INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

COMPARATIVE STUDY ON THE EFFECTS OF AQUEOUS AND ETHANOL LEAF EXTRACTS OF *CASSIA ALATA* LINN ON SOME PATHOGENIC BACTERIA AND FUNGI

Timothy SY[1]*, Wazis CH[1], Bwala AY[1], Bashir HJ[2], Rhoda AS[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria

[2]Pinecastle Hospital and Pharmacy, Mafoni ward, Maiduguri, Nigeria

Article Received on: 06/05/12 Revised on: 22/06/12 Approved for publication: 11/07/12

*E-mail: satiye2002@gmail.com

ABSTRACT

The plant *Cassia alata* linn is a shrub that has various uses ranging from mild to severe infectious and non-infectious diseases. The study was aimed at comparing the antimicrobial activities of the aqueous and ethanolic leaf extracts of *Cassia alata* on bacteria and fungi. The leaf of the plant was collected, dried, macerated and extracted using ethanol and distilled water. The extracts were used for the antibacterial susceptibility testing using agar diffusion. The antimicrobial activity of the water and ethanol extracts against the tested organisms was dose dependent with greater activity against *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Candida albicans* and *Trichophyton mentagrophytes*. The minimum inhibitory concentration of the water extract against the organisms studied was between 1.74 to 7.67 mg, whereas for ethanol extract were 1.26 to 5.23 mg. The ethanol and aqueous extracts of this plant contains phytochemical components which may be responsible for their observed antibacterial effects and this seems to justify the use of the plants in various places for the alleviation of different diseases. The aqueous and ethanol extracts of *Cassia alata* leaves has been found to exhibit more antimicrobial activities against fungi than bacteria with aqueous leaf extract having better antibacterial activity against *Klebsiella pneumoniae*, while ethanol leaf extract was on *Pseudomonas aeruginosa*.

Keywords: Antimicrobial, Aqueous, Ethanol, *Cassia alata*

INTRODUCTION

In the recent years, research on medicinal plants has attracted a lot of attention globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternative systems of treatment of human diseases [1]. The development of resistance to most antimicrobial agents and the high costs of treatments have necessitated the search for new, safe, efficient and cost effective ways for the management of infectious conditions [2, 3]. *Cassia alata* Linn belonging to a family caesalpiniaceae is a pantropical ornamental shrub distributed from tropical America to India and Africa [4]. It is commonly known as "Rai dore" in Hausa, "Asuwon oyinbo" in Yoruba, "Omirima" in Igbo and "Whu shil-shili" in Kilba[3]. *Cassia alata* obtained from Nigeria has shown to contain some phytochemical groups in their leaves and roots that have been reported to be useful in treating diverse medical ailments [5-8, 3]. There was no study reported in our environment that outlined the scientific rational for the appropriate traditional use of this plant in disease management. Therefore, this study seeks to compare the antimicrobial activity of ethanol and aqueous leaf extract of this plant on bacterial and fungal diseases.

METHODOLOGY

Source of Plant Material, Collection and Authentication

The leaf of *Cassia alata* Linn was collected in the month of September, 2011 from Hong, Hong local government area of Adamawa state, Nigeria and was identified by a Taxonomist of the Department of forestry and Wild life, University of Maiduguri at which the voucher specimen number was assigned and deposited in the Department.

Preparation of the Leaf Extracts

The fresh leaves of *Cassia alata* were air dried at room temperature and were then ground into powder using pestle and mortar and were sieved to obtain a fine powder. Two hundred grams each of the powder was weighed into containers labelled A and B. The two samples were subjected to maceration using 800 ml of ethanol and 1500 ml of water so as to obtain the ethanol and aqueous extracts respectively. The mixtures were both stirred and kept for 24 hours at which it was filtered to obtain residues. The residues were soaked in 300 ml and 500 ml of ethanol and water respectively and were kept for another 24 hours. This procedure was repeated three times, and the combined filtrate was transferred to a rotor-vapour machine to obtain the crude ethanol and aqueous extracts. The crude extracts were then evaporated to dryness. The weights of the ethanol and aqueous extracts obtained were 16.4 g (8.2%) and 12.5 g (6.25%) respectively.

