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ISOLATION OF 3,5,7,4'- TETRAHYDROXYFLAVONE FROM BARK OF SUDANESE ACACIA TORTILIS (FORSK) HAYNE AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT

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ABSTRACT

A flavonoid compound was isolated from the ethanol extract of *Acacia tortilis* stem by thin layer chromatography and its structure was partially characterized on the basis of its spectral data (UV, IR and NMR). The ethanolic extract of *Acacia nubica* was screened for antimicrobial activity against five standard human pathogens: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and the fungal species *Candida albicans*. The ethanolic extract exhibited significant antibacterial and antifungal activity against test organisms.

INTRODUCTION

Natural products have long served as a rich source of therapeutic agents and continue to play an indispensable role in drug discovery and development. In recent years, interest in plant-derived compounds has intensified due to the global rise in antimicrobial resistance, which threatens the efficacy of conventional antibiotics and poses a major public health challenge worldwide.[1] As microbial pathogens increasingly evolve resistance mechanisms, there is an urgent need to explore new, effective, and affordable antimicrobial agents from natural origins.[2]

Medicinal plants contain a diverse array of phytochemicals—including flavonoids, alkaloids, tannins, saponins, and essential oils—that have demonstrated significant biological activities.[3] Among these compounds, flavonoids represent one of the largest groups of

secondary metabolites and are known for their antioxidant, anti-inflammatory, and antimicrobial properties.[4,5] Flavonoids are widely distributed in the plant kingdom and play essential roles in plant defense, pigmentation, and signaling.

Acacia tortilis (Forsk.) Hayne, a member of the Fabaceae family, is widely distributed across arid and semi-arid regions of Africa and the Middle East. It has been used traditionally to treat various ailments including infections, gastrointestinal disorders, and inflammatory conditions.[6] Previous studies have reported the presence of bioactive compounds such as phenolics, flavonoids, sterols, and terpenoids in different parts of *Acacia* species, contributing to their notable pharmacological activities.[7,8]

Seed, bark, and stem extracts of various *Acacia* species have demonstrated promising antimicrobial activity against a broad range of Gram-positive, Gram-negative bacteria, and pathogenic fungi.[9] Despite this, the chemical characterization and antimicrobial potential of ethanol extracts of Sudanese *Acacia tortilis* remain insufficiently explored. Flavonoids isolated from *Acacia* species, particularly flavones and flavonols, have shown potent antimicrobial behavior attributed to their ability to disrupt microbial membranes, inhibit nucleic acid synthesis, and interfere with energy metabolism.[5,10]

Analytical tools such as UV-Visible spectroscopy, infrared spectroscopy (IR), and nuclear magnetic resonance (NMR) provide efficient means for structural identification of flavonoids. These techniques allow rapid confirmation of functional groups and conjugation systems characteristic of flavonoid structures.

Given the rising need for new antimicrobial agents and the limited data available on Sudanese *Acacia tortilis*, this study aims to isolate a flavonoid compound from the bark of *A. tortilis*, characterize its structure using spectroscopic methods, and evaluate the antimicrobial activity of its ethanol extract against selected pathogenic microorganisms.

MATERIALS AND METHODS

MATERIAL

Plant material

Stem of Acacia tortilis w as collected from Gezira state(Sudan). The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute(Sudan).

INSTRUMENTATIONS

UV spectra were run on a Shimadzu 2401PC UV-Visible Spectrophotometer. The IR spectra were run on a Perkin- Elmer 1310 Infrared Spectrophotometer. NMR spectra were performed on a Joel ECA 500MHZ NMR Spectrophotometer.

Test organisms

The antimicrobial activity of the ethanolic extract was evaluated using the following standard microorganisms: Bacillus subtillus (Gram +ve), Staphylococcus aureus (Gram +ve), Pseudomonas aeroginosa (Gram -ve), Escherichia coli (Gram -ve) and the fungal species Candida albicans.

