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Short Communication

ANTIMICROBIAL ACTIVITY AND POTENCY OF *CASSIA ABBREVIATA* OLIV STEM BARK EXTRACTS

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ABSTRACT

Objective: To evaluate the potential antimicrobial activity and relative potency of aqueous and organic extracts of *Cassia abbreviata* Oliv stem bark against clinical isolates of *Neisseria gonorrhoeae (NG), Pseudomonas aeruginosa (PA), Klebsiella pneumoniae (KP)* and *Candida albicans (CA)*.

Methods: Six extracts of *Cassia abbreviata* Oliv stem bark were prepared as follows: Four extracts were prepared following Soxhlet extraction procedure using ethanol, water, trichloromethane (TCM) and dichloromethane (DCM)+ethanol (1:1) as solvents, respectively. Two extracts were prepared by soaking the powdered stem bark in ethanol and water, respectively, and subjected to mechanical shaking for 8 hours. Antimicrobial activities and minimum inhibitory concentrations (MIC) of extracts were evaluated *in-vitro* using agar well diffusion assay.

Results: All extracts except TCM were active against *NG* and *PA* with an average MIC of 78.8 µg/ml. The cold aqueous extract had a lowest MIC (46.88 µg/ml) against *PA,* whereas both hot and cold ethanol extracts had a lowest MIC of 46.88 µg/ml against *NG.* Only hot extracts (ethanol, DCM+ethanol and TCM) were active against *KP* with a lowest MIC of 46.88 µg/ml of TCM extract. Both extracts of cold ethanol and DCM+ethanol were active against *CA* with cold ethanol extract having a lower MIC of 93.75 µg/ml compared with DCM+ethanol.

Conclusion: There were variations in *in-vitro* antimicrobial activities (microbial growth inhibition) of *Cassia abbreviata* Oliv stem bark extracts which depended on the solvent used. Organic extracts were more potent against *NG, PA, KP* and *CA* compared with aqueous extracts.

Keywords: Cassia abbreviata, Potency, Antibacterial, Antifungal.

Infectious diseases are a challenge, especially in the developing countries [1]. Eighty percent of the population in Africa depend on traditional medicinal plants to meet primary health care needs. As an actual fact, the numbers of traditional practitioners exceed that of allopathic practitioners in sub-Saharan Africa by 100 to 1 [2].

The use of medicinal plants to manage different ailments is a common practice among rural and urban populations of Zambia. Furthermore, more than 60% of Zambian children with high fever are treated with herbal medicines at home [2, 3]. *Cassia abbreviata* Oliv is an indigenous plant found in mixed woodlands of Zambia [3, 4]. It is often prescribed by most traditional healers in the treatment of leprosy, syphilis and toothache by use of aqueous decoction prepared from roots or stem barks. The leaves are also smoked as a remedy for haematuria [3]. Traditional herbalists and diviners use a decoction of *Cassia abbreviata* Oliv to treat sexually transmitted infections clinically manifested as urethral discharge and swellings in the groin [5]. *Cassia abbreviata* Oliv was reported to have been used in the management of *Candida* infections by some traditional healers [6].

Traditionally, the decoction of *Cassia abbreviata* Oliv is prepared by soaking fresh or dry stem bark/roots in water "overnight" (at least 12 hours) or boiled for some time. Some bioactive phytochemical compounds may be more soluble in aqueous than in organic solvent or vice versa [7]. It is, therefore, possible that extracts of the same plant obtained using different solvents and methods can exhibit variations in the bioactivity and potency. Plant extracts of proven bioactivity and potency are the most desired in effective and successful treatment of bacterial and fungal infections. Studies that compare bioactivities of the same plant are scarce in the literature.

This study evaluated the potential antibacterial and antifungal activity of *Cassia abbreviata* Oliv stem bark extracts against known pathogenic organisms. Antibacterial activity was assessed against *Neisseria gonorrhoeae (NG), Pseudomonas aeruginosa (PA) and Klebsiella pneumoniae (KP)* whereas antifungal activity was assessed against *Candida albicans (CA)*. Extracts of *Cassia abbreviata* Oliv stem bark were obtained using cold and hot aqueous and organic solvents. Relative potencies were compared among the bioactive extracts.

The plant parts of *Cassia abbreviata* Oliv (stem bark, branches and fruits) were collected from its natural habitat in the outskirts west of Lusaka in Zambia. The bark was separated from the wood using a knife and a clean brush was used to remove insects and foreign matter from the bark. A few branches with leaves and fruits were taken for botanical identification and classification at the University of Zambia, Department of Biological Sciences. Voucher of the plant specimen was deposited at the Herbarium of the University of Zambia.

