



EVALUATION OF QUALITATIVE PHYTOCHEMICALS AND ANTIFUNGAL ACTIVITY OF AQUEOUS LEAF EXTRACT OF *Senna tora* ON DOWNY MILDEW OF CABBAGE

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ABSTRACT

This study was embarked upon to evaluate the efficacy of aqueous extracts of Senna tora leaf against Peronospora parasitica, causing downy mildew on cabbage. Phytochemical analysis of the leaf extracts revealed the presence of some secondary metabolites (Tannins, Saponins, Flavonoid, Glycosides, Alkaloids, Phenolic compounds and Carbohydrates) which have been reported to have antimicrobial effects. Assays were performed using extract concentrations of 1000, 500, 250, 125 mg/ml by agar well diffusion technique. Results obtained, revealed a significant difference in diameter of mycelial zone of inhibition between the concentrations. Highest zone of inhibition was recorded at 1000 and 500 mg/ml (20 and 15 mm) respectively. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined and recorded at 250 mg/ml. The antifungal screening revealed that the extract exhibited inhibitory activities at varying concentrations against the downy mildew pathogen. These activities observed could be attributed to the presence of active metabolites contained in the extract.

Keywords: Aqueous leaf extract, Downy mildew, *Peronospora parasitica* and *Senna tora*,

INTRODUCTION

The development of resistance to current antibiotics by disease causing microbes has reinforced research for discovery of new ones (Kunle and Eghareva, 2009). Continuous and overuse of synthetic antibiotics often produce tolerance in human and create resistance in microbes for a particular antibiotic. Current trends in drug development process are focused on natural sources, especially source of plant origin due to some proven correlation between the folk medicinal uses of some of these plants to biological activity. Therefore, the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientists worldwide (Kunle and Egharevba, 2009).

Medicinal plants are gifts of nature to cure limitless number of diseases. The abundance of plants on the earth surface has led to an increasing interest in the investigation of different extracts obtained from the traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar and Farrokhi, 2004). In recent years, there has been a gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries in utilization to develop antimicrobial drugs, because plant- drive drugs have been reported to be safe and without side effects (Rai *et al.*, 2003).

Senna tora L. (leguminosae) is widely distributed in tropical and Asian countries. The seeds are reputed in Chinese medicine as vision improving, antiasthenic, aperient, and diuretic agents. *Senna tora* have shown to possess various biological and pharmacological activities including antiepatotoxic, antiallergic, antimutagenic, antifungal, radical scavenging, and antimicrobial (Maity *et al.*, 1998).

The leaves of *Senna tora* are used as laxative in the form of decoction. Both leaves and seeds constitute a valuable remedy in skin diseases, chiefly for ringworms and itch. In china the seeds are used externally for all sorts of eye diseases. In indo-china, the pods are used in dysentery and diseases of eye. In Nigeria, the leaves are used as mild laxative. The weed is used in various gold coast medicines, chiefly as purgative. In Madagascar and La reunion, the root is considered bitter, tonic, stomachic. The leaves are used as an anti-periodic, asperients, anthelmintic they are given to children with intestinal wounds. The plant and seeds are edible. The edible part of the plant varies from 30-40 percent. Young leaves can be cooked as vegetables while the roasted seeds are a good substitute for coffee. It is used as a natural pesticide in organic farms and its powder is most commonly used in the pet food industry. Alternatively, its mixed with guar gum for use in mining and other industrial applications (Benzie and Strain, 1996).

The aim of this study was to study the qualitative phytochemical contents and antifungal activity of aqueous leaf extract of *Senna tora* on downy mildew of cabbage

MATERIALS AND METHODS

Collection and Identification of Plant Material

The leaves of *Senna tora* were collected randomly from Bayero University Kano old campus. The plant was identified and authenticated in the herbarium unit of Department of Plant Biology BUK with accession number BUKHAN 0307.

Preparation of the Leaf Extracts

The leaves were air dried at room temperature and grounded into powder using wooden pestle and mortar. The powdered plant material was stored in an air tight container prior to extraction.

