



Comparison of the Antimicrobial Activity of Leaf Extracts of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides*

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Abstract

The use of plants and their products for different purposes has been with man from the beginning. *Ficus exasperata* (Sand paper Leaf), *Boerhaavia diffusa* (Hog weed), and *Ageratum conyzoides* (Goat weed) have been used for infections including wound infections in many West African countries like Nigeria. The phytochemical and antimicrobial activity of ethanolic leaf extracts of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* was investigated in an attempt to evaluate their therapeutic efficacy in the treatment of wound infections. The phytochemical screening revealed the presence of the active medicinal constituents analysed. The extracts had varying degree of inhibitory effects on the isolates tested. *Ficus exasperata* and *Ageratum conyzoides* extracts had the highest antimicrobial activity compared to *Boerhaavia diffusa* extract. Gentamicin also had good inhibitory effects on the isolates used. This study show good antimicrobial effects of the extracts on pathogenic bacteria causing wound infections especially *Ficus exasperata* and *Ageratum conyzoides* extracts. This study also supports the folkloric use of the plants. These plants can be useful in the production of antimicrobial agents that could be used in treating wound infections caused by these bacteria and suggests further advance investigations.

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Introduction

The use of plants and plant products for different purposes has been with man from the beginning. Aside food, plants are often used as medicines. Plants used as medicine or for healing processes are usually referred to as MEDICINAL PLANTS [15]. The plants parts used are often the bark, seeds, flowers, root, leaves and the whole plant in most cases [1,15]. These plant parts are often isolated and characterized to investigate their active ingredients which can be used for therapeutic purpose. In most cases, medicinal plants often exhibit a wide range of

biological and pharmacological activities such as anti-inflammatory, anti-bacterial and anti-fungal properties [40]. Extracts, syrups, and concoctions often prepared from plants can be used to treat different ailments which may include anaemia, malaria, etc.

Medicinal plants have been identified and used throughout human history [28]. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform im-

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portant biological functions. At least 12,000 of such compounds have been isolated so far, which is estimated to be less than 10% of the total chemical compounds in plants which mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs [14]. Thus, herbal medicines do not differ greatly from conventional drugs in terms of their mode of action. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [34]. Between 70% and 80% of people living in the developing countries especially in Africa and Asia depend on herbal medicine for their health needs including wounds, infectious and metabolic diseases [20]. The use of plant extracts of known antimicrobial properties can be of great importance in the therapy of infections. Plant extracts are employed for their antifungal, antiviral and antibacterial activities. It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine [22]. *Ficus exasperata*, *Ageratum conyzoides* L. and *Boerhaavia diffusa* L. are important plants in many countries such as Asia, West Africa, and South America respectively and these plants have been used for ages to treat various ailments [12,27].

Ageratum conyzoides L. (goat weed) belongs to the family Asteraceae. In Nigeria and other African countries, *Ageratum conyzoides* (goat weed) have been used to treat wound, skin diseases, digestive disturbances etc. [33]. *Boerhaavia diffusa* L. (hog weed) belongs to the family Nyctaginaceae. It has been used to treat jaundice, hepatitis, oedema, oligurea, anemia, inflammation, eye diseases etc [6]. *Ficus exasperata* (sand paper plant) belongs to the family Moraceae. Leaf pulp or sap of *Ficus exasperata* is externally applied for the treatment of rash, wounds, leprosy sores, fungal infections etc. Leaf preparations are taken for the treatment of cough, colds, flu and asthma, and they are a mouthwash against thrush, inflammation of the gums and other mouth and throat ailments [4]. *Ficus exasperata*, *Boerhaavia diffusa* L. and *Ageratum conyzoides* L. has been known for their strong and effective medicinal properties. These plants have shown to possess pharmacological and physiological properties [12,27]. These plants have been used in traditional medicine as household remedies. This work was carried out to investigate the phytochemical and antimicrobial activity of ethanolic leaf extracts of *Ficus exasperata*, *Boerhaavia diffusa* L. and *Ageratum conyzoides* L. in an attempt to evaluate their therapeutic efficacy in the treatment of wound infections.

