

Full Length Research

Antimicrobial efficacy of *Acacia saligna* (Labill.) H.L.Wendl. and *Cordia sinensis* Lam. leaves extracts against some pathogenic microorganisms

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Acacia saligna and *Cordia sinensis* were used traditionally as medicine and food additives in Saudi Arabia. The antimicrobial activities of leaves ethanol extracts of both species were investigated against 7 medically important bacterial strains, namely: *Bacillus subtilis*, MRSA, *Micrococcus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsella pneumoniae*, and four fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida albicans*). The antibacterial activity was determined by agar well diffusion method. The most pronounced effect was shown by that of *A. saligna*. The most susceptible bacteria was *K. pneumoniae*, followed by *Micrococcus*, while the most resistant bacteria was MRSA followed by *B. subtilis*. The most pronounced effect on fungi was shown by that of *Acacia cyanophylla*. The most susceptible fungi was *A. fumigatus*, while the most resistant fungi was *A. niger*. The HPLC analysis indicated the presence of 8 phenolic compounds as major active constituents (gallic, protocatechuic, chlorogenic, syringic, p-Hydroxy Benzoic, p-Coumaric, vanillic and salicylic acid). The result obtained indicated variable differences in compounds concentration of *A. saligna* leaves extract. Results of HPLC showed gallic, p-Coumaric and syringic in high concentrations of 54.31, 8.27 and 3.71 µg/g respectively. The concentrations of other phenolic compounds ranged from 0.44 - 2.01 µg/g. However, the least concentration was chlorogenic with a range of 0.44 µg/g.

Key words: *Acacia cyanophylla*, *Cordia sinensis*, antibacterial, antifungal, phytochemical constituents, crude extract.

INTRODUCTION

Nowadays, there is a need to find naturally occurring substances with antimicrobial activity as an alternative to available antibiotics due to several serious problems such as growing drug resistance of bacteria or undesirable side effects of antibiotics (Ushimaru et al., 2007). Plants have been shown to be a rich source of antimicrobial agents, as they produce a wide variety of secondary compounds as natural protection against microbial attack (Urszula et al., 2010). Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine, and hence the need to look for new molecular structures as lead compounds from the plant kingdom (Sathiya and Muthuchelian 2008; Nair and Kalariya, 2005). *Acacia* is an important plant genera that is commonly used in a

variety of infections. It is widely distributed in Asia, Australia and America and its efficacy has been demonstrated in the treatment of gonorrhoea, leucorrhoea, diarrhoea, dysentery and wounds (Akinsulire et al., 2007). *Acacia* spp (Family-Mimosaceae) is a medium size thorny tree found in the drier parts of India. It has yellow mimosa like flowers and long grey pods constricted between seeds. The leaves are fine and densely hairy with 3-6 pairs of pinnate consisting of 10-20 pairs of leaflets that are narrow with parallel margins and are rounded at the apex with a central mid-rib closely crowded (Mann et al., 2003). The

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bark is used extensively for colds, bronchitis, diarrhea, bleeding piles and leucoderma (Gill, 2009). The genus *Cordia*, one of the most representative in the Boraginaceae family (Arrebola et al., 2004), consists of approximately 320 species, which are presented as trees, shrubs or herbs (Stevens, 2013). Its species are used in folk medicine as diuretics, weight loss agents, healants and emollients (Saito, 1986). Biological studies of *Cordia* spp reported antiviral, cytotoxic, anti-inflammatory (Rapisarda et al., 1992; Ficarra, 1995) spasmolytic and vasorelaxant (Feng et al., 1962) properties. The present study was designed to evaluate the antimicrobial properties of *Acacia saligna* and *Cordia sinensis* and the phytochemical properties of *Acacia saligna* of leaves extracts obtained from Albahah province, Southwest Saudi Arabia.

MATERIALS AND METHODS

Collection and preparation of samples

Samples of *A. saligna* and *C. sinensis* leaves were collected during May, 2014 from Albahah region (19°98' 28°N, 41°52' 50°E), Southwest Saudi Arabia from cool slopes at 2050 m.a.s.l, Saudi Arabia. Species status of this plant was fervid at the Faculty of Sciences Herbarium, King Abdulaziz University, Jeddah. The plant leaves were brought to the laboratory, washed in running tap water to remove debris and dust particles and then rinsed in distilled water for five minutes.

