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Original Research Article

Comparative Analysis of the Antibacterial Effects of *Emilia sonchifolia* (Tassel Flower) and Selected Antibiotics on Ocular Bacteria, *in vitro*

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ABSTRACT

Developments of bacterial resistance to available antibiotics have necessitated the screening of medicinal plants for bioactive compounds which is gaining increasing popularity in the world. *Emilia sonchifolia (Emilia s.)* is a multipurpose plant that has exhibited antimicrobial effects against a wide range of microorganisms. The antibacterial activity of *Emilia s.* (tassel flower) extracts were compared with selected antibiotics against ocular bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*) *in vitro* using the Kirby-Bauer disc diffusion method. Phytochemical screening of the plant in Ethanol, Methanol and Aqueous solvents showed the presence of Saponin, Tannin, Flavonoid, Phenol, Alkaloid, Steroid and Glycoside in all the extracts but Flavonoid was not present in the Aqueous extract. Bacteria used were isolated from the eyes of infected patients that visited the Federal University of Technology, Optometry Teaching Clinic and St Joseph's Eye Hospital Mgbirichi all in Owerri, Imo State, Nigeria and were identified and characterized macroscopically using standard laboratory methods. Diameter of zones of inhibition was measured for the different extracts of tassel flower and selected antibiotics. The data collected were tabulated and analyzed using Paired T-test and one-way ANOVA at 95% confidence interval and at a 0.05 level of significance. Results showed that aqueous, ethanol and methanol extracts exhibited antibacterial activity against the isolates with mean diameter of inhibition zone 13.33±2.89, 9.33±1.16 and 7.67±2.52 respectively. Their effect on the isolates was significant with $p(0.01) < 0.05$ while that of the antibiotics were 12.00±3.46 for gentamicin, 17.00±10.58 for ofloxacin and 5.00±0.00 for erythromycin. A Comparison of the effects of *Emilia s*. and antibiotics on the bacterial isolates using one-way ANOVA showed the effects of *Emilia s*. does not differ significantly from the antibiotics as $p(0.58) > 0.05$. This shows that *Emilia sonchifolia* could become a promising natural antibacterial agent with potential application in pharmaceutical industries for the production of plant-based ocular drugs.

Keywords: *Emilia sonchifolia*, Antibiotics, Ocular bacteria.

INTRODUCTION

Plants are invaluable in the search for new drugs and scientific studies on plants used in ethno-medicine has led to the discovery of many valuable drugs giving a tremendous historical legacy in folklore uses of plant preparations in medicine [1]. According to Odugbemi [2], majority of people in developing countries still depend on the use of traditional medicine for their health care needs and more than 75 percent who reside in rural areas use herbs in one form or the other. Consequently, search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potential useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs has witnessed a tremendous resurgence [3-9].

Antibiotic resistance is reaching crisis point in many hospitals around the world and resistance is increasing in community-acquired infections as well [10], there is an urgent need to replenish our store and supply of anti-infective agents and an essential need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action [11, 12].

Emilia sonchifolia, also known as Lilac tasselflower or cupid's shaving brush is a flowering plant in the genus *Emilia* and belongs to the family *Asteraceae* [12]. It is found in India and in other Asian countries [13, 14], being widely distributed in open places, wastelands, cultivated lands, gardens, *etc.*, in and about towns and settlements at low and medium altitudes throughout the Philippines and other countries of the world [15]. It is used in folk medicine; its clinical applications include inflammation, rheumatism, cough, cuts, fever, dysentery, analgesics, and antibacterial [14]. *Emilia sonchifolia* is edible, the stem and leaves can be eaten at a later stage of growth, but best when cooked. In India, it is used as a salad before flowering while stem and leaves are cooked and eaten as vegetable [16]. The pappus is white, soft and copious [12]. The flower heads can be chewed and kept in the mouth for about 10 minutes to protect teeth from decay.

In Africa, precisely Nigeria, it is known among the Yorubas as *Odundunodo* and the Igbos as *Nti-ele* [17]. It was discovered that the medicinal values of this plant lie in their possession of chemical substances which produce definite physiological action on the human body and are found to serve as defense mechanism against attack by the microorganism; this discovery has led to the investigation of secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities [18]. Thus, it is anticipated that phytochemicals with adequate antimicrobial efficiency will be used for the treatment of microbial infections; this systematic study of higher plants for the purpose of detailing antimicrobial agents in tissues is of comparatively recent origin [2].

