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Full Length Research Paper

Phytochemical Screening and antibacterial activity of extracts from *Pakia Clapperotoniana keay* against human pathogenic bacteria

D. Kubmarawa[1]*, M. E. Khan[2], A. M. Punah[1] and Hassan[1]

[1]Department of Chemistry School of Pure and Applied Sciences Federal University of Technology, P. M. B. 2076, Yola, Nigeria.

[2]Department of Chemistry Gombe State University, Gombe, Nigeria.

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Pakia clapperotoniana keay **has medicinal properties for the effective management of several ailments including tuberculosis. To establish the pharmacological rationale, for its traditional use, the powdered root, stem- bark and leaves were extracted with water and ethanol. All fractions were subjected to phytochemical screening and antimicrobial activity against, Gram-positive and Gram-negative bacteria using the disc-diffusion method. The extracts contained saponnins, tannins, flavanoids, glycosides but no resin. The roots and stem-bark fractions were more active on *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Baccilus subtilis*, than the leaves fraction. These findings support the claim for its treatment of bacterial and fungal infections.**

Key words: *Pakia clapperotoniana keay*, phytochemical, antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Plants serve as the basis of traditional medicine systems for thousands of years in Nigeria, India, China, Indonesia etc. (Hammer, 1999). In Yola, North eastern Nigeria, tuberculosis is a health challenge (FHI, 2001; Nwankwo et al., 2005; Emokpae et al., 2006) and is believed that *Pakia clapperotoniana keay* possesses medicinal properties that are effective in the management of tuberculosis and other ailments. The medicinal flora in the tropical eco-region has a preponderance of plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements. Collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as secondary metabolites (Fatope, 2001).

The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these include: alkaloids, glucosides, Steroids, flavanoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, body building (Chidambara et al., 2003). Biomolecules of plant origin appear as alternatives for the control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis (Mathias et al., 2000; Ganguly et al., 2001; Martino et al., 2002).

The plant *P. clapperotoniana keay* (*Dorawa* in Hausa, *Igba* in Yoruba, and *Ogili* in Igbo) is a savanna tree, easily recognized by its bright red pendulous flowers. It is 21 m high, bole short and crooked with twisted spreading branches forming a very wide crown bark grey very rough with longitudinal fissures, flaking off in small thick, more or less regular patches, slash brown fibrous exuding a little gum (Keay, 1989).

The stem-back of *P. clapperotoniana keay* has been used in Adamawa State in Northern Nigeria in forms of decoction and concoctions for the cure of persistent cough, catarrh, diarrhea tuberculosis and other ailments (kubmarawa et al., 2007).

Hot water extract of the leaves is taken orally as a preventive against leprosy (Arthur, 1954). The leaves mixed with those of *Laranthus* spp. ground with a pinch of

*Corresponding author. E-mail: dkubmarawa@yahoo.com.

potash treat skin diseases (Arthur, 1954). The seed husks are composed of flavones, 2-hydroxyl, 4-5 - 7-8 pentene (Larsen et al., 1996). The plant also contains fucosterol and situsterol (Schinden et al., 1998). The seed is composed of 29.40% proteins (Balogun et al., 1986).

This paper reports the potentials of *P. clapperotoniana keay* for its anti-microbial activity. Also in mind is sourcing of extracts from, *P. clapperotoniana keay* which could provide the expected chemotherapy against human pathogens.

MATERIALS AND METHODS

Sampling

Fresh sample of the roots, stem- bark and leaves of *P. clapperotoniana keay* were collected in June, 2006 from the North Eastern States (Adamawa and Gombe) Nigeria. Identification was by the forestry Department of Federal University of Technology Yola. The samples were air dried in the laboratory before powdering.

Extraction

180 g each of the powdered roots, stem- bark and leaves of the plant were percolated with 2 L of distilled ethanol for two-weeks. After which there was decantation, filtration, and concentration on rota-vapour (Stuart RE 300 series, UK, R110) at 40°C to obtain ethanol soluble fractions, (F_E01), labeled, $F_{E0}R$ (09 g), $F_{E0}S$ (10 g) and $F_{E0}L$ (07 g) respectively. A portion of each was used for the phytochemical screening while the other kept in the refrigerator for the sensitivity test.

The above procedure was repeated, now on 250 g each in order to increase the quantity of the extracts with the use of 2 L of water, giving the following fractions, F_W01, labeled, $F_{W0}R$ (10 g), $F_{W0}S$ (11 g), $F_{W0}L$ (09 g) respectively.

Phytochemical screening

Phytochemical analysis of all the evaporated solvent extracts was conducted in accordance with the standard procedure (Harborne, 1992). Tests for saponins, tannins, flavanoids, volatile oils, glycoside, alkaloids, phenols and resin were carried out in all the fractions.

