

The Use of *Ageratum Conyzoides* L. [Asteraceae] As A Therapeutic Measure In The Treatment Of Breast Myiasis Sores In Rural Women And Associated Bacteria.

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Abstract: This study reports for the first time on the use of ethanolic extract of *Ageratum conyzoides* in women with breast myiasis. A total of fifty rural women were examined [ages 18-62 years old] and 17.25% had breast myiasis at the fishing settlements at Okoikri and Oyorokotor of Rivers State Nigeria. Women with cutaneous breast myiasis were treated with ethanolic leaf extracts of *Ageratum conyzoides*. Different numbers of maggots were extracted from the breasts and 92.7% of nursing mothers had their sores healed after the application of ethanolic extract of *A. Conyzoides*. After the extraction of larvae of *Cordylobia anthropophaga*, the application of ethanolic extract of *A. Conyzoides* extract caused the dryness of the open sores. The microbiological quality of the leaves, stem, flower and consortium of the leaves, stem and flower of *Ageratum conyzoides* was assessed showing the presence of some species of bacteria. Microbial quality of the plant parts was done to assess the presence of bacteria in the leaves, stems and flowers parts. The consortium of leaves, stem and flower had the highest bacterial count followed by the leaves, stem and flower. Total bacterial counts of the leaves, stem, flower and consortium were 2.5×10^7 cfu/g, 1.6×10^7 cfu/g, 6.6×10^4 cfu/g and 1.3×10^8 cfu/g respectively. Bacterial isolates identified included *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The implication of *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* to public health are of great significance.

Keywords: *Ageratum conyzoides*, therapy, breast myiasis sore, rural women, bacteria.

I. Introduction

Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been revealed to have valid utility and in Nigeria, about 80% rural population depends on its efficacy for their primary health care. Scientist from diverse fields in a similar efforts are investigating new flora with an eye to their therapeutic worth. A sense of urgency accompanies the search as the pace of species extinction continues.

Anthropogenic activities as well as urbanization are major factors that are affecting the growth, multiplication of *Ageratum conyzoides*, it is an annual herb with a long history of traditional medicinal uses in many countries of the world, especially in the tropical and subtropical regions. A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from this species. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities [Okunade, 2002].

A. conyzoides has been widely studied. It is believed that a detailed information as presented in this review on its phytochemistry and various biological properties of the extracts and the constituents might provide incentive for proper evaluation of the use of the plant in medicine and in agriculture. Some small companies in Brazil are reported to be using *A. conyzoides* as raw material for phytochemicals. The author has used the leaves to treat fresh wounds with great success. Test conducted in mice and rats for anti-inflammatory, analgesic and antipyretic activities have shown significant results without adverse side effects. Similarly clinical trials with arthrosis patients conducted with the ethanolic extract of the whole plant did not show any side effect [Marques-Neto et al., 1988]. The major constituents of the essential oil have been shown to produce precocious metamorphosis in insect larvae as well as sterility, moribund and dwarfishness in adult insects. Further work, however, still needs to be carried out on the toxicity of the plant and especially on the precocenes, which have been shown in few cases to cause hepatotoxicity in rats [Halpin et al., 1984; Hammond et al., 1995].

II. Materials And Methods

Prior to the commencement of the study we sought and obtained an ethic permit from the Ethics Committee of the Department of Medical Laboratory Sciences of the Rivers State University of Science and Technology, Port Harcourt. A copy of the ethic permit was given to each village head and consent of the patients was sought. The categories of women examined for the study included nursing mothers [NM]; Child-bearing women [CBW]; these two categories of women were between 18 and 38 years old, also the teenage [TNG] the other category of women covered those that were already into menopause [MW]. This group served

as the control and received only topical application of distilled water. The other two categories of women received topical application of 40ppm ethanolic extract of *A. conyzoides*. The number of sores associated with breast myiasis was counted and recorded per breast. Breasts of women infected by the myiasis agents were periodically [at 4 hourly interval] wiped with clean hand towels dipped in 40ppm ethanolic extracts of *A. Conyzoides* four times daily. Women that had myiasis infection with open sores were divided into three groups and the number of women in each group depended on the number with myiasis infection. The trial was replicated five times.