Preparation of *Acacia saligna* and *Cordia sinensis* extracts

Ten grams of dried *A. saligna* and *C. sinensis* leaves were thoroughly washed in running water prior to cutting into small pieces by blender 1-2 mm. Extraction was done by adding 100 ml of distilled water and organic solvent ethanol extract (1:10W/V). Under cold conditions for 48 h, the solvent extract was filtered through a filter paper. The extracts solutions were evaporated under reduced pressure at 40°C until dryness. Subsequently, the extract was diluted by dimethyl sulfoxide (DMSO) and stored in 20°C until analysis according to Boeru and Derevici (1978).

Phytochemical screening

Phenolic extraction and hydrolysis

Phenolic compounds in plant were extracted as described by Mattila et al. (2005) with some modifications. Approximately, 15 ml of 4N NaOH was added to 200 ml of each concentration of water extract in 50 ml Pyrex centrifuge tube purged with nitrogen and shaken for 2 h in the dark with a wrist - action shaker. After phenolic acid was liberated by alkaline hydrolysis, samples were acidified with ice - cold 6 N HCl to reduce

pH to between 1 and 2. Samples were centrifuged at 3000 g and the supernatant was decanted into a 250 ml separator/funnel. The supernatant was extracted with ethyl acetate (3 × 50 ml) with shaking for 10 s and the mixture was allowed to settle for 5 min between extractions. Ethyl acetate fractions were collected and pooled. The remaining pellet was diluted with 15 ml of distilled H₂O, vortex distributed and re-centrifuged at 3000 g. The second supernatant was re-extracted with ethyl acetate (30 × 50 ml) as before and all ethyl acetate fractions were pooled. The phenolic acids-rich ethyl acetate fraction was dried by addition of anhydrous sodium sulfate and concentrated using a rotary vacuum evaporator at 35°C to dryness. The phenolic acids rich residue was re-solubilized in 2.5 ml of MeOH and stored in a dark prior to separation and quantification by HPLC within 24 h of extraction.

HPLC analysis

Phenolic acids were separated by Shimaduz (Kyoto, Japan) HPLC apparatus (model, LC-4A) equipped with visible / UV detector (model, SPD-2AS) at 280 nm and stainless steel column (25.0 cm × 4.6 mm i.d.) (Phenomenex Co., USA) coated with ODS (RP-18). An aliquot of the sample suspended in MeOH was diluted with 10 mM phosphoric acid buffer (PH 3.5) to the same concentration as initial mobile phase (15% MeOH). Samples were next filtered through a 0.2 μ m poly (tetrafluoroethylene) (PTFE) filter prior to injection. The two solvent systems consisted of MeOH (A) and 10 mM phosphoric acid buffer, pH 3.5 (B), operated at a rate of 1.5 ml / min. The phosphoric acid buffer consisted of 10mM NH₄H₂PO₄ adjusted to pH 3.5 with 10mM H₃PO₄.

Bacterial and fungal strains

Cultures were prepared for *in vitro* antibacterial assay of seven bacteria, three Gram negative: *Escherichia coli* (ATCC8739), *Klebsiella pneumoniae* (ATCC700603) and *Pseudomonas aeruginosa* (ATCC27853) and four Gram positive: *Bacillus subtilis* (ATCC11774), Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC977) and *S. aureus* (ATCC29213) *Micrococcus luteus* (ATCC4698). Those strains were provided by Microbiologics® USA. For the antifungal assay, four fungi (*Aspergillus flavus* (ATCC200026), *Aspergillus fumigatus* (ATCC204305), *Aspergillus niger* (ATCC1015) and *Candida albicans* (ATCC10231) were used. The tested organisms were subcultured on nutrient agar medium (Oxoid laboratories, UK) slopes for bacteria and Saboroud dextrose agar slopes (Oxoid laboratories, UK) for fungi, which were the media used. These stock cultures were stored in the dark at 4°C during the survey.