Two hundred grams of the powdered leaf material was soaked in distilled water (200 g/1.5 L) and the extract decanted at an interval of 24 hours. The filtrate was then evaporated to dryness in a water bath at 40°C giving a dark green solid with a yield of 13.2 g (Palanichmy and Nagarajan, 1990).

Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the leaf extract of *Senna tora* was carried out in extracts using the standard procedures as described by Harbone (1973), Trease and Evans (1989), and Sofowara (1993).

Test for Tannins

Few drops of 0.1 % of FeCl₃ were added to 0.01g of the aqueous extract and boiled in 20 ml of water in a boiling tube. Formation of brownish green or a blue black colouration indicated the presence of tannins.

Test for Saponins

The aqueous extract (0.01 g) was boiled in 20 ml of distilled water in a water bath. Then it was mixed with 5 ml of distilled water and it was shaken well. Stable persistent froth indicated the presence of saponins.

Test for Flavonoid

A few drops of 1% NH₃ solution was added to the aqueous extract of the plant sample in a test tube. A yellow coloration is observed if the flavonoid compounds are present.

Test for Glycosides

The aqueous extract (0.01g) was dissolved in 2 ml of ethanol and then 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added with 1 ml of concentrated H₂SO₄. Appearance of brown ring indicated the presence of the glycosides.

Test for Alkaloids

Crude extract (0.01 g) was dissolved in ethanol and was divided into two parts. Few drops of Mayer's reagent were added to one part. A creamy white precipitate indicated the presence of alkaloids. Few drops of Wagner's reagent was added to other part. A red-brown colour precipitate indicated the presence of alkaloids.

Test for Phenolic Compound

The extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicate the presence of phenolic compound.

Test for Carbohydrates

Extract was individually dissolved in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Benedict's test: The filtrate was treated with benedict's reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars.

Collection of the Diseased Plant Samples (Cabbage)

Samples of cabbage naturally infected with downy mildew were collected from Tarauni market (Tarauni L.G.A) and Na'ibawa market (Kumbotso L.G.A) in Kano state (Singh *et al.*, 2002).

Sterilization of Apparatus and Preparation of Culture Media

All the apparatus involved in this experiment were sterilized using an autoclave machine, all the solvent also were sterilized using an oven machine.

Culture media was prepared according to the manufacturers instruction and autoclave at 121°C for 15minutes. The media was amended with 0.5mg of streptomycin as antibiotic (Singh *et al.*, 2002).

Isolation and Identification of Fungal Isolates

The isolation and identification technique used by Chiejni, (2008) was employed. Thin section 2mm diameter) was cut from the periphery of deteriorating / diseased plant and sterilize with 1% sodium hypo chloride solution for 2minutes. The section is rinsed in 3 changes of sterile distilled water, and plated on Potato Dextrose Agar (P.D.A) plates .The plates were then incubated at room temperature (27°C ± 2°C) for seven days. The pure cultures were then obtained by several transfer of colony growth from P.D.A plates aseptically (i.e. sub-culturing to obtain axenic cultures). A small portion of fungal growth were picked carefully with aid of a pair of sterile dissecting needle and placed on a drop of lacto phenol in cotton blue on a slide covered with a cover slip. The slide under the microscope were examined first with (×10) and then with (×40) objective lens to observe the spores and some structures of the fungi .The fungal isolates were identified based on microscopic appearance which comprised pigmentation, colour of aerial and substrate hyphae and some kind of sexual spores.

Inoculum Preparation

The isolates were sub-cultured on Potato Dextrose Agar plates and incubated at 27°C ± 2°C. Inoculum suspension were prepared from fresh, mature (3-5 days old) cultures, in some cases an extended incubation was required for proper sporulation of the isolates, the fresh colonies were covered with 10ml of distilled sterile water. The conidia were rubbed carefully with a sterile cotton swab and transferred to a sterile tube (Petrikkou *et al.*, 2001)

Preparation of Extract Concentrations for Sensitivity Test

Extract concentration of the plant material was prepared using serial dilution method as described by Aliyu and Sani (2011). Two gram (2g) of the plant extract (*Senna tora*) was dissolved in 2ml of appropriate diluent (DMSO) to arrive at 1.0g/ml, which serves as stock solution from which appropriate quantity of *Senna tora* leaf extract were dissolved in 1ml of Dimethyl Sulphur Oxide (DMSO) to arrive at four different concentrations: 1000, 500, 250 and 125 mg/ml respectively.

