International Journal of Biomedical and Health Sciences
Vol. 5, No. 3, September 30, 2009
Printed in Nigeria

0794-4748/2009 $12.00 + 0.00

http://www.asopah.org

IJBHS 2009106/5306

Studies on brine shrimp lethality and activity of stem bark extract of *Acacia senegal* l. On respiratory tract pathogenic bacteria

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(Received September 11, 2009)

ABSTRACT: The crude ethanol extract from the stem bark of *Acacia senegal* was macerated with 60% aqueous methanol and partitioned into n-hexane, chloroform, ethylacetate and methanol soluble fractions. The fractions were tested for antibacterial activity using disc agar diffusion technique. All the fractions showed good activity against some respiratory tract pathogenic bacteria particularly in n-hexane soluble fraction at 1000 µg/ml , 3000 µg/ml and 5000 µg/ml concentrations. The Brine shrimp test showed highest toxicity in n-hexane soluble fraction with LC_{50} value of 6.7674 µg/ml. Phytochemical analysis of the fractions revealed the presence of alkaloids, steroids, cardiac glycosides, Tannins, reducing sugars and flavonosides.

Keywords :*Acacia senegal*, Brine Shrimp, Antibacteria properties, Respiratory pathogens, Phytochemicals.

Introduction

Lower respiratory tract infections are infections caused by bacteria (such as *Klebsiella pneumonia, Streptococcus pneumonia, H. influenza,* and *Mycobacterium tuberculosis*), viruses and fungi (Patrict, 2006) which usually affect the lungs. The infections caused by these micro organisms include bronchitis, bronchiolitis, Tuberculosis, Pneumonia etc. Furthermore, bacterial resistance to antibiotics in community acquired respiratory tract infections is a serious problem and increasing in prevalence world wide at an alarming rate (Kohno *et al*., 2008) *streptococcus pneumoniae*, one of the main organism implicated in respiratory tract infections has developed multiple resistance mechanisms to combat the effects of most commonly used classes of antibiotics, particularly the beta lactams and macrolides. Therefore, continued search for effective antibiotics through screening of bioactive plants is very essential and one of the intensive area of natural product research today.

Acacia Senegal belongs to the family Fabaceae (mimosaceae).(Sidi , 2006) The leaves of the plant is used in traditional medicine to treat illness such as Dysentery, diarrhea, gonorrhea, cough, gastric disorder and Nodular leprosy (Iwu, 1993).

The stem bark extract is commonly used as remedy for respiratory tract infections (Maydell H., 1990). This study is planned to investigate the bioactivity of the stem bark extract of this plant against respiratory tract pathogenic bacteria such as *Klebsiella pneumoniae, Streptococcus pneumoniae, Pseudomonas aueroginosa, Sstaphylococcus aureus and Escheria coli*. Brine shrimp lethality test was employed as an alternative method to investigate the toxicity of the plant extract (Meyer *et al*., 1972)

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Material and Methods

The stem bark of Acacia Senegal collected from Tiga village, Rano, Kano, Nigeria. was air dried and ground into fine powder. A voucher specimen was deposited at the herbarium. The plant material was identified and authenticated by Dr. Sidi B.A of Biological Science Department, Bayero University, Kano,

Extraction and Partitioning

The powdered plant material (200g) was percolated in redistilled ethanol 800ml in a 1000ml conical flask and stoppered for two weeks. Thereafter, the percolate was filtered with whatman's No 1 filter paper. The ethanol extract was concentrated at 40^{o}C under reduced pressure using rotary evaporator. The crude ethanol extract (8g) was dissolved in 60% aqueous methanol (200ml) in a separatory funnel and partitioned with 100ml x 3 of n-hexane, chloroform, ethylacetate sequentially. The afforded fractions obtained were concentrated using rotary evaporator, weight and labeled AS1-01 (n-hexane soluble fraction), AS1-02 (chloroform soluble fraction), AS1-03 (ethylacetate soluble fraction), and AS1-04 (methanol soluble fraction).respectively. Each fraction was screened for phytochemicals and antimicrobial activity.

Phytochemical Analysis of the Fractions

Phytochemical analysis for qualitative detection of secondary metabolites were performed on the afforded fraction as was described by Harbone, 1975, Evans, 1995, Brain and Tunner, 1975, El-olemy *et al*,1994, Sofowora , 1984 and ciulei, 1994.

Sources of Microorganisms

Pure cultures of *staphylococcus aureus, klebsiella pneumoniae, streptococcus pneumoniae, E. coli, salmonella typi and pseudomonas aueroginosa* were obtained from Microbiology Laboratory, Department of Biological Sciences, Bayero University, Kano. These bacterial cultures were maintained in nutrient agar slant.

Antibacterial Susceptibility Test

Disc agar diffusion technique described by Bauer and kirby (1966) was employed for antibacterial bioassay. Three concentrations for each fraction of the plant extract were prepared such as 5000 $\mu g/ml$, 3000 $\mu g/ml$ and 1000 $\mu g/ml$. These concentrations of the plant extract were subjected to antimicrobial susceptibility test against the selected organisms.

Preparation of Inoculum

The inoculum was prepared from the stock cultures which were maintained in nutrient agar slant at 40^{o}C and subculture in nutrient broth using a sterilized wire loop. The density of suspension to be inoculated was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Elmer *et al*., 1997).