Materials and Methods

Plant collection and authentication

The fresh leaves of the plant *Ficus exasperata*, *Ageratum conyzoides*, and *Boerhaavia diffusa* were collected from the Pharmacognosy Departmental garden College of Pharmacy, Igbinedion University Okada Edo state. The plants were authenticated at the Forest Research Institute (FRIN) in Ibadan, Oyo state. The leaves of each plant were sorted and thoroughly washed with clean water and room dried. After drying they were pulverized into powder form and afterwards subject to organic solvent extraction.

Organic solvent extraction

The soxhlet extraction method was used for extraction. Powdered *Ficus exasperata* was placed inside a thimble of a soxhlet apparatus. 500mls of ethanol (extracting solvent) was taken into

a distillation flask, and the soxhlet extractor was placed on the flask. The solvent was heated and the solvent vapour produced was cooled by the condenser which dripped back down into the chamber housing the powdered plant thus dissolving it. When the soxhlet chamber was almost full, it was emptied by a siphon side arm with the solvent running back down to the distillation flask. The cycle was repeated many times for three days. Finally, the extract was concentrated in the distillation flask. The same procedure was repeated for *Boerhaavia diffusa* and *Ageratum conyzoides*. Ethanol was removed using an evaporator. Weight of total pulverized leaves of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* was 250 g, 220 g and 300 g. Percentage yield of extracts of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* samples was 22.14%, 18.23% and 19.45% respectively.

Microorganism

Different isolates of *Pseudomonas spp* and *E. coli* from wound infections were collected from the University of Benin Teaching Hospital (UBTH), Edo state Nigeria on sterile nutrient agar slants. Isolates were maintained on nutrient agar slants.

Phytochemical screening

The methods described by Harborne (1973) [13] and Trease and Evans (1989) [36] was used.

Antibacterial screening of the crude extracts

The antibacterial activity of the Leaf extracts was evaluated by agar well diffusion [5]. Wells were made (8 mm diameter) on each culture plate of the organisms with the aid of a sterile cork borer. The 3 extracts were reconstituted to 1000mg/ml and 500mg/ml respectively with Dimethyl sulfide (DMSO₄). Aseptically each well was filled up with 0.5 ml of each extract using a pasteur pipette. Gentamicin 40 µg/ml and Dimethyl sulfide was used as positive and negative control respectively. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured and recorded in millimeters.

Results

Plants were authenticated as *Boerhaavia diffusa* Linn, *Ficus exasperata* Vahl, and *Ageratum conyzoides* Linn, (Family: Nyctaginaceae, Moraceae, Asteraceae, respectively.) at the Forest Research Institute of Nigeria (F.R.I.N) Ibadan, Oyo state. A voucher specimen of the plants *Boerhaavia diffusa* Linn, *Ficus exasperata* Vahl, and *Ageratum conyzoides* Linn with reference no. FHI 109883, 109884, 109885 respectively has been kept in the FRIN herbarium for further reference. The results of the phytochemical screening showed that the ethanolic extract of *Ageratum conyzoides* Linn leaves contained flavonoids, phenols, cardiac glycosides, alkaloids, saponins, and tannins respectively (Table 1). Alkaloids was the only constituent not detected in *Ficus exasperata* leaves extract while *Boerhaavia diffusa* Linn leaves extract had all the phytochemical constituents tested present except for Saponins (Table 1).

The results of the antimicrobial activity of the ethanolic extracts of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* leaves on the *E. coli* and *Pseudomonas spp* isolates show *Ficus exasperata* and *Ageratum conyzoides* extracts had the highest antimicrobial activity compared to *Boerhaavia diffusa* extract (Table 2). Gentamicin the positive control also had activity on the bacterial isolates (Table 2).

Table 1: Phytochemical screening of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides*.

	Phytochemical constituent		
	<i>Ficus exasperate</i>	<i>Boerhaavia diffusa</i>	<i>Ageratum conyzoides</i>
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	-	+
Glycosides	+	+	+
Alkaloids	-	+	+
Phenols	+	+	+

Key: (-) Negative, (+) Positive

Table 2: Antimicrobial activity of the extracts.

