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Antimicrobial Activity of *Bridelia micrantha* and *Grewia plagiophylla* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Author KD designed the study, wrote the protocol and wrote the first draft of the manuscript. Author KD managed the literature searches, analyses of the study and performed the spectroscopy analysis. Author AG managed the experimental process. Author KD identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Escalation in occurrences of resistant microorganisms especially to synthetic drugs, has obliged the need to search for new bioactive compounds having natural origin. *Bridelia micrantha* and *Grewia plagiophylla* are medicinal plants whose roots, bark and leaves have been used by traditional herbalists to treat various diseases, but the antibacterial activity of the Kenyan ecotype has not been determined. Among the diseases treated by extracts of these plant parts are bacterial diseases such as gonorrhea, syphilis, leprosy etc. Use of herbal medicine has been with disbelief and fear since people are not sure of their effect. This reserach sought to determine the antibacterial activity of crude leaf extracts of *Bridelia micrantha* and *Grewia plagiophylla.* The phytochemicals present in ethyl acetate and methanol extracts was also determined. *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were used as the test organisms. Extraction of crude extracts was done by cold extraction. Test organisms were inoculated in Muller Hilton media and disks impregnated with sample placed on the surface of the media. The diameter of zone of inhibition was measured in mm for every treatment using a ruler. The methanol and ethyl acetate

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leaf extracts from the test plant had considerable antibacterial effects on *S. aureus*. Most of the extracts tested positive for tannins, terpenoids, flavonoids and carotenoids. Alkaloids were absent in all the plants extracts. All the plant extracts showed no inhibition against *E. coli*, Methanol extract of *Bridelia micrantha* and, *Grewia plagiophylla* exhibited a zone of inhibition of 19 mm and 20 mm against *Staphylococcus aureus* and *Salmonella typhi* respectively. Ethanol extracts was inactive against all test organisms at various concentrations. Antibacterial activity difference was based on type of solvent used for extraction. The results gives scientific validity for the use of the plant as a medicine source.

Keywords: Bridelia micrantha; Grewia plagiophylla; phytochemicals; antibacterial activity.

ABBREVIATIONS

ATCC, American Type Culture Collection; BCC, Belgian Coordinated Collection Microorganisms; MH, Mueller Hinton TLC, Thin Layer Chromatograph; WHO, World Health Organization.

1. INTRODUCTION

Medicinal plants promote healthy life of a country. They play an important role by providing preliminary health care services to both urban and rural people. They also serve as an important therapeutic agents as well as important raw materials for manufacture of both traditional and modern medicine. This can serve as a source of foreign exchange for a country by exporting medicinal plants to other countries. Hence, indigenous medicinal plants play significant role in the economy of a country. They have been identified and used throughout human history because they have the ability to synthesize a wide variety of chemical compounds [1]. These compounds are used to perform important biological functions, and to defend the medicinal plants against attack from predators such as insects, fungus and herbivorous mammals [2]. At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10% of the total [3]. According to World Health Organization report, 80% of the world's population, mostly from developing countries still rely on traditional medicine for the treatment of common ailments [4,5]. A research done by Gebremedhin [6] in Ethiopia also revealed that communities depend largely on their traditional knowledge in use of medicinal plants and prefers to keep it secret. In traditional systems, plant and plant products are used for the cure of diseases. However, in modern science the bioactive compounds of the plants are identified. These plant materials are being used for the development of modern drugs [7]. The presence of active antimicrobial compounds in plants represents a useful area for development of natural products that can be used as substitutes for antibiotic resistance to pathogenic bacteria and fungi. They provide the foundation for development of new antimicrobials [8].

An antimicrobial agent is a compound or substance that kills or slows down the growth of microorganism. Antibacterial agent inhibits bacteria growth and the term is often used synonymously with the term antibiotic. With increased knowledge of the causative agents of various infectious diseases, antibiotic has come to denote a broader range of antimicrobial compounds, including antifungal and other compounds [9].

