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Diagnostic study of some Fatty acids and Antibacterial activity of Bauhinia variegate leaves extracts in some Gram positive and Gram negative bacteria

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Abstract
Antibacterial activity of methanol and equeous extracts of leaves of Banhinia variegata was determined employing the disc diffusion technique, against Escherichia coli (gram negative bacteria) and Staphylococcus aureus (gram positive bacteria). Gentamicin was used as a reference standards for comparism with the extracts activities. The results were appeared with the two extracts owns antibacterial activity against the bacteria that been tested. The methalonic extract of B.variegata was showed highest zone of growth inhibition in staph. aureus was (20 mm) , while the zone of growth inhibition in E.coli was (16 mm). The activity at aqueous extract showed (18 mm) in growth inhibition in staph. aureus and (14 mm) in growth of E. coli .The methanolic extract has much more activity compared to aqueous solutions, in both bacteria, and staph that been tested. aureus was more sensitive than E.coli to both extracts.

1. Introduction

The necessity of information comes by assessing botanical information at a (microlevel) its achieved particular noticeable, and as a uresult, new surveys must be attitude to understand the floristic richness area and the practices of the ethno-medicinal. Since the dawn of time, plants have been an important part of the daily life support system for humans. the contemporary drive to integrate natural resource knowledge for their scientific and commercial exploitation for a variety of applications (1).Plants are the most prolific source of pharmacological intermediates, nutritional supplements, herbal treatments, and chemical building blocks for synthetic pharmaceuticals in both traditional and modern medical systems (2). Strong antibiotics are available, yet multi- or resistance-resistant microorganisms are continuously evolving. As a result, it is important to thoroughly As a kind of safe green medicine, consider the plant utilized in

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traditional medicine (3). Because of their potency, low toxicity, and the availability of natural anticancer drugs manufactured from these plants, the use of medicinal plants is expanding (4). There is a lengthy history of traditional medical usage for several Bauhinia species. There are trees, climbers, and shrubs in the relatively large Bauhinia genus, which may be found all over the world (5). In Hindi, B. variegata L., a family member of the leguminosae, is also known as Kanchanar. a medium-sized tree that grows in the Sub-Himalayan area, which stretches east to Assam and south to India (6). The chemical components identified include tannins, kaempferol-3-glucoside, lupeol, and -sitosterol (7). Extract from B. variegata leaves includes calcium, phosphate, amides, decreasing sugars, (vitamin-C), (crude-protein), and fibers (8). (9) On a dry matter basis, Izatnagar and Palampur leaves were observed to have crude protein contents of 15.80 and 13.18%, respectively (10). From the leaves of B. variegata, quercetin, rutin, apigenin, quercitrin, and apigenin-7-0-glucoside were extracted (11). Heptatriacontan - 12 , 13 - diol and dotetracont - 15 - en - 9 - ol, two novel long-chain substances, were isolated from the leaves of B. variegate (l2). The plant's leaves are frequently used to treat stomatitis (13) and they also stop the action of peptic enzymes (14). Therefore, the current work is being conducted to examine the antibacterial activity of extracts to (leaf) from B. variegata L. Using gentamycin (300 mg/ml) as the standard, methanol and equeous extracts were tested against staph. aureus (gram positive) and E. coli (gram negative).*S. aureus* by detecting the specific *mecA* gene for MRSA.

2. Materials and methods

1- Collecting bacterial samples: Thirteen urine and blood samples from Yarmook Hospital's outpatients were taken. The samples were transported at frigid temperatures. Samples of blood and urine were streaked on nutritional and mackoncy agars and let to sit for twenty-four hours about 1 day at 37 C.

2- Labeling of bacterial isolates the bacterial isolates were identified in accordance with (15) and then described based on morphological, cultural, and biochemical testing.

3- Preparation of plant : The dried leaves of B. variegata were purchased from a local market and ground into a fine powder using an electric grinder. The Department of Plant Protection in College Agriculture of Baghdad University classified the plants.