Varieties of plants are now known to contain substances like Terpenes, Tannins, Steroids, Alkaloids, Flavonoids, Saponins *etc*. that have been confirmed to possess therapeutic values [19, 20]. These natural phytochemicals are also known to contain substances that can be used for therapeutic purposes or as precursor for the synthesis of novel drugs [21]. Nearly 50 percent modern drugs are of natural products origin and as such these natural products play an important role in drug development in pharmaceutical industry, plants actually remain the most common source of antimicrobial agents [21]. Fact remains that research needs to be carried out to improve on the quantity and safety of uses of medicinal plants as widely practiced in various parts of Nigeria [2].

Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases [22]. Most of these agents are of microbial origin and the antibacterial properties of plant-derived compounds are attracting increasing attention [10]. This is in part attributed to the fact that plants can be rationally selected for antibacterial testing based on ethno-medicinal use [23].

Based on the fact that the knowledge of these active constituents has become unveiled by the development of organic chemistry and pharmacology, man can now determine the principle for the therapeutic action of plants as well as to modify the drug's effectivity [24]. Some of the important constituents in plants that are active includes; Alkaloids, Quinones and Quinines, Phenol and Phenolic acids, Flavones, Flavonoids and Flavonols, Tannins, Coumarins, Terpenoids and Essential oils, Lectins and Polypeptides, and Saponin [25, 26].

Despite several ethno botanical and ethno pharmacological investigation on the therapeutic potential of this plant, laboratory data on their bioactivity is still in paucity. Therefore, the present study cannot be over emphasized.

Many bacteria causing diseases are becoming resistant to the commonly used antibiotics necessitating the need for more potent broad spectrum antibiotics [27]. Over the last three decades, antibiotic resistance has become a major problem in hospitals all over the world, with most disease causing microbes developing resistance to the drugs used in killing them or inhibiting their growth [28-30]. Today, 50 percent of the world's blindness rate is largely caused by infectious agents. New, emerging, effective antibiotics are produced every now and then but are not very affordable due to the high cost of these synthetic drugs, especially in resource-poor communities where poverty, ignorance to good hygienic practices, circulation of drugs of questionable qualities and counterfeit pharmaceuticals combine to worsen the plight of the less privileged.

There is growing interest in exploiting plants for medicinal purposes especially in Africa. It is hence very necessary to investigate the claims of the traditional healers about some of the herbs treating a wide array of diseases. Exploiting and confirming the potentials of plants may therefore lead to the development of effective plant-based drugs which will be quite affordable as well as able to combat the rising cases of antibiotics/drug resistance.

MATERIALS AND METHOD

The test plant *Emilia sonchifolia* were procured from fallow experimental fields of National Root Crops Research Institute, Umudike, Abia State, Nigeria and its botanical identity was authenticated by the herbarium officer. Laboratory and other facilities were sourced from the Federal University of Technology Owerri, Imo State, Nigeria, Medical Laboratory. Swabs were collected from infected eyes of patients that visited the Optometry Teaching Clinic, Federal University of Technology Owerri, and St Joseph's Eye Hospital Mgbirichi Owerri, Imo State, Nigeria.

The freshly collected plant was screened for extraneous materials, weighed and oven dried at 55$^{\circ c}$ for 36 hours. The dried leaves were milled into powder using Arthur Thomas laboratory mill. The powdered leaves were weighed using the electronic weighing balance and found to be 232.6 grams. The weighed sample was put into a sterilized container and covered to avoid contamination then stored in a cool dry place.

Qualitative analysis was carried out to ascertain the presence of the different phytochemicals in the samples. Aqueous, ethanol and methanol screening of the plants were done to determine the presence of constituents like Tanin, Saponin, Flavonoids, Alkaloids, Steroids, Phenols, etc.

Different Media were prepared for the inoculation of microorganisms isolated from infected eyes. The microorganisms were identified using their colonial and morphological characteristics in accordance with the standard methods [31] Records of characteristic features of the isolates were matched against those of existing taxa as illustrated in Bergey's manual of systematic bacteriology and Cowan [24]. Identification of isolates in each case was based on the matching characteristics. Gram staining and biochemical tests were also done to further identify the organisms.

Plant extracts were prepared and the tests for antibacterial activity (In Vitro) were conducted using the extracts and drugs to show their respective ability to inhibit the growth of the bacteria isolates using the Kirby-Bauer disc diffusion method.