Antimicrobial activity

Antimicrobial activity was determined, using the agar well

diffusion assay method as described by Holder and Boyce (1994). DMSO was used as a negative control and streptomycin and ciprofloxacin (10 mg/disc) were used as a positive control for bacteria amphotericin B and Nystatin were used as a positive control for fungi. The plates were done in triplicate. Bacterial cultures were incubated at 37°C for 24 h, while the other fungal cultures were incubated at 30-32°C for 48 h. Solution of 10 mg/ml of streptomycin, ciprofloxacin, nystatin and amphotericin B were used as standard for comparison. Antimicrobial was determined by measurement zone of inhibition (Agwa et al., 2000).

Statistical analysis

For each experiment, three replicates and three determinations were conducted. Means of variable, standard error and least significant differences were carried out using SPSS, to detect any significant differences between pathogenic microorganisms and extract type.

RESULTS AND DISCUSSION

Antimicrobial activities

Table 1 presents the results of the antimicrobial influence of ethanol extracts on *A. saligna* and *C. sinensis* leaves which was determined by using disc diffusion method against four Gram +ve bacteria (*B. subtilis*, MRSA, *S. aureus* and *M. luteus*), three Gram -ve bacteria (*E. coli*, *K. pneumonia* and *P. aeruginosa*) at concentrations of 200 mg/ml. *A. saligna* leaves extract showed higher activity against tested organisms than the *C. sinensis* leaves. The antimicrobial influence of leaves extracts obtained by using two different plant extracts appeared to be very different in terms of effectiveness since some bacterial species are highly resistant and some others are more susceptible to the extracts. In this study, the most pronounced effect was shown by that of *A. saligna*. The most susceptible bacteria was *K. pneumoniae*, followed by *Micrococcus*, while the most resistant bacteria was MRSA followed by *Bacillus subtilis*. The main diameter of inhibition zones of the extract against these bacterial strains were 29.33 mm, 27.66 mm, 22.66 mm and 23.33 mm, respectively (Table 1). The present results coincided with those of Raghavendra et al. (2006) whom reported that the antibacterial activity of aqueous extract, different solvent extracts and isolated constituents of leaves of *A. nilotica* were evaluated and found to have high antibacterial activity. Extracts of *A. nilotica* were found effective against gram positive cocci and less effective against gram-negative bacilli (Mustafa et al., 1999). The present results go in line with those of Abd el Nabi et al., 1992; Kambizi et al., 2001) whom reported that the methanol extracts of *A. nilotica* showed significant inhibition against Gram-positive and Gram-

negative bacteria including a moderate antimicrobial activity against multi-drug resistant *Salmonel typhi* (Rani and Khullar, 2004). In another study, Korukluoglu et al. (2010) investigated the effect of the extraction solvent on the antimicrobial efficiency of *S. aureus*, *E. coli*, *S. enteritidis*, *S. thypimurium* and some others. They reported that solvent type affected the phenolic distribution and concentration in extracts, and antimicrobial activity against tested bacteria. This study examines antibacterial activity of *A. saligna* and *C. sinensis* leaves extract in comparison to selected antibiotics. Antimicrobial activities of standard antibiotics control positive showed an inhibitory effect against all the tested bacteria. The results showed that ciprofloxacin is more effective than streptomycin. The present results go in line with those of Shital (2010). The mean diameters of inhibition zones against all bacteria tested were mentioned in Table 1. Antimicrobial activities of standard antibiotics control positive showed an inhibitory effect against all the tested bacteria. Nada et al. (2013) reported on the antibacterial activity of *Acacia nilotica* extract in comparison to selected antibiotics against different serotypes of Salmonella including *S. gombe*, *S. potsdam* and *S. bonariensis* and tested for antimicrobial susceptibility pattern against antibiotics and extract of *A. nilotica* fruit. *A. nilotica* showed good antibacterial activity with 24.4 average zone of inhibition, while inhibition zones of gentamicin, neomycin, colistin and tetracycline was 13.8, 14, 11 and 10 mm respectively. In other studies, antimicrobial activity of the stem bark extracts of *A. nilotica* was assayed against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei*. The plant extract exhibited antimicrobial activity against all the tested microorganisms (Banso, 2009). Deshpande and Kadam (2013) reported on the antimicrobial activities of the stem bark extracts of *A. nilotica* against *Streptococcus mutans*. In present studies, the extract obtained with *A. saligna* leaves showed good effectiveness against the tested bacteria as compared to those obtained with *C. sinensis* leaves. The present results coincided with those of Hatil and Ehsan (2009) who reported that the antibacterial activity of ethanol extracts of *Acacia nilotica* sp pods and *Lawsonia inermis* leaves showed high antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa* and *K. pneumonia* compared with ethanol extract of *C. sinensis* stem bark which showed promising result against *K. pneumoniae* and *P. aeruginosa* only.