The emergence of resistance of bacteria to antibacterial drugs is a common phenomenon. Emergence of resistance often reflects evolutionary processes that take place during antibacterial drug therapy. The antibacterial treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of antibacterial. Under certain conditions, it may result in preferential growth of resistant bacteria, while growth of susceptible bacteria is inhibited by the drug [10]. The emergence of resistant bacteria to common antibiotics has necessitated the search for other antibiotics. Antibiotics from plants are less prone to resistance as is the case with those obtained from other sources.

Bridelia micrantha is a tree belonging to the family Euphorbiaceae. The genus Bridelia is made up of about 60 species scattered throughout Asia, Africa and Australia [11]. They are used medicinally in the treatment of many different ailments*. B. ferruginea Benth* is used in African traditional medicine as a decoction of the stem back to treat diarrhea, dysentery, gastro-

intestinal disorders, gynecological disorders (including sterility), and rheumatic pains. A decoction of the leaves is used to treat diabetes. It is also used as a purgative and a vermifuge. A decoction of their leaves, along with other parts of the plant, is used to treat high fever. Their roots are taken the first three days after childbirth. The chemical constituents of Blidelia have not been thoroughly investigated. From B. monii, the triterpenesfriedelin, friedelan-3a-ol, friedelan-3β-ol, sitosterol and glochidone have been isolated. Trans- triacontyl-4-hydroxy-3-methoxycinnamate has been isolated from B. ovate Dene, along with various triterpenes. The indoleglycocyde, (-) ovatolide has been isolated from B. ovata Dene and B. siamensiscraib. This plant mainly shows antimicrobial, antioxidant, antitumor, and anti HIV activities. Decoction of *Bridelia micrantha* is taken as a remedy for stomach-ache and tapeworm. The back is also boiled to make a soup for treating diarrhea in childrens.

Grewia plagiophylla, belongs to a family of Tiliaceae, comprises 150 species, small trees or shrubs distributed in subtropical and tropical regions, represented in Pakistan by ten species [12]. It is one of the most important ingredients of many medical prescriptions in traditional medicines. It has been successfully, developed into a medicine to treat cough and sore throats. The root and stem bark of these plants have been used in folk medicine for the treatment of malaria, diarrhea, dysentery, typhoid, fever, small pox ,cough, irritable conditions of intestine and bladder. In-vitro studies indicate that they have anti-oxidant, antibacterial, hepaprotective [13]. and antimalarial activities. Plant compounds such as steroids glycosides, flavones, triterpenes and lignins have been isolated and characterized from this plant. These two plants are widely distributed in Kenya (Kilifi, Kwale, Meru and Kakamega district). They are recognized as medicinal plants only in kilifi and kwale district.

2. MATERIALS AND METHODS

2.1 Plant Material

Plants were collected in kilifi district. The species that showed great potential of inhibiting bacterial and fungal growth was sustainably harvested in a manner to protect the habitat. The plants were botanically identified by a botanical taxonomist while in the field prior to collection. Upon transportation to the screening center, they were assigned voucher specimens numbers and voucher specimens deposited at East Africa Herbarium, Nairobi. The leaves of *Bridelia micrantha* and *Grewia plagiophylla* were used in this study. Leaves were collected from lower branches of the tree.

2.2 Extraction

The air dried at room temperature and ground plant materials of the plants (1000 g leaves) were sequentially extracted with organic solvents in the order of increasing polarities including; ethyl acetate and methanol, using cold extraction for 24 hours in each case. The extracts were then concentrated under a reduced pressure to a minimum volume using a Rotavapor. They were then allowed to evaporate to dryness to a solid mass.

2.3 Phytochemical Analysis

Qualitative tests for terpenoids, tannins, flavanoids, flavones, carotenoids and alkaloids were carried out by standard method as described by Edeoga [14]. The tests were based on the visual observations of color change or formation of a precipitate after addition of specific reagent.

2.4 Flavones

Flavones were tested by adding 5 mL of ammonium solution to 1 mL of aqueous filtrate of the plant extract followed by addition of 2 mL sulphuric acid. A yellow coloration indicated the presence of flavones.

