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Antimicrobial Activities of the Leaves and Fruits of (*Cassia fistula*)

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ABSTRACT

The biological activity of the extracts of the leaves and fruits of C. *fistula* tree, was tested against both fungi (*Aspergillus niger and Penicillium digitatum*), and two bacteria (*Staph. aureus* and *E. coli*). From the results it is clear that the leaf extracts were effective against the radial growth of the two fungi. For *P. digitatum* it was 1.0 mm at 100% concentration compared to 2.2 mm at the control treatment (0.0% concentration) at the 8th day. However, for *A. niger* it was only 0.5 mm at 100% concentration compared to 2.3 mm at the control treatment (0.0% concentration) at the 8th day. The results also showed that the fruit extracts of *C. fistula* were also effective in inhibiting the radial growth of both fungi, although the extracts are more effective against *A. niger* than *P. digitatum* , they gave complete inhibition at all of the concentrations The growth was inhibited even from the second day. The effects of the leaf and fruit extracts of *C. fistula* were also examined on the fresh and dry weights of both fungi (*P. digitatum and A. niger*). The leaf extracts were found very effective at their higher concentration compared to the control treatment. However the leaf extracts are better compared to the fruit extracts. The inhibition zone tests were also included in the present study, to evaluate the effect of the leaf and the fruit extracts of the *C. fistula*

tree on inhibiting the growth of two bacteria (the Gram positive, *Staph. aureus* and the Gram negative, *E. coli*). The results indicated that the extracts were very effective in inhibiting the growth of the bacterium both bacteria. They gave 10.0 mm 9.0 mm inhibiting zones, for *Staph. aureus* and 13.0 mm and 11.0 mm inhibiting zones for *E. coli*, respectively.

Key Words: The golden flower (*Casia fistula*), Antifungal and antibacterial activities.

INTRODUCTION

Traditional medicine is an inherited human knowledge improved through experience to cure human beings and their domestic animals (Fabricant and Fransworth, 2001). Plants have played a significant role in maintaining human health as food material, additives and medicines (FAO, 2018). Extracts of different plant parts have been reported to exhibit a variety of pharmacological activities such as antioxidant, anti-inflammatory, anti-tumor, anti-diabetic, antimicrobial, anti-ulcer and wound healing effects (Afolayan and Meyer, 1997; Batista e*t al.*, 1994). Many modern drugs had got their origin from plant extracts (Craig, 1999). The Chinese book on roots and grasses treat 365 drugs (dried parts of medicinal plants), many of which are used even nowadays. The medicinal value of plants lies in some definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds, as well as Glycosides and Trepenes (Edeoga *et al*., 2005). However, about 80% of the world population use medicinal plants as an alternative therapeutic prescriptive.

The present study was aiming to investigate the phytochemical, the antioxidant and the antimicrobial activities of the leaf and the fruit extracts of *Cassia. fistula*. The tree *C. fistula* is a

flowering plant in the family Fabaceae. The species is native to the Indian and adjacent regions (Tucakov,1971). It is found in east Africa and Sudan as an introduced tree. According to Heuzé *et al*. (2018) the tree is medium in size, growing up to 10–20 m in length. The deciduous leaves are 15–60 cm long, and pinnate with three to eight pairs of leaflets, each leaflet is 7–21 cm long and 4–9 cm broad. The fruit is a legume, 30–60 cm long and 1.5–2.5 cm in diameter, with a strong odour and containing several seeds (Thiollet *et al*., 2018). The tree *C. fistula* is in use in herbal, folk and traditional medicine in India. Its phytochemical properties make it a potential candidate in the health management (Murali, 1993). Pole and Sebastian (2012) studied the antifungal activity of methanol extracts of the leaves of *Cassia alata* L., *Cassia fistula* L. and *Cassia tora* L. against *Microsporum gypseum*, *Trichophyton rubrum* and *Penicillium marneffei* fungi. They found that *C. alata* was the most effective leaf extract. In-vivo antimalaria activity was also detected in C. *fistula* extracts.

In Sudan, *C. fistula* is not widely used in traditional medicine. Unpublished information indicated that in some areas of the Ingasana Mountains the fruit is crushed and used as a breast purgative by lactating mothers to facilitate milk production from their mammary glands. The fruit is soaked in water and the aqueous extract is taken orally to cure stomach troubles by some tribe in the Nubba Mountains. However, some residents in Khartoum use the extracts to wash the females' genitalia to accelerate wound healing after birth.

