|---|---|---|---|---|---|
| | CK | CKA | CKM | PE | CHL | ME | | |
| Stem | $0.0^{a}$ | $12.0^{bc}$ | $14.2^{d}$ | $29.6^{d}$ | $72.4^{de}$ | $21.5^{d}$ | 32.1 | |
| Seed | $0.0^{a}$ | $14.1^{bc}$ | $0.0^{de}$ | | $46.9^{fg}$ | $0.0^{hi}$ | $14.2^{h}$ | 19.5 |
| Mean | 0.0 | 10.0 | 7.2 | 57.3 | 36.2 | 16.0 | | |

| | Part | Treatments | Interaction (PxT) |
|---|---|---|---|
| SEM | 0.2 | 0.4 | 0.6 |
| CD(1%) | 0.7 | 1.1 | 1.8 |

**Discussion:-** Present study, it was found that extraction of Cassia farmasiana in various polar and nonpolar solvents resulted in high amount of extractives in polar solvent than those obtained in organic solvents individually. While testing the efficacy of Petroleum Ether extract of seeds of Cassia farmasiana against C. gloeosporioides (table 4) it was observed that irrespective of treatments, the 3.0% extract solution significantly inhibited the growth of test fungi (56.0%) than 2.0% (40.2%). The Chloroform extract of the Cassia farmasiana seeds was found to have more antifungal potency (78.9%) than Petroleum Ether (75.4% respective of difference in concentrations. Similar results were found for Ethanol and chloroform extracts of Sapindus mukorossi against clinical isolates of Yeast and Candida albicans and C. non-albicans.[6,7] The interaction of concentration of Petroleum Ether as well as chloroform inhibited more than 50% growth of C. gloeosporioides (57.3% and 68.4&, respectively); and the same was true for the lower concentration of 2.0% of chloroform 86.4% thought all these values were statistically exclusive to each other. Irrespective of the treatments, the higher concentration of the extract of Cassia farmasiana inhibited the growth of F. oxysporum significantly more (40.0) than lower concentration of treatment (22.7%) (table 5) similar to the effect on C. gloeosporioides. However,

plant extract in Petroleum Ether proved significantly better (69.2%) than that of chloroform (50.0%), even at lower concentration. The concentration versus Treatment Interaction (C x T) showed maximum inhibition of the F. oxysporum at higher concentration of the chloroform extract (94.7) However, in case of Petroleum Ether extract, no significant difference in arrest of growth of the fungi was observed between lower concentration of 2.0 and higher concentration of 3.0 percent (72.3 and 71.9%) inhibition respectively).

**Table 4-** Precent inhibition of growth of colletotrichum gloeosporiodes with different extract of seeds of Cassia farmasiana.

| Con. (%) | Treatments | | | | | Mean |
|---|---|---|---|---|---|---|
| | CK | CKA | CKM | PE | CHL | |
| 2.0 | $0.0^{a}$ | $27.9^{bc}$ | $0.0^{de}$ | $56.6^{tg}$ | $39.8^{h}$ | 40.2 |
| 3.0 | $0.0^{a}$ | $27.7^{bc}$ | $0.0^{de}$ | $75.4^{fg}$ | $94.7^{9}$ | 56.0 |
| Mean | 0.0 | 27.8 | 0.0 | 69.2 | 86.4 | |

| Con (c) | Treatments | Interaction (CxT) |
|---|---|---|
| SEM | 0.2 | 0.9 0.2 |
| CD (10%) | 0.4 | 0.2 0.6 |

**Table 5** Percent inhibition of Fusarium oxysporum with different extract of seeds of Cassia farmasiana

| Con. (%) | Treatments | | | | | Mean |
|---|---|---|---|---|---|---|
| | CK | CKA | CKM | PE | CHL | |
| 2.0 | $0.0^{a}$ | $29.5^{bc}$ | $14.2^{de}$ | $72.3^{ef}$ | $42.9^{h}$ | 25.7 |
| 3.0 | $0.0^{a}$ | $29.6^{bc}$ | $14.2^{de}$ | $71.9^{e}$ | $82.8^{e}$ | 39.5 |
| Mean | 29.5 | 14.1 | 70.2 | 74.2 | | |

| | Con (c) | Treatments | Interaction (CxT) |
|---|---|---|---|
| SEM | 0.2 | 0.2 | 0.4 |
| CD (10%) | 0.6 | 0.4 | 0.9 |

The concentrations, irrespective of treatments showed similar trends as in previous case, i.e., higher concentration of 3.0% of the Cassia farmasiana seed-extract strongly inhibited growth of G. lucidum (10.0%) than lower concentration (14.2%) (table 6). The chloroform extract of Cassia farmasiana seed-extract had highest and significantly strong antifungal activity (64.2%) than any other treatment and irrespective of seed-extract concentration while studying the C x T, the maximum antifungal activity was detected only in the chloroform extract (54.6% inhibition at 2.0% concentration and 71.4% inhibition at 3.0% concentration). Mukherjee et a. & Jain et al. [8,9] studied that the chloroform fraction of Cassia farmasiana seed-extract showed strong fungicidal activity against Botrytis cinerea, Erysiphe graminis, Phytophthora infestans. Rhizoctnia solani. The mode of antifungal activity of Cassia farmasiana extracts on the pathogenic fungi remains a valid query on these findings. Phongpaichit et al[10] tested the antifungal activities of crude methanol extracts from leaves of

Cassia farmasiana on the pathogenic fungus Microsporum gypseum and on microscopic observations found that the extract affected conidial germination, and the hyphae and macroconidia were shrunken and collapsed, which might be due to cell fluid leakage.[11]

**Table 6-** Percent inhibition of Ganoderma lucidum with different extract of seed of Cassia farmasiana

| Con. (%) | Treatments | | | | | Mean |
|---|---|---|---|---|---|---|
| | CK | CKA | CKM | PE | CHL | |
| 2.0 | 0.0[a] | 0.0[b] | 14.5[cd] | 0.0[ef] | 54.6[g] | 13.9 |
| 3.0 | 0.0[a] | 0.0[ab] | 14.5[cd] | 14.2[e] | 71.4[e] | 19.4 |
| Mean | 0.0 | 0.0 | 14.6 | 6.9 | 66.7 | |

| | Con (1) | Treatments | Interaction (CxT) |
|---|---|---|---|
| SEM | 0.4 | 0.0 | 0.2 |
| CD (10%) | 0.2 | 0.2 | 0.4 |

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