ORGANS MS	A	B	C	D	E	F	G	H
	Fe 1000mg/ml	Fe 500mg/ml	Bd 1000mg/ml	Bd 500mg/ml	Ac 000mg/ml	Ac 500mg/ml	Gentamicin	DMSO ₄
<i>E.coli</i> 1	18mm	15mm	20mm	12mm	20mm	15mm	25mm	10mm
<i>E.coli</i> 2	15mm	22mm	10mm	16mm	13mm	20mm	16mm	18mm
<i>E.coli</i> 3	16mm	-	10mm	-	14mm	-	24mm	15mm
<i>E.coli</i> 4	16mm	15mm	10mm	-	14mm	-	25mm	12mm
<i>E.coli</i> 5	10mm	-	17mm	-	16mm	-	22mm	13mm
<i>E.coli</i> 6	15mm	-	-	-	15mm	-	25mm	-
<i>Pseudomonas</i> 1	14mm	14mm	17mm	-	15mm	-	28mm	15mm
<i>Pseudomonas</i> 2	-	-	-	-	13mm	-	32mm	14mm
<i>Pseudomonas</i> 3	15mm	-	-	-	13mm	-	23mm	-
<i>Pseudomonas</i> 4	13mm	-	-	-	14mm	-	24mm	13mm
<i>Pseudomonas</i> 5	16mm	-	13mm	-	17mm	-	27mm	-
<i>Pseudomonas</i> 6	15mm	-	-	-	15mm	-	30mm	12mm

Key: -= No inhibition; Extract A: *Ficus exasperata* 1000mg/ml; B: *Ficus exasperata* 500mg/ml; Extract C: *Boerhaavia diffusa* 1000mg/ml; D: *Boerhaavia diffusa* 500mg/ml; Extract E: *Ageratum conyzoides* 1000mg/ml; F: *Ageratum conyzoides* 500mg/ml; Positive control G: Gentamicin 40mg/ml; Negative control H: Dimethyl sulfoxide; DMSO₄ (solvent used to dissolve extract).

Discussion

With the emergence of Multiple Drug Resistant (MDR) and Total Drug Resistant (TDR) microorganisms causing infections that are difficult to cure both in animals and human beings [8], medicinal plants have gained attention for effective antimicrobials and lot of research on medicinal plants has been reported from all parts of the world [38]. Recent studies have indicated that antibiotic resistance is not only limited to antibiotics but other antimicrobials too including those of herbal origin [32]. The ethanolic extracts of the plants *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* produced tannins, saponins, cardiac glycosides, alkaloids, flavonoids and phenols. These suggest that the medicinal properties attributed to *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* could be based on the antimicrobial effect of these phytochemicals. The phytochemical screening showed that the plant extracts had chemical constituents of pharmacological importance [18]. Saponins and flavonoids have been reported to possess

wound-healing activity [16]. Terpenoids are also known to promote wound-healing process, mainly as a result of their astringent and antimicrobial activities which seem to be responsible for contraction and increased rate of epithelialization in wounds [31]. Tannins on the other hand have been reported to have astringent properties and hasten the healing of wounds and inflamed mucous membrane [25]. Therefore, the wound-healing potential of *F. exasperata* and *Ageratum conyzoides* as seen from the antimicrobial studies can be attributed to the contributions of individual phytoconstituents.

Ficus exasperata, popularly referred to as "Sandpaper leaf tree" owing to the rough surface of the leaves, is increasingly being used for a number of ailments and hence, studies validating the traditional claims are on the increase. Ogunleye 2011 [23] reported the presence of saponins, alkaloids, tannins flavonoids and cardiac glycosides in the aqueous leaf extract of

Ficus exasperata. Available reports indicate that leaves of *Ficus exasperata* exhibit anti-ulcer, hypotensive, hypoglycaemic, hypolipidemic, anti-inflammatory [41], anxiolytic, oxytocin inhibiting, anticonvulsant, antipyretic [7], anti-microbial [2], anti-candida, insecticidal and pesticidal activities. Umeh et al., 2014 [37] justified the use of the leaf extract of *F. exasperata* in the treatment of cutaneous wounds more than any of its fractions. Evaluation of the extract in clinically isolated microbial contaminants of wounds showed dose-dependent inhibitory activity against some pathogenic micro-organisms including *P. aeruginosa* and *S. aureus*, two organisms mostly implicated in chronic and non-healing wounds [9].