In order to create methanolic extract, 10 grams of powder were weighed and macerated in 50 ml of 95% methanol. In the same way, eqeous extract was created using distilled water in place of methanol. Filtrates from the suspension of two extracts were then supplied into a clean flask and appropriately labeled before being stored at 4 C° in the refrigerator until needed (16). Discs with a diameter of 5 millimeter were prepared by utilize a paper borer; they were then placed in appropriate containers, sterilized in an autoclave at 121 C for 15min, and then let it to cool. The discs were kept for a later analysis and allowed to suction up separately in the extracts filterate. The created disc has a 0.01 ml capacity of absorption.(17)

4- Antibacterial assay: The in vitro antibacterial activity of B. variegata extracts, which were screened using Nutrient agar, was determined using the disc diffusion

technique (18). The plates of N.agar were made by pouring molten material onto sterile petri dishes. The bacterial suspension was streaked uniformly across the plates and allowed to dry while the plates were left to harden. The two extracts were fully put onto sterile discs. The loaded disc was put on the medium's surface, the extract was given five minutes to diffuse, and the plates were then left to incubate for 24 hours at 37C. Inhibition zones that had developed around the disc at the conclusion of incubation were measured in (mm).4- Estimated of some fatty acids Bauhinia variegate seeds extracts.

Fatty acids were measured by high-performance liquid chromatograph (HPLC), and three types of extraction methods (aqueous, methanol and ethanol) were used. The following fatty acids were estimated) Oleic, Stearic, palmitic, linoleic) The complete randomized design (CRD) was used to statistically evaluate the data, and means were used as comparison using (LSD) least significant difference.

3. Results

The results of our study were showed that the methalonic and equeous that appeared by results of of the antibacterial assay revealed that extracts of Broad spectrum activity was shown by B. variegata against the tested isolates. Two extracts have more anti-G+ ve isolate activity than anti-G- isolate. According to our findings, S. aureus was more responsive to both extracts with an inhibitory zone of (20 mm) by methalonic extract and (18 mm) by equeous extract , E.coli was showed inhibition zone of (18mm) by methanolic extract and (14mm) by equeous extracts . The methanolic extracts was more potent then equeous one in tested bacteria

Table 1. Showed the results of extract of B.variegata for antibacterial activity of methanolic comparing with gentimicin (300 mg)

B.variegata leares extract	Tested bacteria	Mean diameter of inhibition zone (mm)
Methanolic extreact	Staphy. aureus	20
	E. coli	16
Gentamicin	Staphy. aureus	24
	E. coli	20

Table 2. Showed the results of extract of equeous extract of B.variegata for antibacterial activity comparing with gentimicin

B.variegata leares extract	Tested bacteria	Mean diameter of inhibition zone (mm)
Equeous extreact	Staphy. aureus	18
	E. coli	14
Gentamicin	Staphy. aureus	24
	E. coli	20