Data analysis

Statistically significant difference between groups was calculated by the application of T-test and one-way ANOVA. P values less than 0.05 ($p < 0.05$) were used as the significance level.

RESULTS

The result of this study showed the presence of phytochemicals in Emilia sonchifolia. Saponin, Tannin, Alkaloid, Steroid, Phenols and Glycosides were present in the aqueous extract, except Flavonoid. The Ethanol and Methanol extract showed the presence of Saponin, Tannin, Flavonoid, Alkaloid, Steroid, Phenols and Glycosides. This is shown in Table 1.

Table 1: Phytochemical Result of *Emilia sonchifolia* Extract

Sample	Saponin	Tannin	Flavonoid	Alkaloid	Steroid	Phenols	Glycosides
Aqueous extract	++	++	-	++	+	++	+
Ethanol extract	+	++	+++	+++	+	++	++
Methanol extract	+	+	+++	++	+	+	+

+ = Present at low concentration based on visuals
++ = present at moderate concentration based on visuals
+++ = present at high concentration based on visuals
- = Not present

Table 2 showed results of characterization and identification of isolates. From a total of 27 bacterial infections (of the lids, conjunctiva and cornea) from which swabs were collected and cultured, 5 showed no growth, while 22 showed growth.

Table 2: Results of characterization and identification of isolates

Organism identified	No of organism identified	Colonial arrangement	Cell arrangement	Gram stain	Catalase	Coagulase	Citrate	Oxidase
Pseudomonas aeruginosa	1	Smooth colonies, shiny with grey hint	Short rods, polar flagella slightly curved	-	+	-	+	+
Staphylococcus aureus	15	White milky tiny colonies	Cocci in clusters	+	+	+	-	-
Neisseria gonorrhoeae	6	Smooth colonies in pairs	Kidney-shaped diplococci	-	+			+

Table 3 showed the diameter of inhibition zones of the extracts of *Emilia sonchifolia* against the ocular bacteria isolates. Inhibition zone is the level of antibacterial activity the extracts have on the isolates, the higher the inhibition the higher the antibacterial activity. These results show that there were variations in the efficacy of the different solvent extracts.

Table 3: Zones of inhibition of *In vitro* antibacterial activity of the plant extracts

Samples	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Neisseria gonorrhea*
Aqueous extract	10mm	10mm	8mm
Ethanol extract	15mm	15mm	10mm
Methanol extract	10mm	5mm	8mm

Figures include the diameter of the paper disc (5mm)
5mm = diameter of the disc and indicates no inhibition

The diameter of zones of inhibition by the selected antibiotics against the ocular bacteria isolates were shown in table 4.

Table 4: Zones of inhibition of *In vitro* antibacterial activity of selected antibiotics

Antibiotics	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Neisseria gonorrhoeae*
Gentamicin	16mm	10mm	10mm
Erythromycin	5mm	5mm	5mm
Ofloxacin	25mm	21mm	5mm

Figures include the diameter of the paper disc (5mm)
5mm = diameter of the disc and indicates no inhibition

Table 5 showed the mean values and standard deviation of the extracts and antibiotics.

Table 5: Mean zones of inhibition values and standard deviation of extracts and antibiotics

Samples	No of organisms tested	Mean	Standard deviation	Standard error mean
Aqueous extract	3	9.33	1.16	0.67
Ethanol extract	3	13.33	2.89	1.67
Methanol extract	3	7.67	2.52	1.45
Gentamicin	3	12.00	3.46	2.00
Ofloxacin	3	17.00	10.58	6.11
Erythromycin	3	5.00	0.00	0.00

Data analysis with SPSS (Version 17) using the one Sample T- test at 0.05 level of significance showed that there was a significant effect of *Emilia sonchifolia* on ocular bacteria. [P (0.001) <0.05] However, data analysis using the one-way ANOVA at 0.05 level of significance showed no significant difference in the effect of *Emilia sonchifolia* and the antibiotics on ocular bacteria. [P (0.58) >0.05]

DISCUSSION

Phytochemical screening tests showed the presence of saponin, tannin, flavonoid, alkaloid, glycosides and phenols. This is in line with several studies [32- 34], that confirmed alkaloid and flavonoid as the major constituents of *Emilia sonchifolia*. The presence of phenols in the plant is in accordance with the work of Couto [35]. The result shows that the plant used possess promising antimicrobial activities against the test organisms.