Table 2 presents the results of the antifungal influence of ethanol extracts of *A. saligna* and *C. sinensis* leaves which were determined by using disc diffusion method against four fungi species (*A. niger*, *A. fumigatus*, *A. flavus* and *C. albicans*) at concentrations of 200 mg/ml. The *A. saligna* leaves extract showed higher activity against tested organisms than the *C. sinensis* leaves. The antifungal activity of ethanol extracts of *A. saligna* leaves extracts compared to standard antibiotics are

Table 1. The antibacterial activity of ethanol extracts of *Acacia saligna* and *Cordia sinensis* leaves compared to antibiotics against different pathogenic bacteria.

| Type of bacteria | Bacterial strains | <i>Acacia saligna</i> | <i>Cordia sinensis</i> | Streptomycin (10 µg/disc) | Ciprofloxacin (10 µg/disc) | The mean of pathogenic bacteria | p-value |
|-------------------------|-------------------------------|-----------------------|------------------------|---------------------------|----------------------------|---------------------------------|--------------|
| Gram positive bacteria | <i>Bacillus subtilius</i> | 23.33±0.33 | 18.77±0.33 | 25.00±0.00 | 34.00±0.00 | 21.05 | p-value=0.00 |
| | MRSA | 22.66±0.33 | 21.33±0.33 | 20.00±0.00 | 36.00±0.00 | 21.49 | |
| | <i>Staphylococcus aureus</i> | 24.66±0.33 | 20.00±0.00 | 19.00±0.00 | 38.00±0.00 | 22.33 | |
| | Micrococcus | 27.66±0.33 | 15.00±0.00 | 27.00±0.00 | 46.00±0.00 | 21.33 | |
| Gram negative bacteria | <i>Escherichia coli</i> | 25.66±0.00 | 17.66±0.33 | 23.00±0.00 | 44.00±0.00 | 21.66 | |
| | <i>Klebsiella pneumonia</i> | 29.33±0.33 | 14.33±0.33 | 25.00±0.00 | 42.00±0.00 | 21.83 | |
| | <i>Pseudomonas aeruginosa</i> | 25.66±0.33 | 15.33±0.33 | 22.00±0.00 | 42.00±0.00 | 20.49 | |
| Mean of type of extract | | 25.56 | 17.49 | 23.00 | 40.29 | | |

Table 2. The antifungal activity of ethanol extracts of *Acacia saligna* and *Cordia sinensis* leaves compared to antibiotics against different pathogenic fungi.

| Pathogenic fungi | <i>Acacia saligna</i> | <i>Cordia sinensis</i> | Amphotericin | Nystatin | The mean of pathogenic fungi | p-value |
|---------------------------|-----------------------|------------------------|--------------|------------|------------------------------|--------------|
| <i>Aspergillus flavus</i> | 21.33±0.33 | 20.00±0.57 | 29.00±0.00 | 32.00±0.00 | 20.66 | p-value=0.00 |
| <i>A. fumigatus</i> | 25.67±0.33 | 14.00±0.57 | 26.00±0.00 | 28.00±0.00 | 19.83 | |
| <i>A. niger</i> | 20.00±0.00 | 18.33±0.33 | 25.00±0.00 | 30.00±0.00 | 19.16 | |
| <i>Candida albicans</i> | 23.33±0.33 | 22.00±0.57 | 28.00±0.00 | 29.00±0.00 | 22.66 | |
| Mean of type of extract | 22.58 | 18.58 | 27.00 | 29.75 | | |