Figure 1. The methanolic extract activity of B.variegate in growth of E.coli

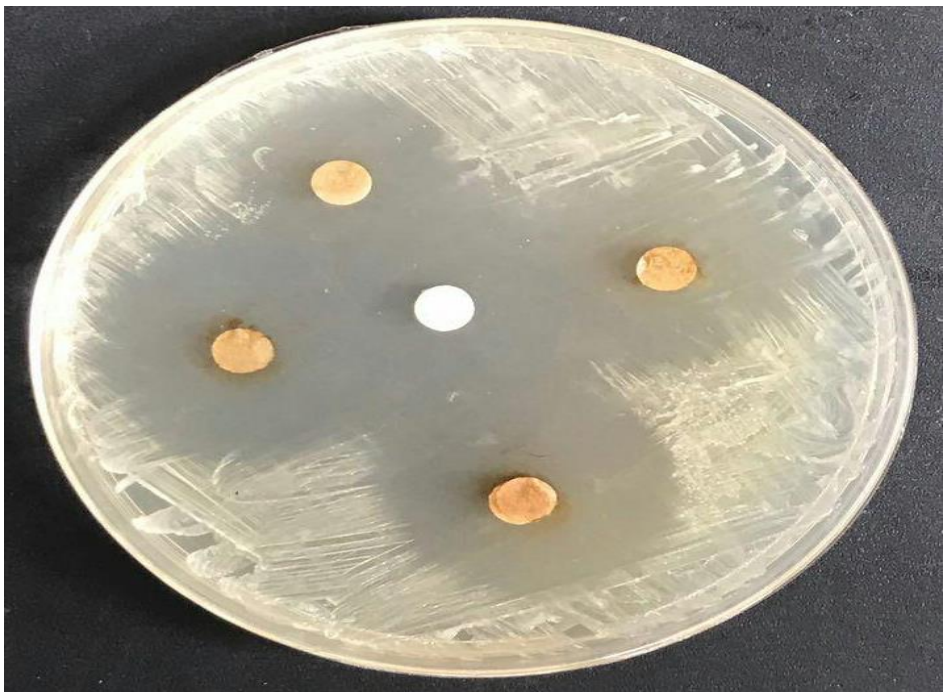

Figure 2. The equeous extract activity of B.variegata in the growth of E.coli

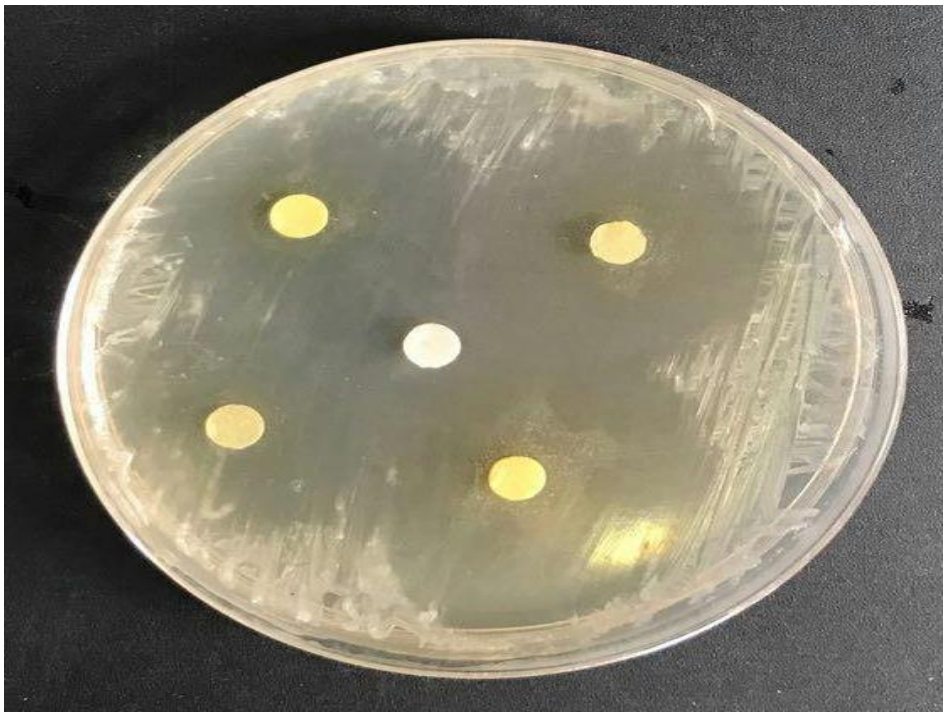

Figure 3. The methanolic extract activity of B.variegate in the growth of Staph.aureus