METHODS

Extraction and isolation of flavonoids

Powdered stem of a Acacia tortilis (1.0 kg) were macerated at room temperature with ethanol (95%) for 72 hours. The solvent was evaporated under reduced pressure to dryness to give a crude product. The crude ethanol extract was fractionated via paper chromatograms were viewed and located under UV light and a flavonoid- compound was eluted from the paper with methanol.

Antimicrobial activity

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of

colony forming units per ml suspension.

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

Testing of antibacterial susceptibility

Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured and averaged.

RESULTS AND DISCUSSION

Characterization of the compound

The compound was isolated by paper chromatography as yellow powder from Acacia tortilis stem and its structure was elucidated via a combination of spectral techniques (UV, IR and ^{1}HNMR). The IR spectrum of the compound (Fig.1) showed v(KBr): 3290.33cm^{-1}(OH) stretching phenolic, 2925.81, 2850.59 cm^{-1} (C-H) stretching aliphatic, 1612.38cm^{-1} (C=O) carbonyl, conjugated ketone, 1460.01, 1421.44 cm^{-1} (C=C Ar.) 1269.07, 1190.00, 1110.92 cm^{-1} (C-O, ether) and 871.76, 725.18, 680.83 cm^{-1} (Ar. bending). This indicate that the flavonoid contains two substituted aromatic ring with tri substitution patterns. The appearance of a carbonyl stretching in the IR spectrum suggests absence of two classes of flavonoids characterized by the absence of a carbonyl function-the flavans and the anthocyanins.[10,11]

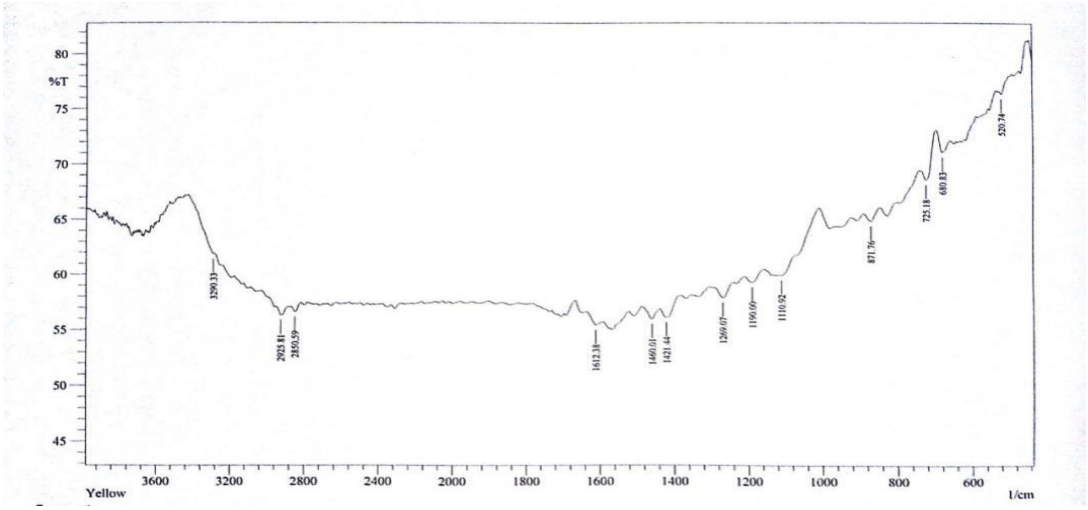


Fig. 1: IR spectrum of the compound.

The UV- spectrum of flavonoid compound in methanol exhibited two characteristic absorption bands: Band II at 232 nm (related to the benzyl system, ring A) and band I at 362 nm (associated with the cinnamoyl system, ring B-C). The position of band II confirms that the compound is a flavone – type.(Fig.2)