shown in Table 2. The ethanol extracts exhibited remarkable antifungal activities against the tested fungi in the order of sensitivity as *A. fumigatus* > *C. albicans* > *A. flavus* > *A. niger*. The antifungal activities of standard antibiotics showed that the Amphotericin is less effective than nystatin. In this study, the most pronounced effect was shown by that of *A. saligna*. The main diameter of inhibition zones of the extract against these fungi strains were 25.67, 23.33, 21.33 and 20.00 mm, respectively (Table 2). Banso (2009) reported that

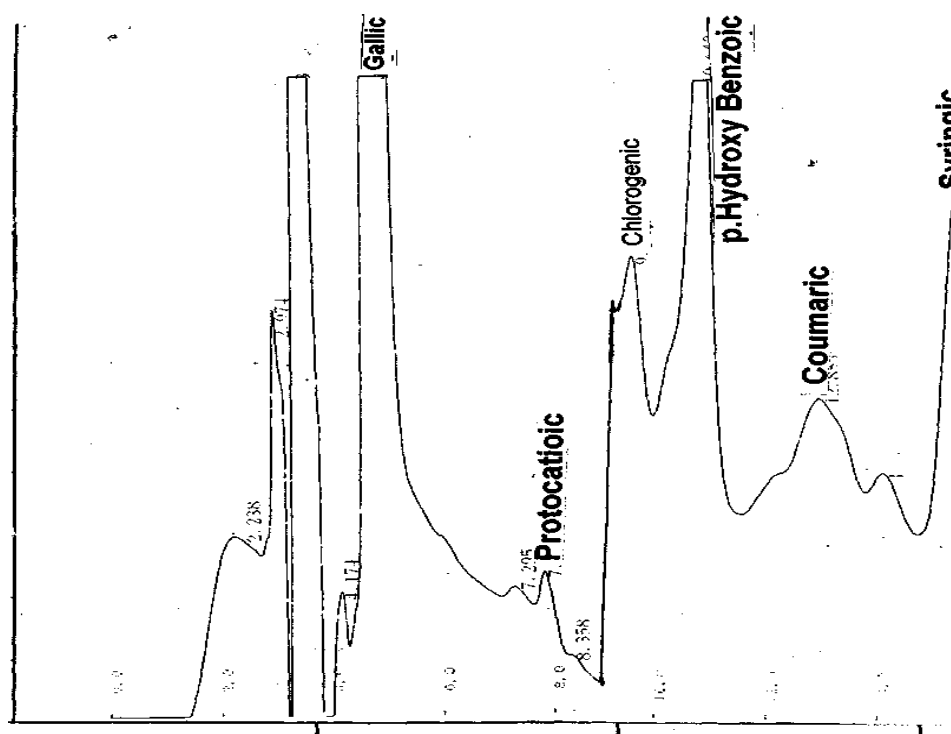
the *A. saligna* extract exhibited antimicrobial activity against all the test microorganisms. *B. subtilis* was the most susceptible to the plant extract, while *C. albicans* was the most resistant. The inhibition zones and MIC revealed antimicrobial activity of the extracts of *A. nilotica* against well characterized bacterial and fungal strains; the antimicrobial effect, expressed as MIC was found at concentrations of 0.05 to 1.6 mg/ml against Gram-positive and Gram-negative bacteria and *C. albicans* (Osman et al., 1992).

Phytochemical screening of *Acacia saligna*

The phytochemical screening of leaves of *A. saligna* presented in Table 3 showed that the HPLC analysis successfully provided the presence of 8 phenolic compounds. The major phenol compounds that were isolated from leaves of *A. saligna* were gallic, protocatechuic, chlorogenic, p-Hydroxy Benzoic, p-Coumaric, syringic, vanillic and salicylic acid. Gallic acid has the highest concentration followed by p-Coumaric, syringic

Table 3. Chemical composition analysis of *Acacia saligna* leaves extracts.

