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ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BACTERIA TO SEED EXTRACTS OF *RICINUS COMMUNIS*: FINDINGS OF A PRELIMINARY STUDY IN NIGERIA

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ABSTRACT
Aim To ascertain the antibacterial properties inherent in seed extracts of *Ricinus communis*.

Procedure Dry seeds of *R. communis* were deshelled, grounded to powder and extracted both with alcohol and water using Soxhlet machine. Different concentrations of the extracts were tested against selected bacteria using diffusion method of susceptibility testing on sensitivity testing agar medium.

Results *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Staphylococcus aureus* and *Enterococcus faecalis* were highly susceptible to both the methanol and water extracts of the seed while *Pseudomonas aeruginosa* showed reduced susceptibility.

Conclusion The active antimicrobial ingredients in *R. communis* should be identified while its medicinal value to humans properly investigated in this regard.

KEY WORDS: *Ricinus communis*, Seed Extracts, Antimicrobial Susceptibility.

INTRODUCTION

Ricinus communis popularly called Castor bean in English is a plant that is widely distributed in virtually in all continents of the world (De Vendra, and Raghavan, 1978). Its leaves are verticulate, long- pertiolate with palmately divided laminae; flowers in terminal panicles are usually overtopped by lateral shoots; male flowers clustered at the bottom, female flowers above, with inconspicuous caduceus perianth. The fruits are globosse, triocular, soft-spiny capsules; the seeds are ovoid, compressed dorsally, thick, shiny pale-grey to almost black with dark mottling (Solaki, *et al*, 2006; Alm, *et al*, 2007; and, Iyothi, *et al*, 2006).

R.communis has found several daily applications in human activity (Challenger, and McCarron, 1990; and, Kinamore, *et al*, 1980): the oil seed is used in coating fabrics and other protective coverings (Venugopal, *et al*, 2006); the hydrogenated oil is utilized in the manufacture of waxes, polishes, carbon paper, candles and crayons (Hugnet-Termes, 2001; Wilcox, and Bodeker, 2004; and, Verscht, *et al*, 2006). It has been used with belief to cure several ailments: arthritis, asthma, boils burns, cancer, carbuncles, catarrh, chancre, cholera, cold, colic, convulsions, and 'craw-craw', to mention but a few (Lakshminaravana, and Sujatha, 2005; and, Korwar, *et al*, 2006).

Several biophysical properties have been associated with *R. communis*. In Jos, Nigeria, whole seeds in stat doses were found to stop pregnancy for about a year in two separate studies among volunteers (Isichei, *et al*, 2000; and, Das, *et al*, 2000). In Norway (Hekland, *et al*, 2000), it was found that the B chain of *R. communis* activates human complement thus boosting immunity; while findings from Sudan (Fakhri, 1989) revealed that, *R. communis* from various plant extracts were found so attain selectively various portions of the cell bodies of hippocampal neurons with defined functions. Also in India (Ilavarason, *et al*, 2006), its anti-inflammatory and free radical scavenging activity by inhibition of lipid peroxidation was well demonstrated.

From the microbiological stand point, there has not been much published data on *R. communis* (Choroma, *et al*, 1985; Villalta and Kierszenbaum, 1984; and, Ng and Ling, 1999) especially as concerns the antimicrobial properties of its various extracts on bacteria, fungi, viruses and parasites.

In view of the fact that bacteria have assumed an unprecedented level of antimicrobial resistance more than ever in the history of modern medicine (Jombo, *et al*, 2006a; Jombo, *et al*, 2006b; and, Jombo, *et al*, 2006c): the continuous search for more reliable antibiotics becomes a worthwhile and noble mission. This study was therefore set up to ascertain the antibacterial properties of the seed extracts of *R. communis*.

MATERIALS AND METHOD

Setting The study was carried out in Jos Plateau state of Nigeria between August and November 2005.

Seed Preparation Seeds of *R. communis* were obtained from Pharmacology Department of the University of Jos. These were deshelled and then crushed into fine powder using laboratory mortar and pestle.

Soxhlet Extraction The solvent used was absolute methanol. Twenty grams of the ground dry sample of the seed was placed in an extracting thimble and placed in the soxhlet apparatus. A water condenser was attached to the soxhlet apparatus at the top. The apparatus was fitted into the neck of a flask containing 250mls of the methanol (solvent) heated on a water bath.

The vapour from the solvent reached the soxhlet apparatus through the side tube and condensed on passing into the condenser. The condensed solvent dropped on the crude substance in the thimble and dissolved the required substance. The solution filtered through the thimble into the flask bearing the solvent. This process continued until the solvent from the thimble was colourless. Extraction was then said to be completed. This continuous extraction method extracted all the components of the plant, which were soluble in methanol. The extract was then evaporated to dryness and a light brown oily extract was collected, weighed and stored by refrigeration at temperature of 4^0C for further susceptibility testing. Similar procedure was carried out for water extraction where water was used in place of methanol.