Preparation of stock solution

Six different extracts ($F_{E0}R$), ($F_{E0}S$), ($F_{E0}L$) and ($F_{W0}R$), ($F_{W0}S$) and ($F_{W0}L$) were used for both analyses. Concentrations (in triplicates) were produced from each extract at 2000, 1500, 1000, and 500 µg / (cm^3) respectively using the dilution formula (Almagboul, 1985).

Antibacterial assay

Six species: *Staphylococcus aureus*, S*treptococcus* spp., *Escherichia coli*, *Pseudomonas aeroginosa*, *Salmonella* spp. *and Baccilus subtilis*, stock cultures were collected from the Specialist Hospital Yola. These organisms were identified in the Microbiology Department Federal University of Technology Yola. The stock was maintained on nutrient agar slant and subculture in nutrient broth for incubation at 37°C prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar-prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using a Whatman (Grade No. 1) filter paper. 100 discs were obtained by punching and putting in vials-bottles and sterilizing in an oven at 150°C for 15 min.

Prepared disks containing the various fractions were carefully placed on the inoculated plates using a sterilized forceps in each case. The plates were then turned upside-down and incubated at 37°C for 24 h in an incubator Genlab Ltd., UK.

The result was taken by considering the zone of inhibition by the test fractions (Mackie and McCartney, 1989). Activity and inactivity were observed in accordance with the standard and acceptable method.

RESULTS

DISCUSSION

Phytochemical screening portrays that most of the natural products tested for were present in the plant material except alkaloids and resin which were not detected in any of the tested fractions. Volatile oils, phenols and glycoside are present only in the ethanol extracts and both absent in the water extracts. This shows the generality of the components in medicinal plants. Biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Mixtures of such chemicals show a broad spectrum of biological effects and pharmacological properties. To a large extent, the phonological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Felix, 1982). (Table 1)

Table 2 shows the zones of inhibition (mm) of the various plant parts against the microorganisms. The stem-bark and the leave extracts of both water and the ethanol fractions are highly efficacious, covering nearly the entire spectrum of organisms. *Salmonella* spp., alone, seemed to have developed resistance against these extracts. However, the roots extracts from both solvents are less effective or have very low activity, with flowering, fresh fruiting and mature fruiting stages (Cuneyt et al., 2007).

Table 1. Phytochemical analysis of extracts from roots, stem-bark and leaves of *P. clapperotoniana keay*.

Phytochemical name	Water extract			Ethanol extract		
	Root	Stem-bark	Leaves	Root	Stem-bark	Leaves
Saponnins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavanoids	+	+	+	+	-	+
Volatile oils	-	-	-	+	+	+
Glycoside	-	+	-	+	+	+
Alkaloids	-	-	-	-	-	-
Phenols	-	-	-	+	+	+
Resin	-	-	-	-	-	-

- Absent; + Present

Table 2. Antibacterial activity of the extracts from roots, stem-bark and leaves of *P. clapperotoniana keay* against human pathogenic bacteria.

Solvent name	Plant parts	Zone of inhibition (mm) ± Standard error					
		S. aureus	***Streptococcus* spp.**	***E. coli***	***P. aeroginosa***	***Salmonella* spp.**	***B. subtilis***
Water	Roots	R	10.5±0.4	R	10.7±0.4	R	14.6±0.2
	Stem-bark	08.2±0.2	12.2±0.2	14.3±0.4	08.5±0.1	12.5±0.3	12.4±0.1
	Leaves	15.1±0.2	14.3±0.1	12.8±o.5	14.5±0.1	R	10.3±0.2
Ethanol	Roots	R	12.4±0.4	R	12.2±0.0	R	16.4±0.2
	Stem-bark	10.4±0.3	14.3±0.0	15.3±0.7	10.4±0.3	14.2±0.2	14.6±0.1
	Leaves	16.1±0.2	15.2±0.3	14.6±0.8	16.6±0.2	R	12.3±0.2
Control	Gentamicin	21.6±0.1	13.8±0.3	14.6±0.1	9.5±0.10	17.0±0,2	11.5±0.2

R = Resistance

However, studies have indicated that certain bioflavonoid have inhibitory activity against human pathogen bacteria (Lin et al., 2001) and other components that acts in similar way as the control, gentamicin. This analysis suggests that, the water and the ethanol extracts of the roots, stem- bark and leaves of *P. clapperotoniana keay* probably contain active agent(s) and this provides the basis for their folkloric use as a cure for some human ailments. This assertion is also confirmed, as their extracts indicate a relatively moderate number of phytochemicals present. It is suggested that more research be conducted that will further elucidate and characterized the active components and possible mechanism involved in the use of this plants in the ethno medical practices.

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