Antifungal Activity/Sensitivity Test

The extract was screened for antifungal property using the agar well diffusion technique. Potato Dextrose Agar (PDA) was used for fungal cultures. Inoculum of test fungus was spread on to sterile Potato dextrose agar plate so as to achieve a confluent growth. The plates were allowed to dry and 6mm diameter wells were punched into the agar using a sterile cork borer and filled with plant extracts of different concentration. A standard antibiotic (Mancozeb, concentration 1g/ml) was used as standard and Dimethyl Sulphoxide (DMSO) as negative control. The fungal plates were incubated for 72hours at 37°C.

The degree of sensitivity was determined by observing and measuring the diameter in millimeter (mm) of the visible zone of inhibition of the microbial growth produced by the diffusion of the extracts (Stockes and Ridgeway, 1980).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration for antifungal activity of *Senna tora* leaf extract by broth dilution technique. Sabourauds Dextrose Broth was prepared and poured 10ml each in the set of six test tube. The tubes were then sterilized by autoclave. The different volumes of this extract was delivered accurately to each test tube using sterile pipette and thoroughly mixed to obtain the concentrations as (500mg/ml, 250mg/ml, 125mg/ml, 62.50 mg/ml).

The tubes were then inoculated with 0.05ml of the standardised inoculum / fungal culture. The inoculated tubes were incubated at temperature 30°C - 32°C and observed for growth by monitoring the turbidity produced. The test procedure was repeated to check the reproducibility of the results. The lowest concentration that inhibits the growth is minimum inhibitory concentration (MIC).

The minimum fungicidal concentration (MFC) was determined by sub-culturing from each well showing no apparent growth. Least concentration of extract showing no visible growth on sub-culturing was taken as MFC (Basri and Fan,2005).

RESULTS

The result showed that the extracts yielded 13.2g. The aqueous leaf extract was found to be dark green

in colour. The texture of the extract was gummy with pungent smell and is soluble in DMSO as shown in Table 1.

Results of phytochemical screening of aqueous leaf extracts indicate the presence of some secondary metabolites such as alkaloid, tannins, flavonoids, glycosides, saponins, carbohydrates, with absence of phenolic compounds as shown in Table 2.

Perenospora parasitica, *Rhizopus* spp, and *Aspergillus niger* were isolated from diseased cabbage. *Perenospora parasitica* is the pathogen causing downy mildew of cabbage and it was found to be predominant pathogen causing downy mildew (Table 3).

Generally, the zone of inhibition increased with increase in concentration of extract as shown in Table 4. Bioassay study showed that the extract of *Sennator* was more active at concentration of 1,000,000 on *Perenospora parasitica* which compares favourably with the standard Mancozeb (20mm) each. No activity against *Perenospora parasitica* was observed at 125 and 250 mg/ml. Similarly, the negative control (DMSO) showed no zone of inhibition.

Results of Minimum Inhibitory Concentration (MIC) shows that the aqueous leaf extract of *Senna tora* against *Perenospora parasitica* at 500 and 250 mg/ml showed no visible growth of microorganism in the test tube after incubation while turbidity was observed within the other test tubes containing the remaining concentrates of 125 and 62.50 mg/ml as shown in Table 5.

Result of MFC showed that the minimum inhibitory concentration value is fungistatic

Table 1: Physical Characteristics of Aqueous Leaf Extract of *Senna tora*

Plant part	Leaves
Solvent	Distilled water
Weight	13.2g
Colour	Dark green
Odour	Pungent
Texture	Gummy
Solubility in DMSO	Soluble

Table 2: Phytochemical Constituents of Aqueous Leaf Extract of *Senna tora*

Phytochemical constituents	aqueous extract
Tannins	+
Saponins	+
Carbohydrates	+
Glycosides	+
Alkaloids	+
Phenolic compounds	-
Flavonoids	+