From the antibacterial screening tests of the crude extracts of *Emilia sonchifolia* carried out on the isolated bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*), the ethanol showed more antibacterial activity on ocular bacteria than the other extracts, this implies that the ethanol extract was more effective in terms of antibacterial activity. The observed difference could be due to the type of solvent used because varying antibacterial activity from plants material is largely dependent on the type of solvent used during extraction. This is in accordance with similar studies [33, 36] were conclusions were made after *Emilia sonchifolia* extraction with methanol, alcohol, acetone, acetic ether and ether that alcohol extract exhibited the finest antibacterial activity. Mamman [37] also noted that the difference in the effect of these extracts within the organisms could be that there are different antibacterial compounds in the plant extracts and that the compound that acted on one may not be same as the one that acted on the others since antibacterial agents have different mechanisms of action. The traditional healers use primarily water and ethanol (Kai Kai) as solvent because water is safe and ethanol possesses preservative property while methanol is considered toxic and as found in this study, the ethanol extract provided more consistent antibacterial activity compared to others. The results particularly do not agree with the work of some researchers[12,37,38] that the methanolic extracts of root plants possess more antibacterial activity. This could be due to the differences in the plant parts used, nature and levels of the antibacterial agents present in the plant parts, mode of extraction and their mode of action on the different isolates [39] and also the specificity of bacterial strains. The observed antibacterial effects on the isolates are also believed to be due to the presence of alkaloids, tannins and flavonoids which has been shown to possess antibacterial properties [24, 40]. Several recent papers has also reported the regular presence of antibacterial activity among flavonoids and alkaloids [41-43]. Specifically the values of the extracts on *Staphylococcus aureus* were shown to be moderately high (10mm-15mm), this has been confirmed by Haraguchi et al [44] on the effect of flavonoids on *Staphylococcus aureus* and the extracts on the same organism by Nwadinigwe [34].

The majority of swabs collected from infected eyes was identified to be *Staphylococcus aureus* (Table 2) it could be inferred that bacterial eye infections is commonly caused by *Staphylococcus aureus*. This is in line with the work of Ubani [45] where isolates from infected eyes were predominantly *Staphylococcus aureus* irrespective of age and sex of the individual.

In the antibiotic sensitivity tests, all the isolates were resistant to erythromycin; gentamicin and ofloxacin showed varying zones of inhibition (Table 4). The varying susceptibility of the various bacterial isolates to commonly used antibiotics could be attributed to indiscriminate and irrational use of these drugs in humans which usually results in resistance developed by the bacteria. *Neisseria gonorrhoeae* was resistant to ofloxacin; this has been confirmed in recent literatures about the recent resistance of *Neisseria* species to quinolones [46]. Several different epidemiological studies track *Pseudomonas aeruginosa* occurrence as a nosocomial pathogen and indicates that antibiotic resistance is increasing in clinical isolates [46]. This could explain why in this work, Gentamicin and Ofloxacin had lower zones of inhibition against *Pseudomonas aeruginosa* than staphylococcus aureus.

In the comparison between the effects of the plant extracts and commonly used antibiotics, it showed that the extracts had similar effects on the bacteria as the antibiotics. The statistical test result of the plant and antibiotic does not differ significantly as ($P>0.05$) this could be as a result of the different mechanism of actions of the phytochemicals working in a synergistic manner against the bacteria isolates. This is in line with Thenmozhi et al [12] as when comparing the antibacterial activity of plants to that of reference antibiotics, the inhibitory potency of the tested extracts could mostly be considered important.

CONCLUSION

The results obtained in this study provide a support to the use of the plant *Emilia sonchifolia* in ethno-medicine in line with similar works. The results also showed no significant difference of the effect of *Emilia sonchifolia* compared to the selected antibiotics, therefore *Emilia sonchifolia* has been shown to possess antibacterial activity against ocular bacteria. Although the mechanisms are not studied in this research, but the active secondary metabolites present in crude extracts of *Emilia sonchifolia* perhaps operate in a synergistic manner to show such effects. The effect of these extracts on the bacteria have also confirmed that bacterial infections of the eye are not self-limiting and when left untreated could lead to blindness. Thus *Emilia sonchifolia* could become promising natural antibacterial agents with potential application in pharmaceutical industries for controlling pathogenic organisms.

Extensive toxicity test run on the plant to determine their toxic effects if any is highly recommended. Further chemical and pharmacological investigations to determine the quantitative constituents in *Emilia sonchifolia* and to screen their individual potential bioactivities is also recommended. These would be useful in determining the principles of their therapeutic action and elucidate their mechanisms of action.

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