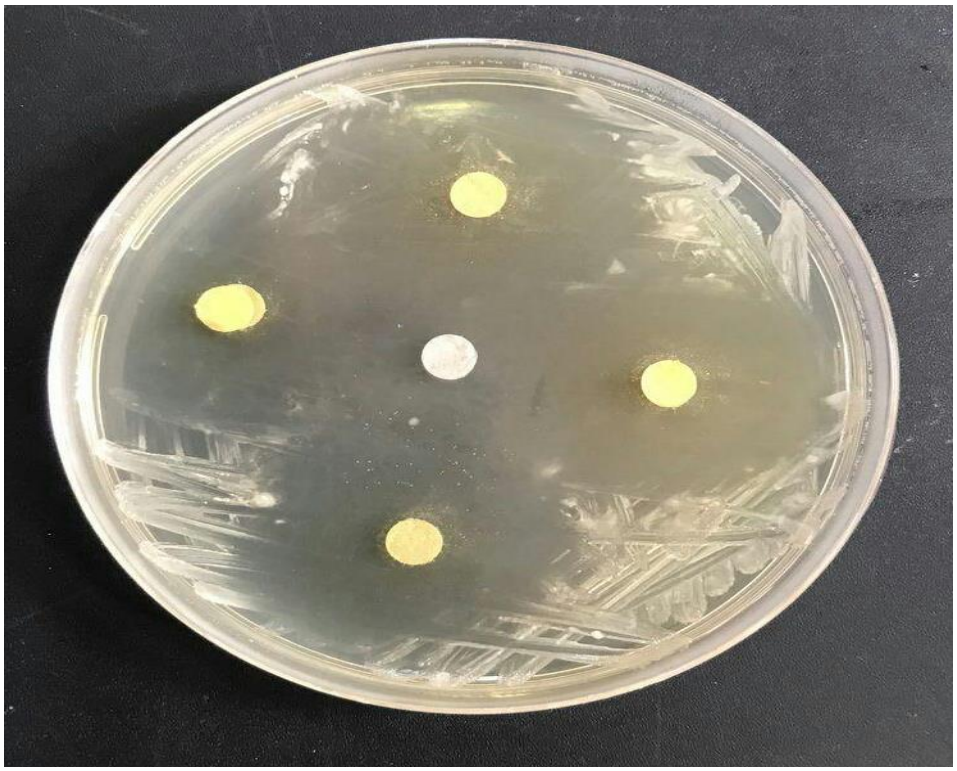

Figure 4. The equeous extract activity of B.variegate in the growth of Staph.aureus

