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ANTIBACTERIAL PROFILE OF FERMENTED SEED EXTRACTS OF *RICINUS COMMUNIS*: FINDINGS FROM A PRELIMINARY ANALYSIS

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Summary: The study was carried out to ascertain the antibacterial properties inherent in fermented seed extracts of *Ricinus communis*. Dry seeds of *R. communis* (Castor oil plant) were deshelled, grounded to powder, fermented, and then 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. *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, and Staphylococcus aureus* were highly susceptible to both the methanol and water extracts of the seed while *Pseudomonas aeruginosa* showed reduced susceptibility. *Enterococcus faecalis* on the other hand was resistant to all the preparations tested. The active antimicrobial ingredients in fermented *R. communis* seeds should be identified while its medicinal value to humans properly investigated.
Key Words: *Ricinus communis,* Fermented, Seed Extracts, Antimicrobial Susceptibility.

Introduction

Ricinus communis is a plant commonly found in both the tropical and temperate climates of the world (Lakshmamma, and Prayaga, 2006; and Raoof, and Yasmeen, 2006). Right from antiquity, the plant has served various purposes to various generations of people to the present day; while presently, a more scientific search light is being thrown at this mystery plant of ages (Armstrong, 1982; Farnworth, *et al*, 185; Estes, 1995; Korwar, *et al*, 2006; and Li, *et al*, 2006).

The seeds of *R. communis* popularly called castor oil plant, is biochemically composed of various macromolecules: the fat which is about 15% to 25% consists of about 40-53% of fixed oil comprising glycosides of ricinoleic, isoricinoleic, stearic and dihydroxy stearic acids (Lin, and Areinas, 2007; Chen, *et al*, 2007). Also the seeds contain about 25% protein with 10-20% carbohydrates, 2.2% ash and 5.1-6.5% moisture (Verscht, *et al*, 2006; Raghavaiah, *et al*, 2006; Guhling, *et al*, 2006; and Devendra, and Raghavan, 1978).

The seeds of *R. communis* have several traditional applications (Devendra, and Raghavan, 1978; and Gaydou, *et al*, 1982). They have been used with arguable success in the treatment of warts, cold tumours, indurations of the mammary glands, corns and moles, to mention but o few (Gibbs, *et al*, 2002; Wilcox, and Bodeker, 2004; Huguet-Termes, 2001; and Sathiyanathan, 2005). The castor oil is also currently used for dyeing cotton fabrics, with alizarin in leather production; dehydrated oil is an excellent drying agent; while hydrogenated oil is used in the manufacture of waxes, polishes, carbon paper, candles and crayons (Rutten, *et al*, 2003; Kumari, *et al*, 2003; Dungarwal, *et al*, 2002; and Reddy, *et al*, 2002).

R. communis has been shown to influence several metabolic as well as histochemical activities in the human body. In Sudan (Fakhri, 1989), its extracts were found to cause proportional increase in mean wheal diameter in skin tests in Castor bean allergic workers; in Japan (Ikeda, *et al*, 1991), the selective binding of its glycoconjugates in gerbil hippocampal neurons with possibly distinct functional delineations was demonstrated. Also in India (Havarasan, *et al*, 2006), the anti-inflammatory and free radical scavenging activity was well demonstrated; while in Jos, Nigeria (Isichei, *et al*, 2000; and Das, *et al*, 2000), and India (Sandhyakumary, *et al*, 2003), its antifertility properties have well been proven among humans.

There has not been elaborate published work on the antimicrobial activity of fermented *R. communis* extracts involving microbes (Sandhyakumary, *et al*, 2003; Choroma, *et al*, 1985; and Villalta, and Kierszenbaum, 1984). Bacteria, in particular, at present, pose serious threat to humanity in the treatment of pyogenic infections (CDC, 2002; and Sule, *et al*, 2002). This is as a result of the unprecedented rate at which bacteria develop resistance against the available antimicrobial agents currently in use (Taiwo, *et al*, 2002). There is therefore the need for a continuous search for newer antibiotics with higher efficacy and comparable advantage over the ones already in use. This indeed forms the basis for the present study.

Materials and Methods

The dry seeds of *Ricinus communis* were obtained from the Pharmacology Department of University of Jos.

Fermentation of the Seeds: Deshelled seeds were weighed 50g and then boiled in plain water for six hours; this was then crushed using laboratory mortar and pestle, and then incubated for two days at 37^0C (Baron, 1994).

Seed Extraction Using Methanol: The fermented seeds were subjected to exhaustive soxhlet extractor using 150ml methanol for three days at 50^0C. At the end of the extraction, the methanol was evaporated at 65^0C using evaporator and a light yellowish oily paste was obtained. This was weighed and stored in the refrigerator at 4^0C (Baron, 1994).

Seed Extraction Using Water: The fermented *Ricinus communis* was mixed with 100ml of sterile distilled water. This was kept on a shaker for three hours. The mixture was then filtered using Whitman no 1 filter paper and the extract was collected into a sterile flask. The extract was evaporated at 65^0C on an evaporator and a light brown oily paste was obtained. This was weighed and stored in the refrigerator at 4^0C (Scott, 1989; and Baron, 1994).