MATERIALS and METHODS

Materials:

Leaves and fruits of the tree (*Cassia fistula*) used in this study, were obtained from Kordofan State. The isolates of both *Aspergillus niger and Penicillium digitatum* fungi were obtained from the food Microbiology Lab, and were tested for their reaction to the extracts of the above tree, using both Potato dextrose Broth (PDB) and Potato dextrose agar (PDA) media. The Bacteria; *E*. co*li* and *Staph aureus*, were obtained from the Faculty of Medical Lab, Univ. of Gezira and grown on Nutrient Agar and Nutrient Broth media.

Prepration of plant part extracts:

The leaves and fruits were washed in tap water, dried for 10 days and blended into a powder using a mortar and pestle. For the preparation of the extracts of each of the dried leaves and fruits 50g were added to 500ml distilled water, left overnight and then filtered through a sterile paper in a Buchner fennel for half an hour before being passed thoubh a membrane filter (0.22mm), and kept in dark bottles before being used. Five concentrations (0.0, 25.0, 50.0, 75.0 and 100.0 mg/ml) were made by serial dilution with the medium. All solutions were sterilized by autoclaving at 121□ C (15/b/in2) for 15 minutes before being cooled to room temperature.

Effect of the extracts on growth of the tested fungi:

Effect on radial growth:

The PDA medium was for this test. The media containing the different concentrations of the each extract were then sterilized and poured in Petri- dish and left to solidify at room temperature (28- 30 ^{0}C). Each solidified Petri-dish was inoculated by a fungal growth disc cut by a sterile cork borer (5.0 mm diameter) from the edge of an actively growing culture of *A. niger* and *P.digtatum* grown on PDA. The inoculated Petri-dishes were then incubated at room temperature for 8 days. All treatments were done in triplicates. The diameter of

growth was measured, every 48 hours. The radial growth was then calculated as a percentage from the diameter of the dish.

Effects of the extracts on fungal mycelial weight

The method used was as described by Abdel-Rahim *et al.* (2002). The Potato Dextrose Broth (PDB) medium prepared was dispensed in 100 ml volume in conical flasks (250 ml). The extract solutions of the leaves and fruits were then added separately to each flask, sterilized in an autoclave at 121^0C (15-Ib/in^2) for 15 minutes, and then allowed to cool at room temperature. Each flask was inoculated by three discs (5.0 mm diameter), taken from an edge of an actively growing culture on a solidified PDA medium. Inoculated flasks were incubated at room temperature (28–30^0C) for 8 days. After incubation mycelia were collected by filtering the culture through a Whatman No. 1 filter paper and the fresh weight was recorded. The mycelia mats were then dried at 80^0C for 24 hours and reweighed to take the dry weight.

The Inhibition Zone Method (Cup Plate)

This method was used for measuring the inhibition zone of the growth of the two tested bacteria (*E. coli* and *Staphylococcus* sp.), using the Nutrient Agar (NA) medium. In this method a standardized cell suspensions of each bacterium were prepared and then added to the solidified medium into sterilized Petri dishes and spreaded using sterile L-shape glass rod. Sterile Whatman glass fiber disks (No.5) were saturated with each extract, then allowed to dry and transferred centrally on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 72 hours and the inhibition zones were measured as described by Barry *et al*. (1970) and Cruickshank *et al*. (1975). The test of the antibiotic compounds was made following the same method. Three replicates were made for each treatment.

EXPERIMENTAL RESULTS

The results of the biological activity of the extracts of the leaves and fruits of *C. fistula* tree, against the two fungi (*Penicillium digitatum* and *Aspergillus niger*) are recorded. The antifungal activity was made by testing the mycelia growth (radial growth fresh and dry weights of mycelia). The antimicrobial activity of the extracts on the two bacteria (*Staph aureus and* E. *coli)* was also measured. Tables (1 and 2) showed the results of the effect of different concentrations (0% - 100%) of *C. fistula* leaf extracts on mycelial radial growth of (*P. digitatum* and *A. niger,* respectively). From the results in Table (1), it was found that the leaf extracts were effective against *P. digitatum*, the radial growth was decreasing with increasing the concentrations of the leaf extract. The radial growth diameter was only 1.0 mm at 100% concentration compared to 2.2 mm at the control treatment (0.0% concentration) at the 8^{th} day. However, all concentrations of the leaf extracts were effective in inhibiting growth of the fungus compared to the control. Table (2) on the other hand is showing the effect of the leaf extracts against *A. niger*. The exracrs gave only 0.5 mm at 100% concentration compared to 2.3 mm at the control treatment (0.0% concentration) at the 8^{th} day.