Source of the Microorganisms

Clinical isolates of the test organisms (*Proteus vulgaris*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, Candida albicans* and *Trichophyton mentagrophytes)* were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH).

Antibacterial Assay of the Plant Extracts

The media used for this research work was a preparative nutrient agar and sabouraud dextrose agar (double layer). Each of the layers contains 30 ml, making a total of 60 ml of for each plate. The media was then sterilized by autoclaving at 121^{o}C for 15 minutes and was allowed to cool at which it was used for the antimicrobial assay. A sterilized cork borer of 6 mm in diameter was used in boring holes on the agar plates, with each media containing 6 holes in which 200 mg, 100 mg, 50 mg and 25 mg of the reconstituted ethanol and aqueous extracts of 500 mg/ml stock solution was poured into the four holes respectively. Distilled water and 10 mg of Amoxiclav were added to the fifth and sixth holes as negative and positive controls respectively. A broad culture was diluted with peptone water to match turbidity of McFarlan standard number 3 which was used as inoculums for the microorganisms. One millilitre of the bacterial inoculums was transferred onto the pure agar plates using a sterile syringe in each case. The agar plates were then slanted to ensure uniform spread on the surface of the plates. Zero point four millilitre of the appropriate diluted extract was

administered into the 4 holes on each plate with varying strength of 200 mg, 100 mg, 50 mg and 25 mg for both ethanol and aqueous extracts. Similarly, 0.4 ml of distilled water was then administered in to the fifth hole on the media plate, while 10 mg Amoxiclav was added into the sixth hole. Five different media plates were prepared for each organism. One hour was allowed for diffusion before incubation of plates at 37^{o}C for 24 hours. The clear zones of inhibition for the respective strength of both extracts were measured in millimetres.

Determination of the Minimum Inhibitory Concentration

The values for the minimum inhibitory concentration of each extract were obtained by extrapolation from the plot of the log strength (per hole) of the extract against the clear zones of inhibition in millimeters [9].

Statistical Analysis

Student t-test was used in the analysis to determine the level of significance of the various bacterial zones of inhibition observed. P-value less than 0.05 were considered significant.

RESULT ANALYSIS

Antimicrobial Screening of Aqueous Leaf Extract of *Cassia alata*

The result from the antibacterial and antifungal assay of the aqueous extract showed a dose dependent effect against all the clinical isolates studied. The effect of the extract at 200 mg was higher on fungi than bacteria, with higher antibacterial activity on gram negative than gram positive organisms. At the highest dose tested the effect of extract is statistically significantly higher than the control against *Candida albicans, Trichophyton mentagrophytes* and *Klebsiella pneumonia* ($p<0.05$) (Table 1).

Antimicrobial Screening of the Ethanol Leaf Extract of *Cassia alata*

The antimicrobial assay of the ethanol extract showed a dose dependent effect against the gram negative bacteria and fungal clinical isolates studied. The effect of the extract at 200 mg was higher on fungi than bacteria, with higher antibacterial activity on gram negative than gram positive organisms. At the highest dose tested the effect of extract is statistically significantly higher than the control against *Candida albicans, Trichophyton mentagrophytes* and *Pseudomonas aeruginosa* ($p<0.05$) (Table 2).

Minimum Inhibitory Concentration of Ethanol and Water Leaf Extract of *Cassia alata*

The minimum inhibitory concentration (MIC) of the aqueous leaf extract of *Cassia alata* for *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Proteus vulgaris*, *Staphylococcus aureus, Streptococcus pneumonia, Candida albicans* and *Trichophyton mentagrophytes* were 5.10 mg, 7.67 mg, 1.74 mg, 3.91 mg, 7.32 mg, 4.90 mg and 5.11mg respectively. The MIC for the ethanolic leaf extract were 3.05 mg, 3.30 mg, 1.26 mg, 2.21 mg, 4.34 mg, 5.23 mg and 3.65 mg against *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Proteus vulgaris*, *Staphylococcus aureus, Streptococcus pneumonia, Candida albicans* and *Trichophyton mentagrophytes* respectively (Table 3).