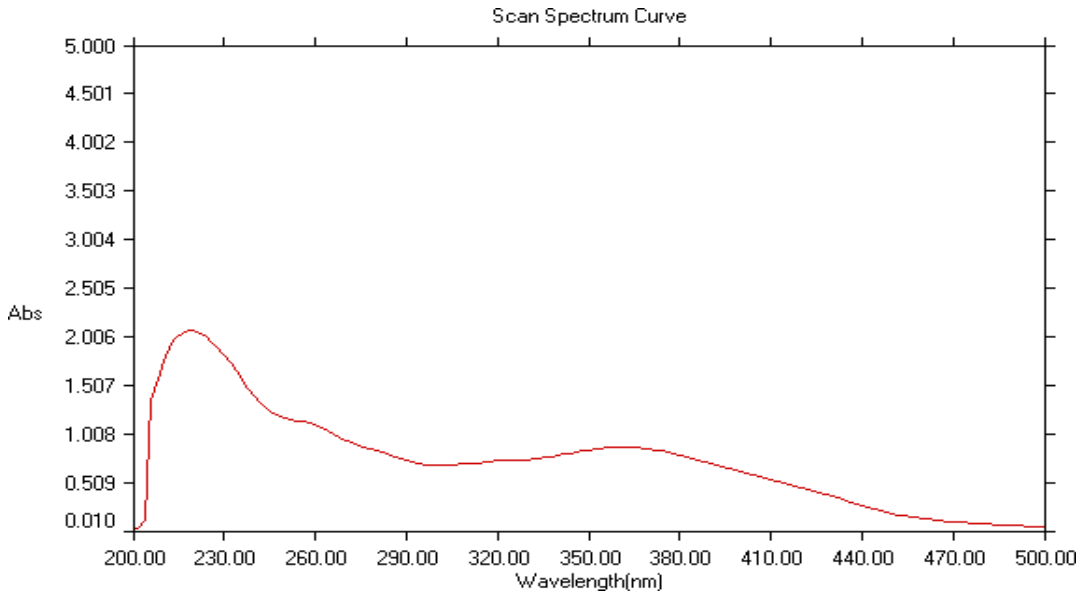


Fig. 2: UV spectrum of the compound in methanol.

After addition of sodium methoxide ($NaOCH_3$), a significant bathochromic shift was observed in band I, which moved from 326 nm to 373-428 nm, accompanied by an increase in absorbance intensity. In contrast band II showed only a minor shift from 232 nm to 272 nm. The spectral change occurs causing deprotonation of the phenolic – OH, particularly at the 4' – hydroxyl position on the B- ring of the flavonoid.(Fig. 3).

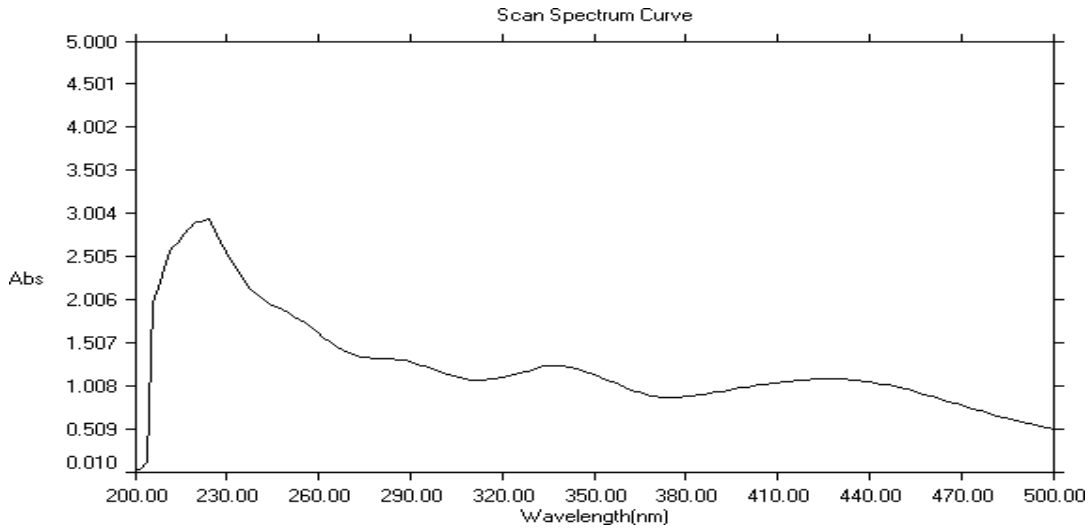


Fig.3: Sodium methoxide UV spectrum of the compound.