Table (1): Effect of different concentration of *cassia fistula* Leaves on radial growth of *P. digitatum*

Leaves Concentration of Extract	**Incubation period (days)**			
	2	**4**	**6**	**8**
0.0	1.5	2.0	2.1	2.2
25.0	1.8	1.7	1.7	1.8
50.0	1.5	1.8	1.9	1.9
75.0	1.0	1.2	1.2	1.3
100.0	1.0	1.0	1.0	1.0

Note: Values in the table are the mean values of three replicates

Table (2): Effect of different concentration of *cassia fistula* leaves on radial growth of the fungus *A.niger* (cm)

Leaves Concentration of Extract	**Incubation period (days)**			
	2	**4**	**6**	**8**
0.0 %	1.5	2.0	2.2	2.3
25.0%	1.0	1.7	1.4	1.6
50.0%	1.0	1.5	1.3	1.3
75.0%	0.9	1.3	1.0	1.0
100.0%	0.3	0.4	0.4	0.5

Note: Values in the table are the mean values of three replicates

The effect the extracts of *C. fistula* fruits on radial growth of *P. digitatum* and *A. niger* are shown in Tables (3 and 4), respectively. The extracts were found effective in inhibiting growth of *P. digitatum* compared to the control, especially at the higher concentration (100%). The extracts of the fruits of *C. fistula* were found more effective against A. niger than *P. digitatum* (Table (4)), they gave complete inhibition at all of the concentrations compared to 2.0 mm at the control treatment at the 8th day. The growth was inhibited even from the second day.

Table (3): Effect of different concentration of *cassia fistula* fruits on radial growth of *P. digitatum*

Fruits Concentration of Extract (100 mg/ml)	**Incubation period (days) Radial growth (cm)**			
	2	**4**	**6**	**8**
0.0 %	2.0	2.0	2.3	2.7
25.0%	1.8	1.7	1.8	1.8
50.0%	1.6	1.5	1.8	2.0
75.0%	1.4	1.5	1.5	2.0
100.0%	0.0	0.3	1.0	1.0

Note: Values in the table are the mean values of three replicates

Table (4): Effect of different concentration of *cassia fistula* fruits on radial grwoth of *A.niger*

Fruits Concentration of Extract (100 mg/ml)	**Incubation period (days) Radial growth (cm)**			
	2	**4**	**6**	**8**
0.0	1.5	1.7	1.8	2.0
25.0	1.4	1.5	1.5	1.5
50.0	1.2	1.4	1.5	1.5
75.0	1.0	1.3	1.4	1.5
100.0	0.0	0.0	0.0	0.0

Note: Values in the table are the mean values of three replicates

The effects of the leaf and fruit extracts of *C. fistula* on the fresh and dry weights of both fungi (*P. digitatum and A. niger*), were also tested in the present study. Table (5) is comparing the effects of leaf and fruits extracts of the *cassia fistula* on *P. digitatum* mycelial fresh weight at th 8^{th} day. The leaf extracts gave 0.44 mm at the higher concentration (100 %) compared to 1.0 at the control treatment (0.0 %). However, the fruit extracts were giving 1.20 mm at the higher concentration compared to 4.82 mm at the control treatment (Table (5)). The effects on the dry weights of the same fungus are shown on Table (6). While the leaf extracts were giving 0.20mm and 0.36 mm, the fruit extracts were giving 0.29 mm and 0.42 mm at the higher concentartion and the control treatment, respectively at the 8^{th} day.

Table (5): Comparison between the effect of leaf and fruits extracts of the *cassia fistula* on *P. digitatum* mycelial fresh weight in 8 days.

Concentration 100mg/ml	Leaf	Fruits
0.0	1.00	4.82
25.0	0.84	3.99
50.0	0.59	1.46
75.0	0.45	1.20
100.0	0.44	1.17

Note: Values in the table are the mean values of three replicates

Table (6): Comparison between the effect of leaf and fruits extracts of the *cassia fistula* on *P.digitatum* mycelial dry weight in 8 days .