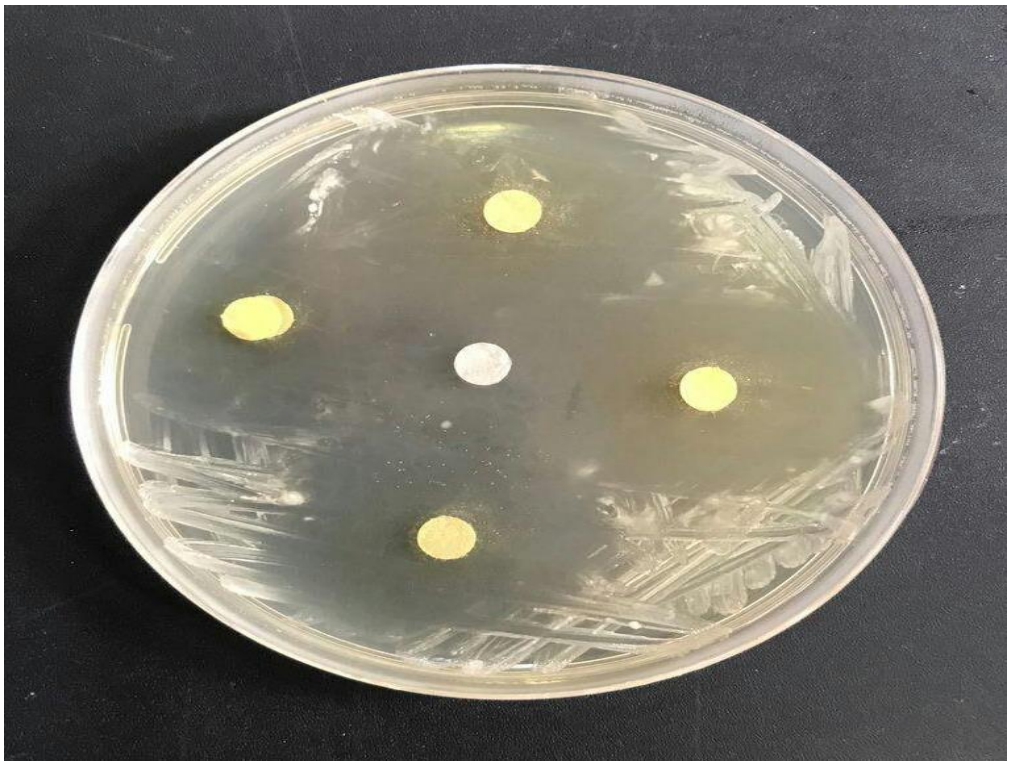

Table (3) show results that there are significant differences in the existence and content of B.variegate leaves that came from some Fatty acids(%)caused by the

effect of the extraction method. The methanol extraction method significantly outperformed the other methods and recorded the highest means of (Oleic,Stearic,palmitic,linoleic) reached(24.51, 17.03, 36.70, 37.32) % sequentially.

Table 3. The effect of the extraction method on the content of B.variegate leaves from some Fatty acids(%)

Extract Method	Oleic	Stearic	palmitic	linoleic
Water	18.24	11.76	30.60	29.64
Methanol	24.51	17.03	36.70	37.32
Ethanol	21.98	14.54	32.97	34.65
L.S.D	2.561	1.986	1.765	2.119

4. Discussion

The major public health concern is the evolution of bacterial resistance to antibiotics, hence it is essential to create new antibiotics with novel modes of action (19). Traditional guarantees of hope for innovative therapeutic molecules come from plants. the application of high importance to therapeutic treatment (20). The extraction method's choice of solvent has a significant impact on the subsequent separation of botanical components from plant material.

Antibacterial activity was detected at varying degrees in equeous and methanol extracts. Additional experiments utilizing solvent of different polarity will investigate the impact of solvent composition on extract effectiveness (21). We tested B. variegata's methanolic and equeous extracts in this investigation because they have potential biological activity, as seen by the results in agreement with .(22)

Antibacterial testing results showed that both had broad spectrum activity against the tested isolate, with G+ ve isolates having higher activity than G- ve isolates. It has already been established that G+ ve bacteria are more vulnerable to both extracts than G- ve bacteria (23). These discrepancies may be caused by the G+ ve bacteria's single-layered cell wall. Contrarily, the cell wall of the G-ve has many layers (24), The presence of prion in the outer membrane of G-ve bacteria prevented the diffusion of many antibiotics, and the multidrug efflux pumps at the trans membranes would also pump out the antibacterial agent through the active efflux processes, giving G- (-) ve bacteria a higher intrinsic resistance, even though the passage of active compound through the G- ve cell wall may be inhibited (25). Many research have demonstrated the antibacterial potency of various Bauhinia species, and it is believed that the presence of flavonoids and phenolic chemicals is what causes this activity.(26)

Enzyme inhibition by oxidizing chemicals, presumably by reactivity with sulphur group, or through more general contact with protein are among the possible mechanisms of phenolic activity (27). It's possible that the active components are absent from the crude extract in insufficient amounts for the dosage level being used to produce activity (28) Potential antibacterial action seems to be significantly influenced by the polarity of the slovent. With regard to bacterial strains with musical significance, both extracts demonstrated exceptional activity.

These findings support the long-standing use of B. variegata. Additional phytochemical investigations to identify and clarify the active component in plant material evaluated are required to serve as a starting point for the creation of new bioactive antimicrobial compounds. The methanol extraction method significantly outperformed the rest of the methods .Results acheived are in compatible with the findings. [29] [30] [31] [32].

5. Conclusion

In light of the obtained results, we can conclude that the methanolic extract was has much more activity compared to aqueous solutions, in both bacteria, and staph that been tested. aureus was more sensitive than E.coli to both extracts.

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