Ageratum conyzoides has been known for its curative properties in the treatment of fever, leprosy, boils, wounds, ulcers, diarrhoea, eye inflammation and it can also be used as a purgative, antipyretic [30]. Extracts from this plant have been found to possess pharmacological and insecticidal activities. *Ageratum conyzoides* contains many bioactive compounds including flavonoids, alkaloids, coumarins, essential oil, chromenes, benzofurans, terpenoids and tannins. *Ageratum conyzoides* leaves are used by the Fipa in South Africa and Central Africa for the treatment of fresh wounds and burns [39]. Okwori et al., 2006 [24] investigated the in-vitro antibacterial activities of *Ageratum conyzoides* extracts on some selected bacterial pathogens. Results obtained from the study [24] showed that the hexane extracts of the leaf, stem and root had 100% susceptibility to all the bacterial isolates used. The aqueous leaf extracts gave 75% susceptibility, while methanolic leaf extract gave 50% susceptibility. The extracts inhibited the growth of the bacterial isolates in a concentration dependent manner. Phytochemical analysis of the dried leaves revealed the presence of resins, alkaloids, tannins, glycosides and flavonoids. This correlates with results obtained in this study. Odeleye et al., 2014 [21] showed that *Ageratum conyzoides* extract had a broad spectrum of antibacterial activities, inhibiting *P. aeruginosa*, *E. coli*, *S. dysenteriae* and *S. aureus* at concentration of ≥ 50 mg/ml. The antimicrobial activity of *A. conyzoides* could be due to the abundant presence of phytochemicals [29] which include alkaloids, flavonoids, tannin, saponins and phenol. In this study, the presence of tannins and saponins in *A. conyzoides* and its antimicrobial activity on the wound isolates supports its use in treating wounds [19]. The presence of phenol in the plant extracts further explains the antibacterial properties of the plants as phenols and phenolic compounds have been extensively used in disinfection and remain the standard with which other bactericides are compared [26].

The *B. diffusa* plant is also reported to possess many pharmacological, clinical, and antimicrobial properties. Thakur and Pathak, 2016 [35] reported a high nutritive and pharmacological value of the plant based on the result of its phytochemical and nutritional evaluation. Das (2012) [10] evaluated the antimicrobial activity of ethanolic extract of *Boerhaavia diffusa* by disc diffusion method and according to her, growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi* was inhibited at 2000 μ g/ml concentration and for *E. coli* minimum inhibitory concentration was 4000 μ g/ml. The minimum inhibitory concentration was far higher than the one employed in this study in the antimicrobial screening of the plants against the isolates. The antimicrobial screening results of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* against microorganisms isolated from wound infections especially *Escherichia coli* and *Pseudomonas aeruginosa* showed that the *Ageratum conyzoides* and *Ficus exasperata* extract at a concentration

1000mg/ml had the highest antimicrobial activity compared to *Boerhaavia diffusa*.

Conclusion

Our results supports earlier reports by various researchers on the potential uses of plants in traditional medicine which include *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* [11,17]. The significant inhibitory potentials displayed by ethanolic leaf extract of *Ficus exasperata*, *Boerhaavia diffusa* and *Ageratum conyzoides* especially *Ficus exasperata* and *Ageratum conyzoides* against susceptible strains of *Escherichia coli* and *Pseudomonas spp* isolated from wound infections establish the scientific rationale for the use of these plants in folk medicine. Phytochemicals detected in the plants shows that if the plants are properly screened, they could probably yield drugs of pharmaceutical importance. Further advanced work to isolate, purify and characterize these bioactive constituents is therefore recommended.

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