Antimicrobial Susceptibility Testing Bacteria used for the study were obtained from the Microbiology laboratory of the Jos University Teaching Hospital (JUTH), Jos. Organisms tested were: *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis*. Whitman no 1 filter paper was used to prepare susceptibility discs of 4mm in diameter, which were sterilized in hot air oven. Commercially prepared discs of ceftriaxone (30ug) were used as positive control, which was susceptible against all the organisms, tested, while either sterile distilled water or methanol was used as negative control. The refined oil extracts obtained was either mixed with methanol or warm sterile distilled water in varying concentrations. With a fine pipette, 0.02 mls of each concentration was impregnated with a sterile sensitivity disc; similar preparations were done with methanol and sterile distilled water. Sensitivity testing agar media were dried for 30 minutes at 37^0C and then flooded with about 0.5 McFarland's broth culture equivalent of the selected organisms. Using sterile forceps, the commercially prepared ceftriaxone discs and discs impregnated with appropriate concentrations of methanol and water extracts along with the negative controls were carefully placed on the flooded agar media.

The preparation was incubated overnight at 37^0C and the radiuses (in millimeters) of zones of inhibition were measured using vernier calipers (Scott, 1989).

Interpretation of Results The sensitivity report was interpreted as Sensitive (S), Intermediate (I) and Resistant (R) as follows:

Sensitive (S) Zone radius of inhibition wider than, equal to, or not more than 3mm smaller than the positive control.

Intermediate (I) Zone radius of inhibition is more than 3mm smaller than the positive control but not less than 3mm.

Resistant (R) No zone of inhibition or zone radius measures 2mm or less.

Analysis of Results Results obtained were analysed using simple descriptive methods.

RESULTS

All the organisms tested were Resistant to both the methanol and water extracts at 5mg/ml concentrations except *Proteus mirabilis* which was Intermediate against the water extract. Similarly, most organisms were Resistant to the extracts at 6mg/ml strengths except the water extracts against *Proteus mirabilis* (Intermediate), and methanol extracts against *Staphylococcus aureus* (Intermediate).

For the 7mg/ml concentrations, *Proteus mirabilis* and *Staphylococcus aureus* were Intermediate for both forms of extracts; *Klebsiella pneumoniae* Intermediate and Resistant for the methanol and water extracts respectively; *Pseudomonas aeruginosa* Resistant and Intermediate for the methanol and water extracts respectively. Both extracts were Resistant against *Enterococcus faecalis* but Intermediate and Sensitive against *Escherichia coli* respectively.

Most of the organisms were Intermediate to Sensitive for the 8mg/ml extracts of both methanol and water except *Enterococcus faecalis* which was Resistant to both extracts as well as the methanol and water extracts of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively.

Both the methanol and water extracts at 9mg/ml strengths of the extracts were Intermediate to Sensitive against all the organisms tested, while the 10mg/ml of both extracts were Sensitive against all except the water extracts against *Pseudomonas aeruginosa* which was Intermediate.

Table1Antimicrobial susceptibility patterns of bacteria to various concentrations of the seed extracts of *R. communis.*

Concentration of extracts inside disks* used (volume =0.02mls)

	5mg/ml		6mg/ml		7mg/ml		8mg/ml		9mg/ml		10mg/ml	
	A	B	A	B	A	B	A	B	A	B	A	B
Klebsiella pneumoniae	R	R	R	R	I	R	I	R	I	I	S	S
Proteus mirabilis	R	I	R	I	I	I	S	I	S	I	S	S
Staphylococcus aureus	R	R	I	R	I	I	I	I	I	S	S	S
Pseudomonas aeruginosa	R	R	R	R	R	I	R	I	S	I	S	I
Enterococcus faecalis	R	R	R	R	R	R	R	R	I	I	S	S
Escherichia coli	R	R	R	I	I	S	S	S	S	S	S	S

Key: A= Methanol Extracts
B= Water Extracts
S= Sensitive
I= Intermediate
R= Resistant
*Disk Diameter= 4mm

DISCUSSION

Generally all the bacteria tested (*Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis*) had a steady increase in susceptibility patterns from almost all resistant at 5mg/ml concentration through intermediate/sensitive at 9mg/ml to virtually all sensitive at 10mg/ml. This finding points to the fact that, there is a possibility to exploit the antibacterial properties inherent in the seed extract of *R. communis* for large scale medicinal uses. The finding is beneficial as it also heralds probably the emergence of a new antibiotic with such a wide spectrum of activity as found in the study being added to the existing list of them.

The active antibacterial ingredients in the Castor seed extracts should be identified and processed in possibly commercial quantities in order to seek its relevance in the current war against antimicrobial resistance. This no doubt poses a serious challenge to the modern day practice of medicine (Albertin, *et al,* 2002; Krishna, *et al,* 2004; Cuevas, *et al,* 2004; and, Ishikawa, *et al,* 2004). The fact that treatment of infections caused by organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis* are increasingly becoming difficult (Cuevas, *et al,* 2004; and, Ishikawa, *et al,* 2004) further strengthens the importance of these present findings and the need for a continuous search of antibiotics with comparative advantage.

Pseudomonas aeruginosa appeared less susceptible to the extracts compared to the other organisms. The high profile resistance of this organism against several antimicrobials in current use has severally been documented (Bouza, *et al,* 1999; and, Chastre, and Trouillet, 2000).

Further work should be carried out to identify the active ingredients with the antibacterial properties as well as the tolerable human dose range vis-à-vis the minimum inhibitory concentration (MIC).

In conclusion, seed extracts of *R. communis* of both ethanol and water preparations were found to be highly active against several bacteria tested. Hence, active ingredients of these seed extracts should be identified and consequently its medicinal benefits to humans exploited.

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