The UV- spectrum of the flavonoid in methanolic solution with $AlCl_3$ show characteristic Band II at 230 nm and band I at 348 nm. The appearance of an additional band at 430 nm indicates the formation of a flavonoid – Al^{3+} complex, confirming the presence of hydroxyl groups at position 5 and 4' in the flavonoid structure.(Fig. 4)

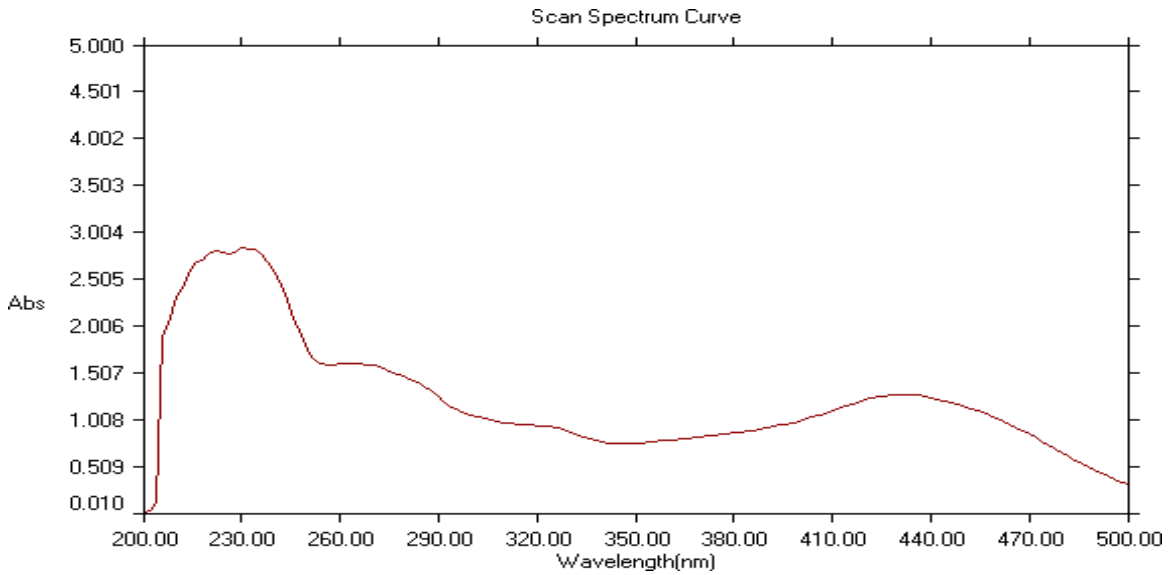


FIG. 4: Aluminum chloride UV spectrum of the compound.

The marked bathochromic shift Band I suggests the presence of hydroxyl groups at positions 5 and / or 4' that can chelate with Al^{3+} ions, confirming that the compound is a flavonoid with hydroxyl substituents capable of metal complexation. (Fig. 5).