The test for flavonoids was carried out according to the method described by Geissman [15], whereby 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of the leaves powder extracts. This was followed by addition of concentrated sulphuric acid. Yellow coloration indicated the presence of flavonoids.

2.5 Alkaloids

Alkaloids were tested for by mixing 5 g of the powder sample extract with 25 mL of 1% sulphuric acid. It was then allowed to stand and then filtered. About 10 mL of the filtrate was shaken and Meyer's reagent added. Formation of a white precipitate indicated the presence of alkaloids.

2.6 Saponins

Tests for saponins was carried out according to the method described by Evans, [16]. About 2 g

of the leaves powder extracts were boiled in 20 mL of distilled water on a water bath and then filtered. About 10 mL of the filtrate was then mixed with 5 mL of distilled water and shaken vigorously to give a stable persistent froth. Three drops of olive oil were then added and the mixture shaken vigorously, then observed for the formation of emulsion.

2.7 Carotenoids

Leaves extracts were subjected to a test for carotenoids whereby 1 g of each extract was boiled in 20 mL water. The filtrate was then treated with concentrated sulphuric acid. Blue color indicated the presence of carotenoids.

2.8 Terpenoids

Test for terpenoids was carried out by adding 1 mL chloroform to the 1 mL of extract and then an equal volume of concentrated sulphuric acid was added. Formation of the bluish red coloration indicated presence of terpenoids.

2.9 Tannins

Tannins were tested by mixing 0.5 g of the sample with 20 mL of water in a test a beaker. About 2 mL of 0.1M $FeCl_3$ was added. Formation of blue black coloration indicated presence of tannins.

2.10 Biological Studies

Antibacterial activity of the extracts were determined by micro-broth dilution assay as described by Buwa and Staden, [17]. The three preserved strains of bacteria used were *Escherichia coli ATCC 25922*, *Salmonella typhi* ATCC 87853 and *Staphylococcus aureus ATCC 25923.* The bacterial strains were cultured for 18 hours at 37℃ and were standardized to a cell density of 1.5×10^8 cfu mgL^{-1} equivalent to 0.5 McFarland Standard. About 10 mL of standardized bacterial strains (*Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 87853, and *Staphylococcus aureus* ATCC 25923) were seeded in Petri-plates containing MH agar by streaking entire MH media surface using sterilized cell spreader under luminous flame. The mixture was then allowed to dry for five minutes. This was followed by the application of plant extract filter paper discs which were placed over the numbered divided parts on culture media. Two-fold Serial dilutions was used. Concentration was made in range of 100 to 5 mgL^{-1}. Plates were covered to avoid contamination and were incubated for 24 hours at 37℃. DMSO impregnated discs were used as the negative control. Zones of inhibitions of growth were measured in millimeters. The minimum inhibitory concentration (MIC) was described as the lowest concentration of the test compounds that completely inhibited the growth of microorganisms.

3. RESULTS AND DISCUSSION

Phytochemical tests of the extracts revealed the presence of terpenoids, tannins, carrotenoids and flavones in *Bridelia micrantha* and *Grewia plagiophylla* (Tables 1 and 2). Alkaloids were absent in all the leaves extract. Various solvents used produced crude extracts with varying antibacterial activities. Some extracts resulted in the formation of clear inhibition zones and their diameter was indicative of the extent of antibacterial effect on *S. typhi*. *Escherichia coli* (Gram Negative) were completely resistant to all plant extracts at all concentrations and its growth was not inhibited. This is because *E. coli* is known to be resistant against many antibiotics. Crude extracts of both plants leaf extracted using ethyl acetate as a solvent did not display visible zone of inhibition due to lack of antibacterial effect on *E. coli, S. aureus and S. typhi*. Negative control disks using DMSO failed to inhibit bacterial growth while positive control disks using chloramphenical showed clear inhibition zones. To facilitate extraction the solvents diffused inside plant cells and efficacy of extraction depended on solubility of plant's active substances in the various solvents. Highest concentration obtained was equilibrium of concentration between the extract and plant cells. Serial dilution of the crude extracts produced solutions containing lesser concentrations of active substances. This explains the reduction in the diameter of zone with each dilution for example *G. plagiophylla* leaf extract with methanol as the solvent of extraction formed a zone with a diameter of 20 mm without dilution and an almost insignificant zone of 8 mm at lower dilution. The higher the concentration of active substances the larger the diameter of zone was formed.