Plant Material

Conyzoides were collected in the month of March 2014 from the Department of Applied and Environmental Biology of the Rivers State University of Science and Technology, Port Harcourt, Nigeria.

Preparation Of The Extract.

The leaves, stems and flowers of *Ageratum conyzoides* were collected from the Department of Applied and Environmental Biology of the Rivers State University of Science and Technology, Port Harcourt. The plant parts were washed under a running tap to remove the adhering dirt and sand. All the plant parts [4g] were blended using in Moulinex Blender and extracted in 70% ethanol and 40ppm was made from the stock after the method of Orlu and Ogbalu, 2013.

Microbiological Quality Analysis Of Ageratum Conyzoides

The leaves, stem and flower of the *Ageratum* were obtained from the Botanical garden of the Applied and Environmental Biology Department of the Rivers State University of Science and Technology, Port Harcourt, Nigeria. These parts were washed with distilled water, air dried for a few days and ground. They were sent to the Laboratory for culture analysis. The agar used were nutrient agar (Laboratory M. Bury, Lancashire, U.K.), macconkey agar and Potato dextrose agar (PDA, Harrigan and mcccance, 1990).

Bacterial and Fungal Viable Counts

The method used was the 10-fold dilution method of Harrigan and mcccance (1990). Ten grams (10g) each of the ground leaves, stems, flowers and consortium of leaves, stems and flowers were aseptically transferred into 90ml of sterile normal saline in 150ml conical flasks. The flasks were shaken vigorously to dislodge the microbial flora. Further 10-fold dilutions were carried out by adding 1.0ml of the penultimate dilution to 9ml of fresh diluents. Finally, 0.1ml of an appropriate dilution was placed on dried nutrient agar, evenly spread with a sterile glass spreader and incubated at 30°C for 24hrs. At the end of the incubation period, counts were performed for the dilutions with counts between 30 to 300 colonies (Anonymous, 1994). All counts were performed in duplicates and the average taken.

Mould and yeast counts were enumerated by aliquots of appropriate diluted samples on acidified potato dextrose agar containing streptomycin (1mg / 100 ml). The plates were incubated at 30°C and counted after 48 hours for yeasts and 96 hours for moulds.

Similarly, 0.1ml of appropriate dilutions was inoculated on macconkey agar. Mean colony counts were calculated and expressed as colony forming units per gram (cfu/g) of the sample analyzed (Harrigan and mcccance, 1990).

Representative colonies of the ten-fold dilution of the leaves, stems, flowers and consortium of the leaves, stems and flowers were picked and sub cultured on nutrient agar until pure cultures were obtained. The pure cultures were stored on agar slants.

Identification And Characterization Of Isolates

The methods described in Cheesebrough (2000) were adopted in characterization of isolates. Isolates were identified by standard methods (Abbey, 2007).

Statistical tool

Results were subjected to statistical analysis employing the student t-test at 95% and 99% probability levels.

III. Results And Discussion

The total heterotrophic bacteria microscopic characteristics isolated from the leaves, stems, fruits and consortium of the *Ageratum* is shown on table 1. The total bacterial population of the leaves, stem, flower and consortium of leaves, stem and flower were 2.5×10^7 , 1.6×10^7 , 6.6×10^4 and 1.3×10^8 cfu/g respectively. Moulds and Yeasts were not obtained from the samples.

The identification of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was done with reference to Harrigan and mcccance (1990) and Cheesebrough (2000)

Statistical Analysis:

We adopted purposive sampling and we used analysis of variance.....

Results

Out of a total of fifty rural women examined [ages 18-62 years old], 17.25% had breast myiasis at the fishing settlements at Okoikri and Oyorokotor of Rivers State Nigeria.

