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Antifungal and Antibacterial Activities of an Alcoholic Extract of *Senna alata* Leaves

OWOYALE, J A; OLATUNJI, G A; *OGUNTOYE, S O

Department of Chemistry, University of Ilorin, P.M.B 1515, Ilorin, Kwara State, Nigeria. e-mail: stevorol@yahoo.com

ABSTRACT: Methanolic, ethanolic and petroleum ether extracts of *Senna alata* leaves were screened for phytochemicals, antibacterial and antifungal activities. Out of the three crude extracts, the methanolic extract showed the highest activity than the ethanolic and petroleum ether extracts. The unidentified active components purified from preparative thin layer chromatography exhibited low activities against *Mucor, Rhizopus* and *Aspergillus* niger at 70µg/ml while higher activity was exhibited against all the test organisms at 860µg/ml. @JASEM

The use of herbal medicine predates the introduction of antibiotics and predates social, economic and religious barriers (Akinyemi et al., 2000). *Senna alata Linn* (fabaceae) is an ornamental shrub, which grows well in forest areas of West Africa. It is locally used in Nigeria in the treatment of several infections, which include ringworm, parasitic skin diseases (Dalziel, 1956; Palanichamy et al., 1990). The leaves are reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains, oedema and is also used as a purgative (Ogunti et al., 1993). A study in Malaysia (Ibrahim et al., 1995) reported that ethanolic extract of the *Senna* plant showed high activity against dermophytic fungi: Trichophyton mentagrophytes var interdigitale, T. Mentagrophytes var. mentographytes, T. rubrum and Microsporium gypseum (MIC: 125mg/ml) and Microsporium canis (MIC: 25mg/ml). Several studies (Akinsinde et al., 1995; Akinyemi et al., 2000) have been conducted to provide scientific basis for the efficacy of plants used in herbal medicine. It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates. In this study the methanolic, ethanolic and petroleum ether extracts of the leaves of *Senna alata* were investigated for antibacterial and antifungal activity. The phytochemical components were also investigated as a scientific assessment of the claim of therapeutic potency.

MATERIAL AND METHODS

Leaves of *Senna alata* Linn were collected at various locations within Kwara State. They were identified and authenticated at the Herbarium of the Department of Biological Sciences, University of Ilorin, Nigeria.

Test Microorganisms: Five bacterial and five fungal species were employed as test organism. These include:, *Escherichia coli, Bacillus subtilis, Salmonella typhi, Pseudomonas* aeruginosa, *Staphylococcus aureus, Mucor, Rhizopus, Aspergillus niger, Candida albicans* and *Saccharomyces*, which were obtained as fresh pure cultures from the culture collection room of Department of Biological Sciences, University of Ilorin.

Preparation of Extracts: About 15g each of dried powdered leaves of *Senna alata* were separately extracted with three different solvents, namely, petroleum ether, methanol (100%) and ethanol (100%). The crude extract yields were (2.49g) 16.6% (3.99g) 26.6% and (4.05g) 27% respectively. The concentrated crude extracts were tested for antibacterial and antifungal activities. Also, 500g of *Senna alata* leaves dried at ambient temperature (28^0C) were powdered and defatted with petroleum ether. It was then dried and extracted with 100% methanol for five days at 28%. The extract was filtered, and the greenish brown filtrate obtained was concentrated. The concentrated methanolic extract was subjected to thin layer chromatography using 0.25mm thick silica gel/UV_{254} pre-coated plate. After ascertaining the major spots on the chromatographic plate, a column chromatography (cc) (silical gel mesh 60 grade) was run using 2g of the extract. Seven fractions were obtained from the column chromatography they were concentrated and purified using preparative thin layer chromatography (PTLC). Benzene-acetone (3:1) was used as the solvent system. The unidentified fractions were thus separated and tested for antibacterial and antifungal activities.

*Corresponding author: e-mail: stevorol@yahoo.com

Phytochemical Tests: These tests were conducted on the most active fraction. The fraction gave a positive result to shibata's reaction, ferric chloride test, mineral acid test, boric acid reagent and Fehling's solutions A and B test (Harborne, 1965).

Antibacterial Assay: Antibacterial activity of the extracts and fractions were tested using the agar diffusion method described by Collin et al., (1970) Varying concentrations of the extracts and fractions were prepared and tested using Muller Hinton agar. The plates were incubated at 37^0C for 24 hours and the zones of inhibition measured. The Minimum Inhibitory Concentrations (MICs) for the most active component were recorded after 24 hours.

Antifungal Assay: Antifungal activity of the extracts and fractions were tested using the agar dilution method described by Collin et al., (1970). Varying concentrations of the extracts and fractions were prepared and incorporated into Potato dextrose agar. The plates were incubated at 25^0C for 48 hours and inhibition of growth was noted. The Minimum Inhibitory Concentrations (MICs) for the most active component were recorded after 48 hours.