DISCUSSION

The result obtained from this study showed that the ethanol and aqueous leaf extracts of *Cassia alata* was similar to the report presented by Makinde and his colleagues [10] in which the activity on fungi was significantly higher than on bacteria. The discrepancy observed was the absence of activity against *Proteus vulgaris* that was observed in this study. The use of methanol-water extract by Makinde *et al* [10] may be responsible for the observed differences in the antimicrobial activity. The use of methanol and chloroform solvent system by El-Mahmoud and Doughari [2] for extraction could also be the likely explanation for the disagreement in the antimicrobial result. The inhibiting activities exhibited by the extracts on the microorganisms tend to agree with the report of Timothy *et al* [3] that linked antimicrobial properties of plants to the presence of bioactive secondary metabolites like saponins, flavonoids, glycosides, diterpenes etc. The ethanol and aqueous crude leaf extracts of *Cassia alata* inhibited the growth of *Escherichia coli, Salmonella typhi* and *Staphylococuss aureus* to varying degrees dose dependently[3] agree with the result of this finding. The activity of the leaf extracts against both gram negative, gram positive bacteria and fungi is an indication that a broad spectrum antimicrobial compound(s) could be present in the extracts studied. The relatively higher activities of the aqueous and ethanol leaf extracts against fungi and gram negative bacteria could support the traditional use of the decoction of this plant for the treatment of ringworm and typhoid fever in our locality [7]. The activity of both aqueous and ethanol leaf extracts of *Cassia alata* on *Candida albicans* and *Trichophyton mentagrophytes* showing a higher statistical significant difference ($p<0.05$) than the positive control (Ketoconazole) at the highest dose tested strongly agrees with literature reports in which similar result was obtained [7, 8, 10]. The minimum inhibitory concentration (MIC) of the aqueous and ethanol leaf extracts against organisms tested in this study agrees with the reports of Timothy et al [3] and Makinde *et al* [10] in which the effects of most crude leaf extracts vary widely in their degree of susceptibility to antimicrobial agents. In this study, the MIC value for the ethanol extract is relatively lower than the water extract, suggesting higher activity against the test organisms to thus justifying ethnomedical uses.

CONCLUSION

The antimicrobial activity of the aqueous and ethanol extracts of *Cassia alata* leaves has been evaluated. The extracts exhibited more antifungal than antibacterial properties. Aqueous leaf extract had better antibacterial activity against *Klebsiella pneumoniae*, while ethanol leaf extract was better on *Pseudomonas aeruginosa*.

ACKNOWLEDGEMENT

Authors are thankful to staff of Microbiology Laboratory, UMTH for their technical assistance.

REFERENCES

1. Sher A. (2009). Analysis of typhoid ileal perforation. Gomal Journal of Medical Sciences, 7(1): 72-78.
2. El-Mahmoud AM, Doughari JH. Phytochemistry and activity of *Cassia alata.* African Journal of Pharmacy and Pharmacology 2008, 2(7): 124-129.
3. Timothy SY, Lamu FW, Rhoda AS, Adati RG, Maspalma ID, Askira M. Acute toxicity, phytochemistry and antibacterial activity of aqueous and ethanolic leaf extracts of *Cassia alata* linn. International Research Journal of Pharmacy 2012; 3(6): 73-76.
4. Abubacker MN, Ramanathan R, Senthil Kumar T. Invitro antifungal activity of *Cassia alata* Linn flower extract. Natural Product Radiance. 2008; 7(1): 6-9.
5. Zhongguo Z. Studies on chemical constituents from leaves of *C. alata*. Chinese Article 2009, 34(7): 861-3.
6. Adedayo O, Anderson WA, Mooyoung M, Snieckus V, Patil PA, Kolawale DO. Phytochemical and antibacterial activity of *Senna alata* flower. Pharmaceutical Biology 2001, 39(6): 408-412.
7. Timothy SY, Wazis CH, Adati RG, Maspalma ID. Antifungal activity of aqueous and ethanolic leaf extracts of *Cassia alata* Linn. Journal of Applied Pharmaceutical Science 2012; 02(07): 182-185.
8. Sule WF, Okonko IO, Ojezele MO, Nwanze JC, Alli JA, Adawale OG et al. Invitro antifungal activity of *Cassia alata* leaf extract. Advances in Applied Science Research 2010, 1(2): 14-26.
9. Timothy SY, Galadima IH, Wazis CH, Maspalma DI, Bwala AY, Reuben U et al. Antibacterial and Phytochemical screening of N-butanol

and Ethyl acetate leaf extract of *Byrsocarpus coccineus* Schum and Thonn. Sahel Journ of Vet Sci 2011, 10(2): 21-26.