| Phenol compound M9/g | Retention time | <i>Acacia saligna</i> |
|----------------------|----------------|-----------------------|
| Gallic | 4.8 | 54.31 |
| Protocatioic | 6.8 | 1.45 |
| Chlorogenic | 9.4 | 0.44 |
| p.Hydroxy Benzoic | 10.11 | 1.95 |
| p-Coumaric | 11.58 | 8.27 |
| Syringic | 15.07 | 3.71 |
| Vanillic | 18.27 | 2.01 |
| Salicylic acid | 20.15 | 1.25 |
| Total | | 73.41 |

**Figure 1.** Phenol compound of *Acacia saligna* leaves extracts.

and vanillic with 54.31, 8.27 and 3.71 $\mu\text{g/g}$, respectively. The concentration of other phenolic compounds ranged from 1.25 to 2.01. Chlorogenic compound was the lowest phenol compound (0.44 $\mu\text{g/g}$). Deshpande and Kadam (2013) showed the results of phytochemical screening of ethanol and petroleum ether extract of stem bark of *A. nilotica*. Both the extracts contained alkaloids, carbohydrates, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides, while proteins, amino acids, fixed fats and oils were absent in it. These results are consistent with the findings of Banso (2009) who investigated phytochemical screening of the stem

bark of *A. nilotica* revealing that the plant contains terpenoids, alkaloids, saponins and glycosides. Negative results were recorded for steroids and flavonoids which confirm the absence of these active principles. The active principles identified in this study exhibited antimicrobial activity against all the tested organisms (Table 3 and figure 1). These findings correlate with the observations of Gumgumje et al. (2012), Gumgumjee and Hajar (2012) and Hajar and Gumgumjee (2013). Several plants, which are rich in alkaloids, tannins and glycosides, have been shown to possess antimicrobial activity against a number of microorganisms. For example, Adebajo et al. (1983)

investigated the antimicrobial activity of leaf extract of *Eugenia uniflora* and reported that tannins, glycosides and alkaloids were detected and that the ethyl acetate and methanolic leaf extract of the plant were active against *E. coli*, *P. vulgaris*, *K. pneumoniae* and *A. niger*. Olive leaves ethanol extract were also tested against the same strains and the HPLC analysis indicated the presence of 15 phenolic compounds as major active constituents in the leaves (aspartic, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine). Results of HPLC showed that glutamic acid has the highest concentration of 121.17 µg/g, followed by aspartic and lysine with 109.51 and 89.08 µg/g, respectively. The concentration of other phenolic compounds ranged from 17.61 to 77.93 µg/g. Methionine was the lowest phenol compound with 17.61 µg/g (Hajar and Gumgumjee, 2014). In a previous study, Gumgumjee and Hajar (2012) also reported the HPLC analysis of *Casuarina equisetifolia* leaves extract which allowed the identification of seven phenolic compounds: caffeic acid, verbascoside, oleuropein, luteolin 7-O-glucoside, rutin, apigenin 7-O-glucoside and luteolin 4'-O-glucoside. In addition, extracts may be more beneficial than isolated constituents, since a bioactive individual component can change its properties in the presence of other compounds present in the extracts (Borchers, 2004). These findings correlate with the observations of Owen et al. (2003). These results are consistent with the findings by Tenguria et al. (2012). Several plants which contain alkaloids, tannins, glycosides have been shown to possess antimicrobial activity against a number of microorganisms as is investigated by Adebajo (1983). Deshpande and Kadam (2013) reported that the results clearly indicated that using *A. nilotica* had the beneficial effect in controlling the microbial infections. Thus, *A. nilotica* are more suitable in the aspect of antibacterial activity. This inhibitory effect of the extract on the growth of these microorganisms could be attributed to the presence of some phytochemicals that were found present in the leaves of *A. nilotica*.

The demonstration of antimicrobial activity against pathogenic bacteria and fungi may be an indication of the presence of broad spectrum antibiotic compounds (Doughari, 2006). Previously, Deshpande and Kadam (2013) showed that *A. nilotica* is rich in phytochemicals. The phytochemical screening can serve as the basis for preparation of herbal monograph for proper identification and authentication of drug. The present study indicated the therapeutic potential associated with *A. nilotica* leaf constituents.

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