The *Cassia abbreviata* Oliv barks were cut into small pieces, spread on white paper and left to dry in the shade for 21 days. Dried barks were ground and powdered using a domestic grinding machine (Retsch GmbH, Type SM1, German). The powdered bark was stored in an umber container.

Six extracts of powdered bark were prepared using different extracting solvents. The Soxhlet extraction procedure was performed to prepare four extracts using ethanol (95%), distilled water, trichloromethane (TCM) and a mixture of dichloromethane (DCM)+ethanol (1:1), respectively. Each extraction was carried out for 8 hours. The extract solution was evaporated to dryness at 40 °C under reduced pressure using a rotary evaporator (Buchi model No. RE120). The dry residue was collected and stored in an airtight container.

The cold ethanol extract was prepared by mixing powdered bark with ethanol in a conical flask and subjected to mechanical shaking for 8 hours. The contents were filtered on Whatman No.1 filter paper and the filtrate was evaporated at 40 °C using a rotary evaporator under reduced pressure. The dry residue was stored in an airtight container.

The cold aqueous extract was prepared using distilled water by following the cold ethanol extracts procedure. However, the filtrate

was not evaporated but quantified by calculating the difference between the mass of 50 ml of the filtrate and mass of 50 ml of distilled water (50.09g–49.73g = 0.36g/50 ml). This was performed in order to reflect a procedure of how the extract is prepared traditionally in Zambia. The solution was stored in a refrigerator (2-8 °C).

The dry crude extracts obtained from Soxhlet extraction (ethanol, water, TCM, DCM+ethanol) and cold ethanol procedures were each reconstituted with dimethylsulphoxide to the concentration of 1500 µg/ml. The cold aqueous extract solution was diluted with distilled water to a concentration of 1500 µg/ml.

The clinical isolates of bacteria and fungi were obtained from the University Teaching Hospital Microbiology Laboratory in Lusaka. Agar well diffusion assays were performed on chocolate agar, Mueller-Hinton agar and blood agar for *NG*, *PA* and *KP* , respectively. On seven already prepared chocolate agar plates, pure isolates of *NG* were inoculated by streaking with a flame sterilized loop. The seven agar plates were each set up and marked A, B, C, D, E, F and P, respectively. Plate 'A' for ethanol extract, plate 'B' for water extract, plate 'C' for TCM extract, plate 'D' for DCM+ethanol extract, plate 'E' for cold ethanol extract, plate 'F' for cold water extract and plate 'P' for positive control, respectively. A well was made on each agar plate using a sterile borer. To each corresponding well, 0.1 ml of each extract at a concentration of 1500 µg/ml was added, whereas 0.1 ml (100 mg/ml) of Ceftriaxone was added to the well of the agar plate marked 'P' to act as the positive control. The same procedure was performed on Mueller-Hinton agar and blood agar for *PA*and *KP,* respectively. However, Ciprofloxacin (0.1 ml, 2 mg/ml) was used as the positive control.

The procedure for the antifungal assay of *CA* was performed similarly to the antibacterial assay. Fluconazole (0.1 ml, 2 mg/ml) was used as a positive control. All the agar plates were incubated for 24 hours at 37 °C. Clear zones of microbial growth inhibition were observed in comparison with positive controls and taken as indicators of antibacterial and antifungal activity [8].

The MIC was determined for each extract that was active against the micro-organisms. Each bioactive extract was reconstituted to1500 µg/ml and was serially diluted appropriately to six concentrations (750, 375, 187.5, 93.75, 46.88 and 23.44 µg/ml). *NG* was inoculated on seven chocolate agar plates marked C1, C2, C3, C4, C5, C6 and P. A well was made on each agar plate using a sterile borer. 0.1 ml of each diluted extract was added to the appropriate well of the agar plate. 0.1 ml of Ceftriaxone (100 mg/ml) was added to the well on agar plate marked 'P' as a positive control. A similar procedure was performed on *PA, KP* and *CA* with appropriate agar plates and positive controls. Agar plates were incubated for 24 hours at 37 °C. MIC was determined as the lowest concentration at which a clear zone of microbial growth inhibition was observed.

The plant was identified as *Cassia abbreviata* Oliv (Family: Fabaceae, Genus: Cassia, Species: Abbreviata, Author: Oliver). The yields of the extracts are shown in table 1. The results for bioactivities and MICs of extracts are presented in table 2 and **3**, respectively.

Table 1: Yields of extracts

S. No.	Solvent used	Quantity of extracts (g)	Physical state	Powdered bark used (g)	Percent yield (%)
1	Ethanol	13.09	Brown crystals	69	19
2	Water	7.21	Brown crystals	60	12
3	TCM	0.13	Light green crystals	40	0.325
4	DCM+ethanol	4.47	Light brown crystals	60	12.8
5	Cold ethanol	0.13	Light brown crystals	50	8.9
6	Cold water	148 ml (7.2 g/l)*	Brown liquid	50	2.13

*The cold water extract was quantified in the liquid state.