Antimicrobial Susceptibility Testing: Bacteria used for the susceptibility study were obtained from the Microbiology laboratory of the Jos University Teaching Hospital (JUTH), Jos. Organisms tested were: *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes,* Coagulase Negative Staphylococcus (CONS) 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 controls which were active against all the organisms tested, while either sterile distilled water or methanol was used as negative control. The refined oil extracts obtained were either mixed with methanol or warm sterile distilled water in varying concentrations. With a fine pipette, 0.02 mls of each concentration was diffused into 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 (Scott, 1989).

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

Interpretation of Results The sensitivity report was interpreted as Sensitive (S), Intermediate (I) and Resistant (R) as follows (Scott, 1989; and Baron, 1994). *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.

Table 1: Antimicrobial susceptibility pattern of selected bacteria to the fermented seed extracts of Ricinus 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	I	R	I	R	S	R	S	I	S	S
Proteus vulgaris	R	R	R	R	I	R	S	R	S	R	S	I
Coagulase Negative Staphylococcus (CONS)	R	R	I	R	I	I	S	I	S	S	S	S
Pseudomonas aeruginosa	R	R	R	R	R	R	I	R	S	I	S	S
Enterococcus faecalis	R	R	R	R	R	R	R	R	R	R	R	R
Escherichia coli	R	R	I	I	I	S	S	S	S	S	S	S
Streptococcus pyogenes	R	R	R	I	I	S	S	S	S	S	S	S
Staphylococcus aureus	R	R	R	R	I	I	S	S	S	S	S	S

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

Results

Several micro-organisms were tested against both the methanol and water extracts of various concentrations of *R. communis*.

Klebsiella pneumoniae isolates were resistant to most of the preparations of 5mg/ml to 8mg/ml but sensitive at concentrations of 9mg/ml and above. *Proteus vulgaris* was similarly resistant to concentrations 9mg/ml and below but sensitive at concentrations of 10mg/ml. Coagulase negative Staphylococcus (CONS) was intermediate or sensitive to both the methanol and water extracts of *R. communis* at concentrations of 7mg/ml and above. *Pseudomonas aeruginosa* on the other hand showed only appreciable sensitivity at concentrations of 9mg/ml and above. *Enterococcus faecalis* isolates were however resistant to both the methanol and water extracts of all the concentrations tested. *Escherichia coli* isolates were either intermediate of sensitive to the extracts at concentrations as low as 6mg/ml with *Streptococcus pyogenes* showing similar sensitivity patterns. *Staphylococcus aureus* isolates were resistant to the water and methanol extracts of *R. communis* concentrations at 5mg/ml and 6mg/ml, but either intermediate or sensitive at concentrations of 7mg/ml and above.

Discussion

Significant susceptibility was recorded by most of the organisms tested (*Klebsiella pneumoniae, Proteus vulgaris,* Coagulase Negative *Staphylococcus* (CONS), *Pseudomonas aeruginosa*, and *Escherichia coli*) to both the alcohol and water extracts of fermented seeds of *Ricinus communis* except *Proteus vulgaris* which showed a comparatively reduced susceptibility pattern. This susceptibility pattern exhibited by the tested organisms to these fermented *Ricinus* extracts could be exploited for probably medicinal purposes in chemotherapy among humans and other animals.

The findings from this study partly agree with that of an earlier study on unfermented extracts of the same plant where *Klebsielle pneumoniae, Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus* were found to be appreciably susceptible Jombo, and Enenebeaku, 2007). The susceptibility of organisms such as *Staphylococcus aureus* and *Proteus vulgaris* to the unfermented extracts of *R. communis* could be of unique benefit especially in the present wide spate of high resistance currently exhibited by these organisms in the treatment of various infections (Jombo, *et al*, 2006; Jombo, *et al*, 2007a; and Jombo, *et al*, 2007b). Strains of methicillin resistant *Staphylococcus aureus* (MRSA) commonly complicating post burns infections, which at present appear to be resistant to all the available antimicrobials could be tried against this extract in order to assess their usefulness in that regard (Amani, *et al*, 2003; and Christof, *et al*, 2000).

The findings from the present study show fermented R. *communis* extracts to have a wide spectrum of activity involving several gram positive as well as gram negative organisms. This property could be of immense clinical benefit involving clinical trial on other life threatening bacterial infectious agents such as *Salmonella typhi, Bacillus anthracis,* and *Neisseria meningitidis* as well as its activity on fungi and helminths.

Findings from the fermented *R. communis* extracts are partly different from that of the unfermented extracts of the plant where water extracts showed intermediate activity at higher concentrations compared to the present increased activity of the water extracts over alcohol at the same concentrations (Jombo, and Enenebeaku, 2006). The probability of the existence of different antimicrobial agents in the two preparations is likely as well as alterations in concentrations of a similar active ingredient in the two preparations. Further work however may be required to establish the validity of these propositions.

Generally, with the current spread of antibiotic resistance almost at geometric scale (Olayinka, *et al*, 2004) and obvious challenges confronted with by medical practitioners in the treatment of infectious diseases (Taiwo, *et al*, 2002), proper attention should be given to such plants to reap the potential antimicrobial benefits inherent in them. In like manner, the actual antimicrobial ingredients need to be extracted and identified, also its tolerable levels in the human body as well as any toxic effects on humans and animal tissues be investigated accordingly.

In conclusion, both alcohol and water extracts of fermented seeds of *Ricinus communis* were found to be substantially active against several bacteria. Hence, the antibacterial ingredients should be identified and probable medicinal benefits in chemotherapy among humans and other animals exploited. Also its toxic properties as well as its tolerable levels in humans be evaluated as well.

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