10. Makinde AA, Igoli JO, Ta`ama L, Shaibu SJ, Garba A. Antimicrobial activity of *Cassia alata*. African Journal of Biotechnology 2007, 6(13): 1509-1510.

Table 1: Antimicrobial screening of aqueous leaf extract of *Cassia alata* showing the zones of inhibition (mm) (n=5)

Organisms	*Controls*	*Aqueous Extracts (mg)*			
		25	*50*	*100*	*200*
Klebsiella pneumonia	20.00±1.58	15.20±0.84*	18.00±1.58*	19.00±0.58*	25.80±1.48*
Pseudomonas aeruginosa	27.00±1.41	15.20±0.81*	16.25±0.96*	19.60±1.14*	23.80±0.84*
Proteus vulgaris	29.80±1.22	15.20±0.53*	18.40±0.89*	21.40±0.89*	26.40±1.14*
Staphylococcus aureus	25.20±0.84	14.20±0.63*	14.80±0.24*	15.00±1.58*	22.20±0.44
Streptococcus pneumonia	27.20±0.64	18.80±0.34*	20.20±1.74*	17.90±1.28**	20.20±0.84*
Candida albicans	24.00±1.58¥	14.80±0.64*	21.20±0.74	25.80±0.64	33.00±0.81*
Trichophyton mentagrophytes	22.40±0.74¥	15.20±0.84*	24.20±0.41	30.80±0.72*	36.00±0.58*

*indicates a significant P value (T-test) with the control, +ve controls = Amoxiclav 10 mg, Ketoconazole 200 mg¥

Table 2: Antibacterial assay of ethanol leaf extract of *Cassia alata* showing the zone of inhibition (mm) (n=5)

Organisms	*Control*	*Aqueous Extracts (mg)*			
		25	*50*	*100*	*200*
Klebsiella pneumonia	22.00±1.58	15.20±0.84*	18.00±1.58*	20.00±0.58*	24.80±1.48
Pseudomonas aeruginosa	21.00±1.41	14.20±0.74*	16.25±0.96*	25.60±1.14*	26.80±0.64*
Proteus vulgaris	29.00±1.22	16.20±0.24*	17.40±0.89*	21.40±0.89*	25.40±1.14*
Staphylococcus aureus	25.20±0.84	15.20±0.13*	12.10±1.04*	14.10±1.58*	17.20±0.94*
Streptococcus pneumonia	26.20±0.64	19.20±0.74*	13.40±1.12*	14.50±1.58*	19.20±0.34*
Candida albicans	24.00±1.58¥	15.80±0.62*	23.20±0.74	26.10±0.64	31.00±0.81*
Trichophyton mentagrophytes	18.40±0.74¥	17.20±0.81	19.20±0.41	26.80±0.72*	35.00±0.58*

*indicates a significant P value (T-test) with the control, +ve controls = Amoxiclav 50 mg, Ketoconazole 200 mg

Table 3: MIC of ethanol and aqueous leaf extracts of *Cassia alata*

S/No.	*Test organism*	*MIC (mg)*	
		Ethanol	*Water*
1	*Klebsiella pneumoniae*	3.05	5.10
2	*Pseudomonas aeruginosa*	3.30	7.67
3	*Proteus vulgaris*	1.26	1.74
4	*Staphylococcus aureus*	2.21	3.91
5	*Streptococcus pneumonia*	4.34	7.32
6	*Candida albicans*	5.23	4.90
7	*Trichophyton mentagrophytes*	3.65	5.11

MIC = Minimum inhibitory concentration

Source of support: Nil, Conflict of interest: None Declared

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com. All rights reserved.