

Evaluation of Antitrichomonal Activity of Aqueous Extracts of *Pentaclethra Macrophylla* against *Trichomonas Gallinum*

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ABSTRACT

Aqueous extracts of the seed, stem-bark, fruit pericarp and the leaves of *Pentaclethra macrophylla* (Benth), grown in south eastern Nigeria were tested for antitrichomonal activities using *T. gallinum* grown in the laboratory. The leaf extract showed no activity against the bird parasite while the seed, fruit pericarp and bark showed some significant degree of inhibitory activities with Minimal Lethal Concentrations ranging from 100 - 1000µg/ml. The fruit pericarp exhibited the highest antitrichomonal activity of all the three positive extracts, though its activity was significantly ($P<0.05$) lower than that of the reference drug, metronidazole, which recorded Minimal Lethal Concentrations ranging from 10 - 1000 µg/ml. With increasing reports of metronidazole-resistant *Trichomonas* species, the need to identify new candidates that can replace the old drugs with proven parasite- resistance, and which can also be used by local people to treat human trichomonal diseases becomes imperative.

KEY WORDS: *Pentaclethra macrophylla*, *Trichomonas gallinum*, Antitrichomonal activity, ethnobotanicals, South Eastern Nigeria.

INTRODUCTION

Trichomonas gallinum is the etiologic agent of canker in pigeons and flounce in birds of prey (De Carli and Tasca, 2002). It is a flagellated protozoan inhabiting the upper digestive tracts and various organs of different avian groups especially

doves and pigeons (Columbiformes). The domestic pigeon, *Columbia livia*, is the primary host of this parasite. It has also been found in Java sparrows, various raptors, and Sea gulls (De Carli and Tasca, 2002).

Over the years, emphasis on the treatment of *Trichomonas gallinae* has been based on the administration of nitroimidazole, since the discovery of the drug by Stabler and Mellentin in 1951 but in recent times, metronidazole (its derivative), used for the treatment of human *T. vaginalis* (Cosar and Julou 1959), has equally been found very effective (Bussieras *et al.* (1991). Unfortunately, the first report of treatment failure with metronidazole was made in 1962 (Meingassner, *et al.* 1978), and ever since then, more reports have been made (Grossman and Galask, 1990; Sobel and Brown, 2001; Lo *et al.*, 2002; Crowell *et al.*, 2003). This then calls for continuous search for candidatures with anti-trichomonal property, which can substitute old ones with proven parasite -resistance.

Plant-based drugs used in traditional medicine are currently receiving great attention. The reason is because they are accessible, cheap and have little side effects. It has been estimated that about 80% of the world's population rely mainly on herbal remedies (Kumara, 2001). This work then attempted to evaluate the anti-trichomonal activity of *Pentaclethra macrophylla*, which is used locally for some protozoan diseases and very recent work by Oparaocha and Okorie (2009), proved its antiplasmodial activity.

Pentaclethra macrophylla, the African oil bean tree is relatively abundant throughout the tropical forests of Eastern Nigeria where protozoan parasitic diseases like trichomonas and malaria are endemic. It is a large luminous woody plant that belongs to the sub-family, Mimosoidae (Keay, 1989). The raw seed is a potential source of edible protein and calories, containing the twenty (20) essential amino acids and essential fatty acids that make up more than 80% of fatty acids in the oil (Ikediobi, 1981; Enujiugha and Agbede, 2000), and is usually eaten when fermented as salad, a process that increases the nutritional value (Enujiugha, 2003).

Traditionally, the whole fresh leaves of *P. macrophylla* are given to ruminants as feed, but in humans, fresh aqueous extracts of the leaves is taken orally against ailments like diarrhea and dysentery (Aka, *et al.*, 1999). The seeds are used in the preparation of liniments against itching and the oil extract from the seed is used in treating psoriasis, dysentery and helminthiasis (Singha, 1963). The medicinal uses of the bark include treatment for gonorrhea and epileptic fits (Githens, 1948). The antinociceptive and anti-inflammatory properties, as well as antiplasmodial property of the seed and leave extracts have also been demonstrated (Okorie *et al.*, 2006, Oparaocha and Okorie, 2009). There has been no literature on the use of *P. macrophylla* in the treatment of *T. gallinum*. Thus, the successful evaluation of the extract as anti-trichomonal agent will contribute tremendously to the control of the disease in birds and related infections like the widely prevalent trichomoniasis in humans.

MATERIALS AND METHODS

Collection of Plant Materials

The seed, fruit-pericarp, leaf and stem-bark of *Pentaclethra macrophylla* (Benth) were collected from Umuahia, Abia State, Nigeria and were identified by a botanist.

Extraction Procedure:

Four different extracts were prepared; seeds (200g) and grinded pericarp (213g) were each boiled in 400ml of distilled water, filtered and lyophilized to obtain the extracts. The powdered stem bark (225g) and grinded dried leaves (39g) were macerated in 1L of distilled water, filtered and equally lyophilized to obtain the respective aqueous extracts.

Anti-Trichomonal Activity

Preparation of Ringer's Solution

The following salts; 6.5g NaCl, 0.14g KCl, 0.01g NaH₂P₀₄ and 0.2g NaHCO₃ were first dissolved in

small quantity of distilled water. They were then mixed in a liter (1L) of distilled water with 0.12g CaCl₂, which was poured last. Two grams (2.0g) (10%) glucose solution was made separately in distilled water.