RESULTS

The phytochemical screening revealed that most active chromatographic component is a flavonoid glycoside. The activity results obtained are presented in Tables 1 and 2. *Senna alata* leaves crude extract showed both antibacterial and antifungal activities. The methanolic extract was the most active of the three crude extracts tested against bacterial and fungal organisms. This is as shown in table 1.

Table 1: Antimicrobial Activities of the Crude Petroleum ether, Ethanolic and Methanolic Leave Extracts of *Senna alata*

	Diameter of Zone of inhibition (mm)			
Test Organisms	Petroleum ether extract	Ethanolic extract	Methanolic extract	Control (Solvents)
Mucor sp.	2.0	2.0	2.2	Nil
Rhizopus sp.	2.2	1.8	2.4	"
Aspergillus Niger	3.2	3.0	4.0	"
Candida albicans	2.1	2.0	1.0	"
Saccharomyces	1.6	1.4	1.1	"
Escherichia coli	0.0	10.0	8.0	"
Bacillus subtilis	2.0	2.0	6.0	"
Salmonella typhi	5.0	2.2	7.0	"
Pseudomonas aeruginosa	0.1	3.0	17.0	"
Staphylococcus aureus	0.1	5.0	12.0	"

Concentration used 0.125g/ml, Nil: No zone of inhibition. Each value is a mean of two replicates.

However, methanolic crude extract has a narrow spectrum of activity not being able to inhibit successfully *Candida albicans* and *Saccharomyces.* This is as shown in table 2.

Table 2: Antifungal and Antibacterial Activities of the Unidentified Components Purified from Preparative Thin Layer Chromatography (PTLC) on Media Plates.

Test Organisms	Chromatographic fractions							
	I	II	III	IV	V	VI	VII	Control
Mucor sp.	+ve	+ve	+ve	+ve	+ve	-ve*	+ve	+ve
Rhizopus sp.	+ve	+ve	+ve	+ve	+ve	-ve*	+ve	+ve
Aspergillus niger	+ve	+ve	+ve	+ve	+ve	-ve*	+ve	+ve
Candida albicans	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Saccharomyces	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Escherichia coli	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Bacillus subtilis	+ve	+ve	+ve	+ve	-ve*	-ve*	+ve	+ve
Salmonella typhi	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Pseudomonas aeruginosa	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Staphylococcus aureus	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve

+ve = Growth observed -ve = Growth inhibited

-ve* = Highly sensitive growth inhibited Control = 100% methanol

It appeared that the most active chromatographic component is only slightly effective both in degree of inhibition and spectrum of activity on all the test organisms to the methanolic crude extract. The Minimum Inhibitory Concentration (MICs) of the active component on the moulds and yeasts are

70µg/ml and 860µg/ml respectively, while the MIC as shown in Table 3.

Table 3: Minimum Inhibitory Concentration (MIC) of the Active Component.

Test Organisms	Minimum Inhibitory Concentration (MIC, µg/ml)
Mucor sp.	70
Rhizopus sp.	70
Aspergillus niger	70
Candida albicans	860
Saccharomyces	760
Escherichia coli	860
Bacillus subtilis	860
Salmonella typhi	860
Pseudomonas aeruginosa	860
Staphylococcus aureus	860
Control (100% methanol)	Nil

DISCUSSIONS

The results presented in Tables 1 and 2 show that crude methanolic extract was found to inhibit the growth of all the organisms tested except *Candida albicans* and *Saccharomyces*. Out of the seven separated chromatographic components from the crude methanolic extract, the component eluted with Benzene-acetone, 2:1 (30mg, 1.5% yield) proved to be the most active and was showed by chemical test to be a flavonoid glycoside (Harborne, 1965). Similarly, *Cassia alata* has been reported to contain anthraquinone, the principal laxative constituent of many plants used as purgatives (Ogunti et al., 1993). Thus, from literature search, there is no evidence that flavonoid glycoside, a main constituent of the leaf extract of *S. alata* is responsible for antifungal activity. Meanwhile, the methanolic extracts of leaves, flowers, stem and root of *Cassia alata* had been shown to have a broad spectrum of antibacterial activity after fractionating with petroleum spirit, dichloromethane and ethyl acetate. The dichloromethane fraction of the flower extract was found to be the most effective (Khan et al., 2001). In a recent review, the methanolic fraction of the leave has been shown to be active against Trichophyton mentagrophytes at a concentration of 50mg/ml but has no activity against moulds and *Candida albicans* (Villasenor et al., 2002). Much earlier, the antifungal activity of *Cassia alata* leaf extract has been reported (Palanichamy et al., 1990).

Nevertheless, from the biological activity results obtained in this study, the most active component exhibited low MIC against the mould at 70µg/ml while higher activity was recorded against all the test organisms at 860µg/ml. The Minimum Inhibitory of the active component on the bacteria is 860µg/ml

Concentration (MIC) values of the active component in our study were lower than the MIC reported in the literature. Since the higher activity value for the most active component was less than 1mg/ml, it is therefore regarded as a potential candidate for further studies. We believe that the result of this study is an encouragement for further work that will lead to the elucidation of the structure of the active component.

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