KEYS: Present = +, Absent = -

Table 3: Frequency of occurrence of the isolates

Isolates	Frequency	% occurrence
<i>Perenospora parasitica</i>	7	70
<i>Rhizopus</i> spp	2	20
<i>Aspergillus niger</i>	1	10

Table 4: Anti-Fungal Activities of Aqueous Leaf Extract of *Senna tora* on downy mildew disease of cabbage (*Peronospora parasitica*)

Concentrations (mg/ml)	Zone of Inhibition (mm)
125	0
250	0
500	15
1000	20
Standard (Mancozeb)	20
DMSO	0

Table 5: Minimum Inhibitory Concentration (MIC) of Aqueous Leaf Extracts of *Senna tora*

Concentrations (mg/ml)	Turbidity signs
62.50	+
125	+
250	-
500	-
Positive control	+
Negative control	-

KEYS: Turbidity Present = +, Turbidity Absent = -

DISCUSSION

The result of phytochemical analysis of aqueous leaf extract of *Senna tora* indicated the presence of Tannin, Saponins, Carbohydrate, Glycoside, Alkaloids and Flavonoids. This was in consonance with work of Akindahunsi and Olaleye (2003). Some of the metabolites have been reported to be responsible for antimicrobial activity in some ethnomedicinal plants (Kumar *et al.*, 2009^a).

Reports in other studies by Linn *et al.* (1979) on phytochemical screening revealed the presence of certain plant metabolites, which have been known to elicit inhibitory effect on microorganisms. These phytochemicals may have caused the observed inhibitory effect either singly or in synergy with each other, this requires further investigation. Also, Reyes-Chilpa *et al.* (2009), reported that Flavonoids possess antifungal property. Earlier, Baba-Moussa *et al.* (1999) reported that Tannins present in some plant species possess antifungal property. It is hoped that the elucidation of the structure of the active principle(s) and its/their subsequent use in antifungal investigations would give better results.

Tannins also interfere with cell division hence the presence of tannins in *Senna tora* could account for the antimicrobial activity in the study, this is in agreement with the findings of Chewonarin *et al.* (1999). Tannins have been traditionally used for protection of inflamed surface of the mouth and treatment of catarrh, wound and diarrhea.

It was observed that the fungal isolates used in this research exhibited varying degrees of susceptibility to the extracts. Thus, the values obtained for the zones of inhibition differed, for test organism. These results corroborate the findings of Anani *et al.* (2000) and Rajakaruna *et al.* (2002). Results of this study also agree with the report of Kumar *et al.* (2009^b). The difference in susceptibility observed in this study could be attributed to the inherent resistance factor of the test organisms among other factors (Ekpo and Etim, 2009).

The result showed that the MIC of *Peronospora parasitica* is 250mg/ml the antifungal activities observed could be due to the presence of secondary

metabolites which have been reported as active constituents responsible for antifungal activities (Salihu and Garba, 2008).

CONCLUSION

Observations and data collected in the study indicated that fungal species identified to be associated with the diseased symptoms of Downy mildew of Cabbage (*Brassica oleraceae*) were *Peronospora parasitica*, *Rhizopus* spp and *Aspergillus niger*. The predominant species identified was *Peronospora parasitica* and therefore suspected to be the causal agents of the disease symptoms observed on the cabbage plants in the study areas.

The aqueous leaf extract of *Senna tora* is effective or shown good inhibitory activity against *Peronospora parasitica* as compared with standard Mancozeb which is an indication that the parts of plant contain some major bioactive compounds that inhibited the growth of the microorganism thereby proving cheap and effective source of derived drugs.

Recommendations

Attempts should be made to conduct *in vivo* studies with the extracts (such as toxicity of the plant to humans at different concentration is very essential in the use of the plant for treatment and the efficacies of the different concentrations in the *in vivo* control of Downy mildew of Cabbage) so as to confirm the present *in vitro* findings.

Further experimental and clinical trials need to be carried out on the individual isolates or fraction of the aqueous extract of *Senna tora* leaves. This would also help to correlate the antifungal activity with known constituents of *Senna tora* leaves.

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