Data collected showed in Fig.1 wounds of *C. Anthropophaga* larvae in a 38 year-old nursing mother. Fig. 2 showed the number of maggots of *Cordylobia anthropophaga* extracted from breasts of different categories of women in a Fishing settlement at Emanafor, Rivers State. The number of maggots collected from breasts of nursing mothers was significantly higher [13.5 larvae, n=30] than those from the breasts of other categories of women [8.6 larvae in CBW] and [3.4 in MN] [Fig.2], after an interval of three weeks, the sores in the breasts started healing [Fig.3]. In Fig. 4, the assessment on the efficacy of *A. Conyzoides* for the treatment of breast myiasis sores showed more than 92.7% cure in nursing mothers that received the treatment and they significantly differed from the category of women in their child bearing age that had 85.9[approximately 86%] cure [DMRT; P < 0.05].



Fig.1. Wounds Of *C. Anthropophaga* Larvae Prior To The Application Of Ethanolic Extract Of *A. Conyzoides*.

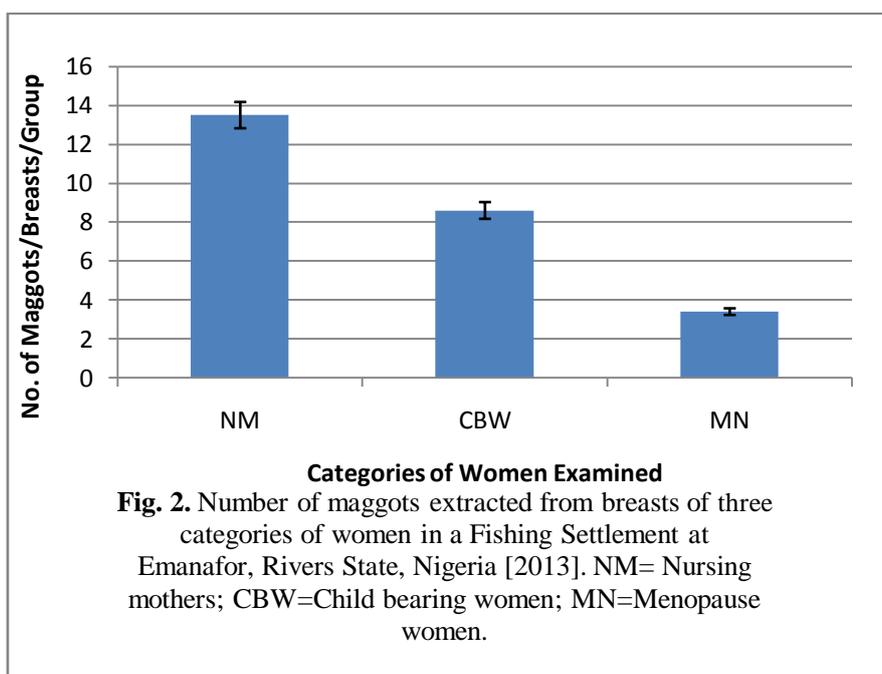
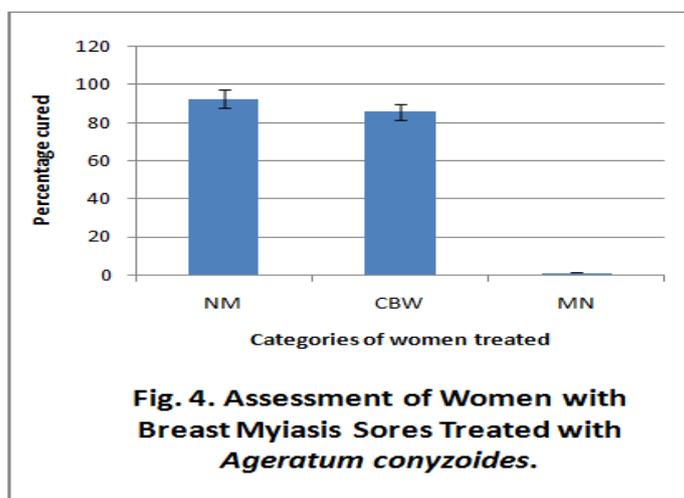




Fig. 3. The Infected Left Breast Of A 38 Year-Old Fish Farmer [A Nursing Mother] With Sores Of *C. Anthropophaga*; Breast Undergoing Treatment With *Ageratum Conyzoides*.