Concentration 100mg/ml	Leaf	Fruits
0.0	0.36	0.42
25.0	0.24	0.42
50.0	0.23	0.39
75.0	021	0.27
100.0	0.20	0.29

Note: Values in the table are the mean values of three replicates

Data on Table (7) is comparing the effects of the extracts on the fresh weight of mycelia of the fungus *A. niger*. The leaf extracts gave 2.89 mm at the higher concentration (100 %) compared to 5.99 at

the control treatment (0.0 %). However, the fruit extracts were giving 4.32 mm at the higher concentration compared to 6.23 mm at the control treatment (Table (7)). The effects on the dry weights of the same fungus are shown on Table (8). The leaf extracts were giving 0.09 mm and 0.43 mm, while, the fruit extracts were giving 0.04 mm and 0.46 mm at the higher concentration and the control treatment, respectively at the 8^{th} day.

Table (7): Comparison between the effect of leaf and fruits extracts of the *cassia fistula* on *A. niger* mycelial fresh weight in 8 days

Concentration 100mg/ml	**Leaf**	**Fruits**
0.0	5.99	6.23
25.0	4.32	6.05
50.0	4.00	5.81
75.0	3.90	5.49
100.0	2.89	4.32

Note: Values in the table are the mean values of three replicates

Table (8): Comparison between the effect of leaf and fruits extracts of the *Cassia fistula* on *A. niger* dry weight in 8 days

Concentration 100mg/ml	Leaf	Fruits
0.0	0.43	0.46
25.0	0.40	0.39
50.0	0.35	0.34
75.0	0.12	0.20
100.0	0.09	0.04

Note: Values in the table are the mean values of three replicates

The effect of the leaf and the fruit extracts on inhibiting the growth of the two bacteria (*Stagh. aureus* and *E. coli*) are shown in tables (9 and 10). Table (9) showed the effect of on the inhibition zone of the bacterium *Stagh. aureus*. From the results it is clear that the extracts of both the leaf and the fruit of *C. fistula* were very effective in inhibiting the growth of that bacterium. They gave 10.0 mm 9.0 mm inhibition zones, respectively. The effects on the ihbiting zones of the bacterium *E. coli*, are shown on Table (10). The leaf and the fruit extracts were found highly effective. They gave 13.0 mm and 11.0 mm inhibition zone, respectively.

Table (9): Effect of different concentration of the aqueous *cassia fistula* extracts on inhibition zone of *Staph aureus* (mm)

Concentration %	**Leaf**	**Fruits**
0.0	0	0
25.0	5	6
50.0	7	8
75.0	9	9
100.0	10	9

Note: Values in the table are the mean values of three replicates

Table (10): Effect of different concentration of the aqueous *cassia fistula* extracts on inhibition zone of *E.coli* (mm).

Concentration %	Leaf	Fruits
0.0	0	0
25.0	7	9
50.0	9	10
75.0	11	11
100.0	13	11

Note: Values in the table are the mean values of three replicates

DISCUSSION

The presence of the antimicrobial substances in plants has been done by many researchers in the Sudan (Ahmed 2004; Abdel Daim, 2001; Sulieman *et al*., 2008; Abdel. Rahim and Idris, 2010). The biological activities of the extracts of the leaf and fruit of *C. fistula* tree were tested against two fungi (*P. digitatum,* and *A. niger*). The study was also investigated the effects of the extracts against two bacteria *(Staph*. a*ureus and E. coli*). The results showed that the extracts were effective against the radial growth of both fungi. However, antifungal activities against many fungi were also reported (Bullerman, 1974; Abdel.Rahim *et al*., 1989 and Al-jali *et al*., 1997). The extracts of both parts of the tree *C. fistula* were also found effective in reducing mycelial weights of *P digitatum,* and *A. niger*. However, the higher concentrations (75, and 100%) were always more effective. Similar findings were also found by Abdel-Rahim *et al*. (2012) and Osman *et al*. (2015) who were investigating the antifungal activity of Garad plant part extracts. Chuang *et al*., (2007) in Taiwan, found that the Moringa extracts have antifungal activities *in-virtro* against dermatophytes such as *Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporium conis*. Nweke (2015) also reported that extracts of leaves and stems of some plants were effective against *Penicillium oxalicum* and *A. niger*. However, Vinoth *et al*. (2012) has reported that the increase in the incidence of fungal infections and the frequent of resistance and therapeutic failure were found with herbal screening for compounds with antifungal properties.

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