Each treatment produced significantly different diameters of zones of inhibition. This indicates that the impact of solvent of extraction used differed. In this study it is possible that the antibacterial activity observed could extend to

other pathogenic microorganisms. *G. plagiophylla* methanol extract exhibited the highest antimicrobial activity (19 -20 mm) while *G. plagiophylla* ethyl acetate extract revealed low activity against both gram positive and gram negative bacteria (8-9 mm). The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. In previous study, the phytochemical investigation of *Grewia. mollis* stem bark indicated the presence of Tannins, Saponins, and Cardiac glycosides, Flavonoids, Steroids, Phenols and Resins. This is in agreement with the work done. These phytochemicals are well known for their pharmacological activities. Saponins are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties. Studies have also shown that saponin as a phytochemical found in plants have antitumor and antimutagenic properties and cann also lower the risk of cancer in humans by reducing the growth of cancer cells [18].

Table 1. Compounds present in leaves of *Bridelia micrantha*

	Leaves extract			
	Hex.	**DCM**	**EtoAc**	**Meth**
Alkaloids	-	-	-	-
Terpenoids	+	+	+	+
Tannins	+	+	+	+
Flavanoids	+	+	+	+
Flavones	+	+	+	+
Carotenoids	+	+	+	+

Key: + denotes present, Hex: Hexane, Meth: Methanol, - denotes absent. EtoAc: Ethyl acetate, DCM; Dichloromethane

Table 2. Compounds present in leaves of *Grewia plagiophylla*

	Leaves extract			
	Hex.	**DCM**	**EtoAc**	**Meth.**
Alkaloids	+	+	+	+
Terpenoids	+	+	+	+
Tannins	+	+	+	+
Flavanoids	+	+	+	+
Flavones	+	+	+	+
Carotenoids	+	+	+	+

Key: + denotes present, Hex: Hexane, Meth: Methanol, - denotes absent. EtoAc: Ethyl acetate, DCM; Dichloromethane

Table 3. The minimum inhibition concentration of methanol and ethyl acetate against *Staphylococcus aureus* and *Salmonella typhi*

Solvent extract	**Concentration in mg/mL**	***Bridelia micrantha* (Zone of inhibition in mm)**		***Grewia plagiophylla* (Zone of inhibition in mm)**	
		Staphylococcus aureus	***Salmonella typhi***	***Staphylococcus aureus***	***Salmonella typhi***
Methanol	100	19	13	20	17
	75	17	11	17	15
	50	11	10	15	11
	25	10	8	18	8
	10	7	7	8	7
	5	-	-	-	-
Ethanol	100	8	8	7	9
	75	-	9	5	7
	50	-	-	5	6
	25	-	-	-	-
	10	-	-	-	-
	5	-	-	-	-

4. CONCLUSION

Methanol and Ethyl acetate appeared to be potential for testing plants of high medicinal values for various microbial activities as well other medicinal activities. All the plant extract had no antibacterial effect on *E. coli*, there was observed inhibition of *S. aureus by* methanol extract of *G. plagiophylla and B. micrantha* and less inhibition by ethyl acetate extract of the same plant. This showed that the active ingredients responsible for the antibacterial effect can be best extracted with methanol and Ethyl acetate. Wide zone of inhibition of *G. plagiophylla* and *B. micrantha* against *S. aureus* and *S. typhi* showed that they have great potential as a remedy for infectious diseases caused by *S. aureus* and *S. typhi.*

A negative attitude towards medicinal plants, their use and their users is widespread. By many, especially educated people, medicinal plants are considered inferior to modern drugs that are produced in factories and are sold in pharmacies. To have a meaningful interaction with users of medicinal plants it is of utmost importance for scientists to have an open mind and attitude. Caution is needed when promoting the use of specific medicinal plants. Recommendations, especially for internal use, should be based on local experience and preferably on scientific confirmation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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