Preparation of Sensitivity Disc and Sample

Discs of about 6mm diameter were made from whatman's No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 110^{o}C for 24hours .The stock solution of 50mg/ml of the plant extract was prepared by dissolving 0.1g of each fraction in 2ml Dimethylsulphoxide (DMSO). 30mg/ml and 10mg/ml were prepared by serial dilution by taking 0.6ml and 0.2ml of the stock solution and then dissolved in 0.4ml and 0.8ml of DMSO respectively. Hence, three concentrations were prepared from the stock solution such that each disc would absorb 0.01ml which is equivalent to 1000 $\mu g/ml$, 3000 $\mu g/ml$ and 5000 $\mu g/ml$ respectively.

Brine Shrimp Lethality Test (BST)

Brine shrimp lethality bioassay was carried out using brine shrimp larvae (Artemia salina) to test the cytotoxicity of the plant extract. Each test material (20mg) was dissolved in 2ml absolute methanol and 500, 50, 5 μl of the solution were transferred using a microsyringe into three separate vials corresponding to 1000, 100, 10 $\mu g / ml$ respectively. Each dosage was tested in triplicate, 500 μl of solvent was also added to a control vial. The control plus the 9 vials were allowed to dry at room temperature. After 48 hours, 4.5ml of sea water and 10 shrimps were introduced into each vial and the volume in each vial was made up to 5ml with sea water. 24 hours after introducing the shrimps, the number of survival at each dosage was counted and recorded. L_C 50 values were determined at 95% confidence interval from the total mortality by analyzing the data using finney software programme (Meyer and Mitscher,1972).

Results

Table 1 Results of preliminary phytochemical screening.

2^0 metabolite group	AS1 01	AS1 02	AS1 03	AS1 04
Saponins	+	-	+	-
Alkaloids	- - -	-	-	+
Phlobatannins	-	-	-	-
Cardiac glycosides	-	+	+	+
Flavonoids	-	-	-	-
Steroids	+	+	+	+
Anthraquinone	-	-	-	-
Tannins	+	+	+	+
Resins	-	-	-	-
Flavonoside	-	-	+	+
Reducing sugar	+	+	+	+

Key + = present
- = Absent
AS1-01= n-hexane soluble fraction
AS1-02= chloroform soluble fraction
AS1-03= ethylacetate soluble fraction
AS1-04= methanol soluble fraction

Table 2 Antibacterial susceptibility Test result

Fraction	Concentration ($\mu g / ml$)	Test organisms with Zone of inhibition in (mm)					
		K.P	S.P	S.A	E.C	S.T	P.A
ASl-01	1000	10	13	10	7	11	11
	3000	19	21	15	10	10	10
	5000	22	24	17	12	12	13
ASl-02	1000	6	7	6	9	7	10
	3000	6	00	6	6	20	22
	5000	6	8	6	11	23	20
ASl-03	1000	11	7	6	6	16	6
	3000	6	7	6	6	26	10
	5000	6	6	6	12	29	11
ASl-04	1000	27	6	6	6	9	6
	3000	6	6	14	6	11	7
	5000	17	7	6	6	10	6

Zone of inhibition for control = 6mm.
Key:
K.P = *Klebsiella pneumoniae*

S.P = *Streptococcus pneumoniae*
S.A = *Staphylococcus aureus*
E.C = *Escheria coli*
S.T = *Salmonella typi*
P.A = *Pseudomonas auroginosa.*

Table 3 Result of Brine shrimp lethality Test (BST]

Fractions	Total larvae used	Total mortality	LC50 value (µg/ml)
AS1-01	90	70	6.7674
AS1-02	90	39	27.2112
AS1-03	90	40	139.76
AS1-04	90	60	27.3830

Discussion

Result of the phytochemical analysis (table1) revealed the presence of the following secondary metabolites in the plant extract, Tannins, steroids, cardiac glycosides, flavonosides, saponins and alkaloids. However, Steroids, Tannins and Reducing sugars were found to be present in all the fractions. While Flavonoids, Anthraquinone, Resins and Phlobatannins were found absent in all the fractions. Antibacterial susceptibility test result (Table 2) showed the zones of inhibition measured in millimetre (mm) on the bacteria susceptible to the plant extract. All the fractions showed good degree of susceptibility against the test organisms. However, n- hexane Soluble fraction (AS1- 01) was found to be more active against the respiratory tract pathogenic bacteria such as *klebsiella pneumonia and streptococcus pneumoniae* while chloroform soluble fraction was found to be inactive against *klebsilla pneumonia, staphylococcus aureus and streptococcus pneumonie* in all the three concentrations prepared (1000 µg/ml, 3000 µg/ml, and 5000 µg/ml). The result of the brine shrimp lethality test (BST) (Table 3) showed good activity in all the fractions with highest toxicity observed in n- hexane soluble fraction with LC_{50} value 6.7674µg/ml.

Conclusion

The promising result displayed by the plant extract both in the antibacterial bioassay and brine shrimp lethality test justified the efficacy of the plant in traditional medicine which indicate that the fractions contained antibacterial agent(s) that could be effective in treatment of respiratory tract infections caused by bacteria whose chemotherapeutic index may exceed the drugs in used. Based on the interesting result displayed by this plant extract, particularly the n-hexane soluble fraction which was found to be the most active fraction. Therefore, activity guided isolation and characterization to uncover the active agent(s) is recommended.

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