IV. Discussion

Over the years, WHO advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non- microbial origins (WHO 1978). Twenty-eight rural women of the Niger Delta were infected with the human warble fly (Tumbu fly) larvae, *Cordylobia anthropophaga*, and of the women examined, 22 (78.5%) were nursing-mothers [Ogbalu et al., 2006].

The therapeutic and protective roles of *Ageratum conyzoides* had been reported [Okunade, 2002; Kokwaro, 1976; Ita et al., 2007; Ita et al., 2009]. In the eastern states of Nigeria, *A. Conyzoides* is taken as a spice in porridges and records showed that spices have also been recognized to possess medicinal properties and their use in traditional systems of medicine has been on record for a long time. With the advancement in the technology of spices and on knowledge of the chemistry and pharmacology of their active principles, their health benefit effects had been reported [Srinivasan, 2005]. These studies documented digestive stimulant action, hypolipidemic effect, antidiabetic influence, antilithogenic property, antioxidant potential, anti-inflammatory property, antimutagenic, and anticarcinogenic potential of spices. Among these, the hypocholesterolemic and antioxidant properties of a few specific spices have far-reaching nutraceutical value. These beneficial physiological effects also have the potential of possible therapeutic application in a variety of disease conditions [Srinivastan, 2005]

In Nigeria and some African countries, the plant has been used in the treatment of scabies, crawl-crawl, ringworm, ulcers and some other skin diseases without any adverse effects. In the eastern states of Nigeria the leaves had been used in wound dressing and a decoction is used in relieving pains associated with omphalitis and colic. Generally, it is used traditional medicine for its antiasmatic activities and for the curing of various

diseases. *Lepidagathis alopecuroides* [Vahl] and *Bryllophyllum* species are some of the Niger delta plants' extracts that had been used in the flushing of the serosanguinous fluid that is associated with *Cordylobia anthropophaga* larval extraction [Ogbalu et al.,2012, 2013]

In the present study, it exhibited its property as an antipyretic agent in the treatment of breast myiasis sores. The application of ethanolic extracts of *A. Conyzoides* resulted in relieving pains associated with breast myiasis sore and initiated closing of wound sites within 72 hours after application. Some women squashed the leaves and spread them on the open sores and claimed immediate and faster relief of breast myiasis pain especially in lactating nursing mothers. The breast myiasis pain was not as painful as in non lactating mothers. The source of breast myiasis of *Cordylobia anthropophaga* is through soiled brassieres or improperly washed brassieres and breast pads as well as hand towels used in cleaning breast milks in rural nursing mothers. Ogbalu, et al., 2006 reported the incidence of breast myiasis in rural nursing mothers.

Some workers [Adisa and Mbanaso, 2004] reported that the lesion of breast myiasis heals rapidly after the larva is removed or it spontaneously exits. Complications include cellulitis, abscess formation, osteomyelitis and tetanus [12]. Methods of removing the larva include obstructing the cutaneous orifice thus suffocating the larva, which forces it to wriggle out. Substances used include oil, petroleum jelly, butter and liquid paraffin [9]. Our current report has shown that the breast myiasis had microbial infections implicating *Staphylococcus aureus*... and other organisms that are associated with skin infections. Also, the wound sites of breast myiasis did not heal rapidly until the application of 40ppm of ethanolic solution of *Ageratum conyzoides*.