Table 2: *In-vitro* antibacterial and antifungal activities of plant extracts

S. No.	Test extract (1500 µg/ml)	*NG*	*PA*	*KP*	*CA*
1	Ethanol	+	+	+	-
2	Water	+	+	-	-
3	TCM	-	-	+	-
4	DCM+ethanol	+	+	+	+
5	Cold ethanol	+	+	-	+
6	Cold water	+	+	-	-
	Ceftriaxone	+	NT	NT	NT
	Ciprofloxacin	NT	+	+	NT
	Fluconazole	NT	NT	NT	+

Note: (+)= active (clear zone of microbial growth inhibition observed),(-)=inactive (no clear zone of microbial growth inhibition observed),(NT) = not tested.

Table 3: MICs of bioactive plant extracts

S. No.	Test extract	MICs of extracts in µg/ml			
		NG	*PA*	*KP*	*CA*
1	Ethanol	46.88	93.75	187.5	NT
2	Water	93.75	93.75	NT	NT
3	TCM	NT	NT	46.88	NT
4	DCM+ethanol	93.75	93.75	93.75	187.5
5	Cold ethanol	46.88	93.75	NT	93.75
6	Cold water	93.75	46.88	NT	NT
	Ceftriaxone	+	NT	NT	NT
	Ciprofloxacin	NT	+	+	NT
	Fluconazole	NT	NT	NT	+

Note: (+)= Active (clear zone of microbial growth inhibition observed);(NT) = Not tested; Number of agar plates for *NG*, *PA*, *KP* and *CA* were 31, 31, 19 and 13, respectively.

The highest percentage yield of extracts was achieved when ethanol was used as the extracting solvent. However, using TCM as solvent achieved the lowest percentage yield. This indicated that the majority of compounds were mostly soluble in hot ethanol and least soluble in hot TCM amongst extracting solvents used.

In the current study, five extracts (water, ethanol, DCM+ethanol, cold ethanol and cold water) showed *in-vitro* antibacterial activity against *NG* and *PA* by bacterial growth inhibition. Furthermore, three extracts (ethanol, TCM and DCM+ethanol) were active against *KP.* Our results further support the use of the decoction of *Cassia abbreviate* Oliv traditionally to treat gonorrhoea [9]. Similarly, *Cassia abbreviata* Oliv extracts (water, methanol and acetone) were found active against some gram positive and negative bacteria with MIC value range of 500–5000 µg/ml[10]. In the literature [11], the extract obtained using acetone demonstrated antibacterial activity against *PA* and *KP* with each MIC value of 80 µg/ml. In this study, the MIC values of active extracts ranged between 46.88 µg/ml and 187.5 µg/ml for *PA* and *KP.*

The differences in the MIC values of active extracts could arise from variations in solubility of specific bioactive compounds in different extracting solvents. The lower the MIC value the better the activity of the extract against micro-organisms [12]. Some scholars suggest the plant extracts MIC value of 100 µg/ml or lower as significant [12]. In this study, the hot ethanol extract had MIC value above 100 µg/ml for *KP*. This implies that the hot ethanol extract of *Cassia abbreviate* Oliv may be inferior in treating *KP* infections compared with other extracts.

In this study, only cold ethanol and DCM+ethanol extracts were found to have *in-vitro* antifungal activity against *CA* with cold ethanol extract being more potent than DCM+ethanol extract (MIC = 93.75 and 187.5 µg/ml, respectively). In a study by Kolaczkowski *et al.* [13], ethylacetate extract demonstrated anti fungal activity against *CA* with MIC of 1200 µg/ml which was less potent than DCM+ethanol extract in the current study. Nevertheless, in another study by Hamza *et al.* [14], methanol extracts of root and stem bark were reported inactive against *CA*. Arguably, the method (maceration in 80% methanol) and duration (24 hours) of extraction that Hamza *et al.* [14]employed could have affected the potency of active compounds. In the current study, Soxhlet extraction method (95% ethanol) was performed and lasted for 8 hours. It was therefore deduced that the cold ethanol and DCM+ethanol extracts of *Cassia abbreviata* Oliv may be useful in the treatment of superficial Candida infections.

The aqueous and organic extracts *Cassia abbreviata* Oliv stem bark have varying demonstrable antibacterial and antifungal activity through microbial growth inhibition. Organic extracts demonstrated more potent *in-vitro* activity than aqueous extracts. The extracts can potentially be applied in the treatment of community-acquired *PA* or *KP* infections and gonorrhoea. The antifungal activity of organic extracts (cold ethanol and DCM+ethanol) can be utilised in the management of *CA* infections like oral thrush.

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CONFLICT OF INTERESTS

Authors have declared no conflict of interest

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