Preparation of Culture Medium

The albumin of an egg is mixed with 12.5ml of Ringer's solution in a neat cup using a small fork. Two milliliter (2ml) each of this solution was dispensed into the small cotton-stoppered test tubes. They were then removed and placed in a slanting position on a flat plate, autoclaved for 20 minutes at 15lb pressure; to sterilize the egg slants. Ringer's solution and 10% glucose were equally treated in like manner; 1ml of 10% glucose was pipetted into 50ml of Ringer's solution, the resultant mixture was warmed for some minutes till it was as hot as the hand could bear. Horse serum (1ml) was pipetted into it. This becomes the overlay. The overlay is used to top the egg slant. This mixture (overlay and the egg slant) becomes the culture medium (pH 7.2), which was maintained at a temperature of 37°C.

Culturing Trichomonas:

Trichomonas gallinum was isolated from the crop of domestic pigeon – *Columbia livia* by introduction of moistened, sterile cotton swab stick into the crop and rolling the stick round the walls of the crop to collect any parasite lodging there. On removing the swab stick from the pigeon's crop, it was dropped into a test tube containing normal saline and rolled round to dislodge any parasite collected. A drop of the saline solution was observed under a microscope to confirm the presence of trichomonas. The saline solution was then dispensed into small test tubes containing the culture medium and incubated at 37°C. No antibiotics was added into the culture medium because previous studies revealed that the presence of antibiotics in the medium affected the pathogenicity level and decreased the hemolytic activity of *T. gallinae* isolates (Stabler, 1954). A daily check of the growth of the parasites was made under the microscope.

Screening For Anti-trichomonal Activity of the Plant Extract:

The stock concentration consisted of 40mg/ml of each extract dissolved in 1ml of distilled sterile water. One hundred (100µl) microlitre of each stock was taken and diluted in 900 µg of overlay; in serial dilution tubes previously labeled according to the extracts and the controls used. The above dilution was done based on the following concentration in (µg/ml): 1000, 100, 10

and 1. Metronidazole (0.04g) was dissolved in 1ml DMSO. Metronidazole served as a reference drug (positive control), while the overlay served as a negative control. The following concentrations ($\mu\text{g/ml}$) of metronidazole were used: 5, 2.5, 1.25 and 0.625. Fifty microlitre of each stock solution previously diluted with 900 μl of overlay was pipetted into the microtitre plates. A mixture of the overlay and stock solution formed the new culture solution. One hundred and fifty microlitre (150 μl) of the test substance (cultured trichomonae) was used to top each of the stock solution (50 μl) in the microwells. They were incubated in a steam incubator at 37°C. Checks were made at 24 hrs for growth of trichomonas using microscope.

Results and Discussion

The minimal lethal concentration (MLC) of *Pentaclethra macrophylla* (Benth) aqueous extracts on *Trichomonas gallinae* is shown in (Table 1). The extract from the leaf showed no effect against the parasite (*T. gallinae*), while the bark, fruit-pericarp and seed, showed inhibitory impact at high concentrations. The fruit pericarp showed the highest impact with MLC ranging from 100 -1000 $\mu\text{g/ml}$, while the others were at 1000 $\mu\text{g/ml}$. The MLC for the reference drug (metronidazole) is from 1 - 1000 $\mu\text{g/ml}$, while the overlay which served as negative control showed no inhibition.

The minimal lethal concentration (MLC) for the reference drug (metronidazole) was similar to that obtained by Müller *et al.* (1988). According to Meingassner and Palla (1986), MLC values exceeding 25 $\mu\text{g/ml}$ indicate a reduction in parasite sensitivity. But since the active extracts showed concentration-related inhibitory actions, isolating and purifying the active ingredient(s) in each and then concentrating it might give a good result. The extract from the leaf showed no evidence of antitrichomonal activity. This also gives credence to earlier findings that the leaf extract is not toxic (Okorie *et al.* 2006), and the traditional use of the leaves as feed for ruminants is quite alright.

The result from this study indicated that the extracts (seed, bark and fruit-pericarp) of *Pentaclethra macrophylla* contain active agents which can be used against trichomonae indicating a possible candidate that may be tried also against the human *T. vaginalis*. Githens (1948), equally observed that the bark was active against gonorrhoea; another venereal disease. It is however suggested that the flavonoidal constituents of the fruit-pericarp and seed are responsible for the observed activities but that of the bark is yet to be elucidated.

Table 1: Minimal Lethal Concentration (MLC) in $\mu\text{g/ml}$ of *Pentaclethra macrophylla* (Benth.) aqueous extracts

| Conc. $\mu\text{g/ml}$ | F.P | B | L | S | 0 | M | Conc. For M $\mu\text{g/ml}$ |
|---------------------------|----------------|----------------|----------------|-----------------|-----------------|---|------------------------------------|
| 1000 | - | - | 500 \pm 50.1 | - | 2000 \pm 50.1 | - | 5.000 |
| 100 | - | 200 \pm 10.3 | 200 \pm 16.7 | 500 \pm 35.1 | 1600 \pm 87.6 | - | 2.500 |
| 10 | 400 \pm 10.3 | 500 \pm 20.1 | 800 \pm 20.4 | 1000 \pm 16.1 | 5900 \pm 44.6 | - | 1.250 |
| 1 | 400 \pm 20.1 | 500 \pm 44.1 | 700 \pm 25.7 | 900 \pm 50.1 | 7300 \pm 48.4 | - | 0.625 |

Values are means \pm S. E. M

Key

| | | |
|-----|---|------------------|
| F.P | = | Fruit – Pericarp |
| B | = | Bark |
| L | = | Leaf |
| S | = | Seed |
| 0 | = | Overlay |
| M | = | Metronidazole |

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