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Polyphenolic phytochemicals, flavonoids and phenol have been reported from *Ageratum conyzoides* which is suggested to have multiple biological effects, including antioxidant activity (Kahkonen et al.,1999; Kamboj and Saluja, 2008; Juliana et al., 2010; Nyuna et al., 2010). Phenolic compounds are implicit to induce the cellular antioxidant system by approximately 50% cellular glutathione concentration increase[Kamboj and Saluja, 2008; Juliana et al., 2010]. Flavonoids possess a wide range of biological activities [Treutter, 2006], one of them is their contribution to human health; many flavonoids are active principles of medicinal plants which exhibit pharmacological effects [Kong et al., 2003; Marles et al.,2003; Yilmaz and Toledo, 2004]. The results obtained in the present study demonstrated that the role of *A. Conyzoides* ethanolic extracts on breast myiasis sores was therapeutic. In the rural settings of Nigeria, people have relied on the traditional systems of medicine for the treatment of many skin diseases. Many plants of the Niger Delta have therapeutic potentials and are used against different ailments. Some of the indigenous medicinal plants exhibit digestive stimulant action, hypolipidemic effect, antidiabetic influence, antilithogenic property, antioxidant potential, anti-inflammatory property, antimutagenic, and anticarcinogenic potentials.

The total heterotrophic bacteria microscopic characteristics isolated from the leaves, stems, fruits and consortium of the *Ageratum* is shown on Table 1. The total bacterial population of the leaves, stem, flower and consortium of leaves, stem and flower were 2.5×10^7 , 1.6×10^7 , 6.6×10^4 and 1.3×10^8 cfu/g respectively. Moulds and Yeasts were not obtained from the samples.

The identification of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was done with reference to Harrigan and mcccance (1990) and Cheesebrough (2000)

Table 1: Microscopic characteristics of bacterial isolates

Code	Mot	gram	rxn	Spore	Flagella	capsule	cell morphology	Probable identity
Le1	-	+	+	-	-	-	Large short single rods	<i>Bacillus</i> sp
Le2	-	+	+	-	-	-	Large short single rods	<i>Bacillus</i> sp
St1	+	-	-	+	-	-	Rods in singles, few in chains	
St2	+	-	-	+	-	-	Short, small single rods	<i>Pseudomonas</i> sp.4r
F1	-	+	-	-	-	-	oval cells in cluster	<i>Staphylococcus</i> sp.
F2	-	+	-	-	-	-	oval cells in cluster	<i>Staphylococcus</i> sp.
Con1	-	+	-	-	-	-	oval cells in cluster	<i>Staphylococcus</i> sp.
Con2	-	+	-	-	-	-	oval cells in cluster	<i>Staphylococcus</i> sp.

Mot- motility; Gram rxn- Gram reaction, Le1-Leaves 1, Le2-Leaves 2, St1-Stem1, St2-Stem2, F1-Fruit1, F2-Fruit2, Con1-Consortium1, Con2-Consortium 2.

Table 2: Biochemical Characteristics of Bacterial Isolates

Cat	oxi	Coag	In	MR	VP	Cit	No ₃	H ₂ S	Ure	Glu	Suc	Mal	Lac	Mann	Identity of isolate
+	-	-	-	+	-	+	+	+	-	+	+	+	+	+	<i>Bacillus subtilis</i>
+	-	-	-	+	-	+	+	+	-	+	+	+	+	+	<i>Bacillus subtilis</i>
+	-	-	+	+	-	+	-	-	-	+	+	+	+	+	<i>Escherichia coli</i>
+	+	-	-	+	-	+	+	+	-	+	+	+	-	-	<i>Pseudomonas aeruginosa</i>
+	-	+	-	+	-	+	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>

+	-	+	-	+	-	+	+	-	-	+	+	+	+	+	Staphylococcus aureus
+	-	+	-	+	-	+	+	-	-	+	+	+	+	+	Staphylococcus aureus
+	-	+	-	+	-	+	+	-	-	+	+	+	+	+	Staphylococcus aureus

Cat- catalase; Oxi-oxidase; Coag-coagulase; In- indole; MR-methyl red; VP- Voges Proskauer; NO₃. nitrate reduction; H₂S- hydrogen sulphide; Cit- citrate; +- positive; -, negative Glu- glucose; Suc-sucrose; Mal- maltose; Mann- mannitol.

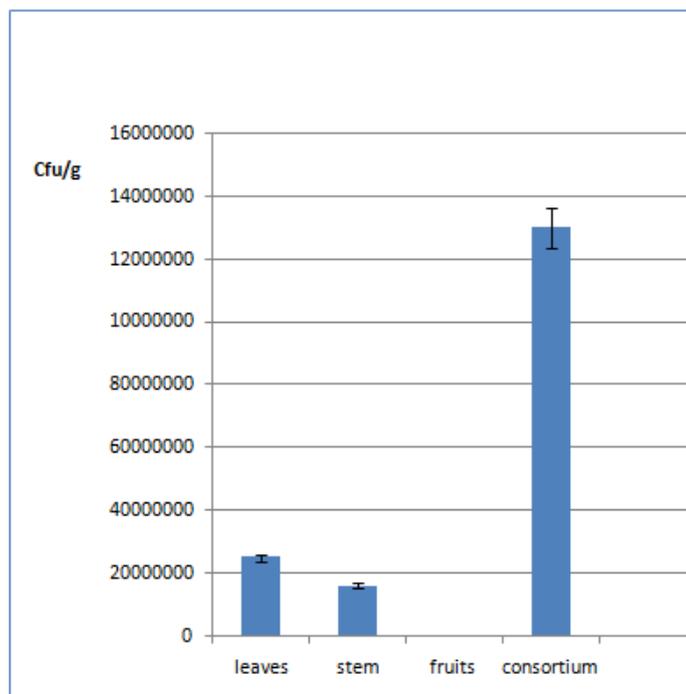


Fig.1: Total Bacterial Count In Different Parts Of Ageratum

Herbal plants such as *Ageratum conyzoides* contain some phytochemical components like saponins, tannin, alkaloids etc. These phytochemical components are known to have antimicrobial effects. (Oyetayo and Oyetayo,2006). For these phytochemical components to be extremely effective in antagonizing the pathogenic activities of invading micro organisms, a large quantity of the plants must be applied. The presence of microorganisms of public health significance like *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* may be as a result of improper handling of the plants by passer-by and contaminated equipment (Frazier and Westhoff,2003). *Pseudomonas aeruginosa* is a ubiquitous microorganism found in environments like soil, water, humans, animals, plants etc Also the unhygienic lifestyles and habits of passers-by such as spitting of saliva, coughs and even defecation will introduce microorganisms on *A. Conyzoides* that grows in the garden, roadside and parks from where the plants were harvested.

A comparative study was done to observe the bacterial load present in the leaves, stem, fruits and consortium. From figure 1, it was shown that the Consortium had the highest bacterial load. In the descending order, it is as follows: Consortium>leaves>stem>fruits. From the students' T-test table, there was significant difference at 0.05 and 0.01 probability levels between the consortium and the fruits also, between the stem, leaves and the fruits.

V. Conclusion

This study has demonstrated that ethanolic plant parts of *Ageratum conyzoides* possess some cytotoxic and therapeutic properties and it has added to the knowledge and evidence for its potential benefits in patients with breast myiasis sores. Although our sample size was small; it targeted only a cross section of the community. It provided pain relief in patients that applied it to wound sites and we recommend that it can be used to clean wounds of myiasis after extraction of myiasis-causing larvae in the rural villages where there might be no access to hospitals or health care centres. We recommend that further investigations be done to validate our findings in order to assess other potential benefits of the plant.

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