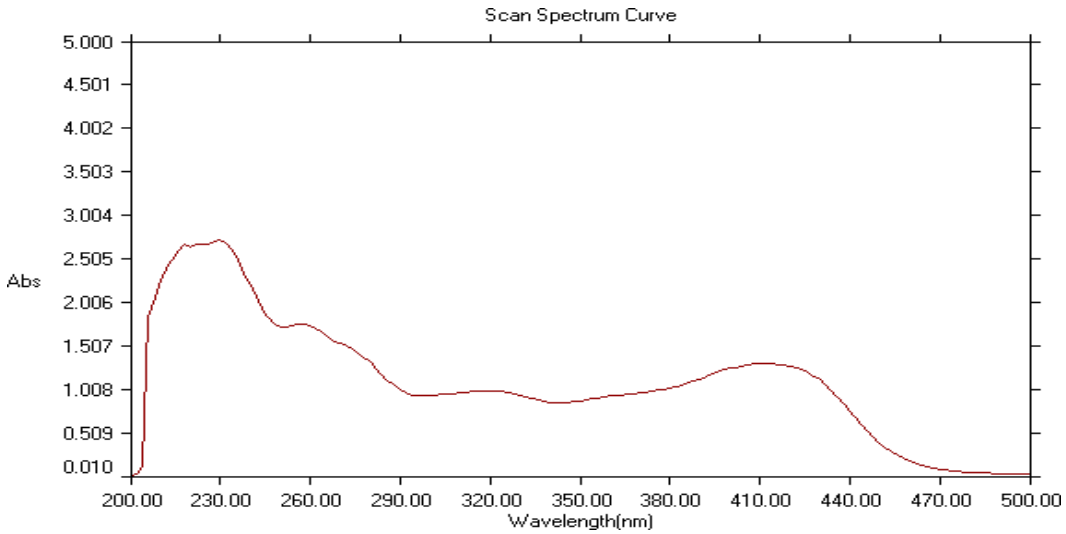


Fig. 5: Aluminum chloride/HCl UV spectrum of the compound.

When a methanolic solution of the compound was treated with excess powdered sodium acetate the UV spectrum supports the presence of hydroxyl groups (OH) in the flavone structure (likely at the positions 7,3, and 4').

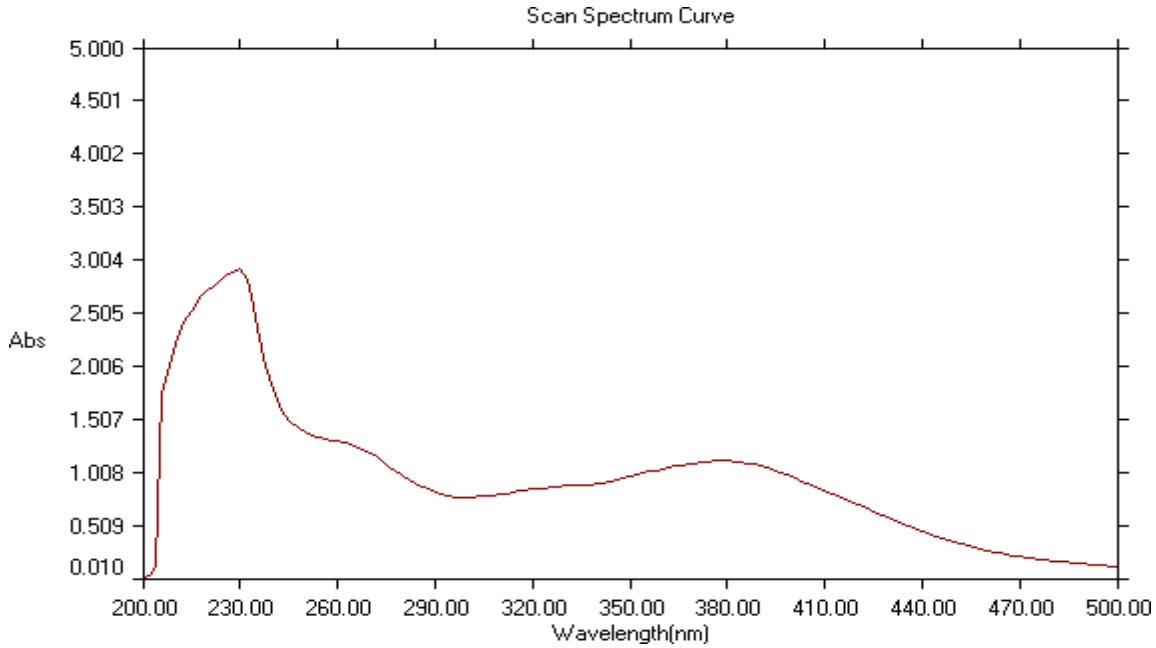


Fig.6: Sodium acetate UV spectrum of the compound.

Fig. 7 show the spectrum confirms that the flavonoid interacts with boric acid, indicating the presence of hydroxyl group (OH) (at positions 3 and 7), leading to spectral changes due to borate complex formation.

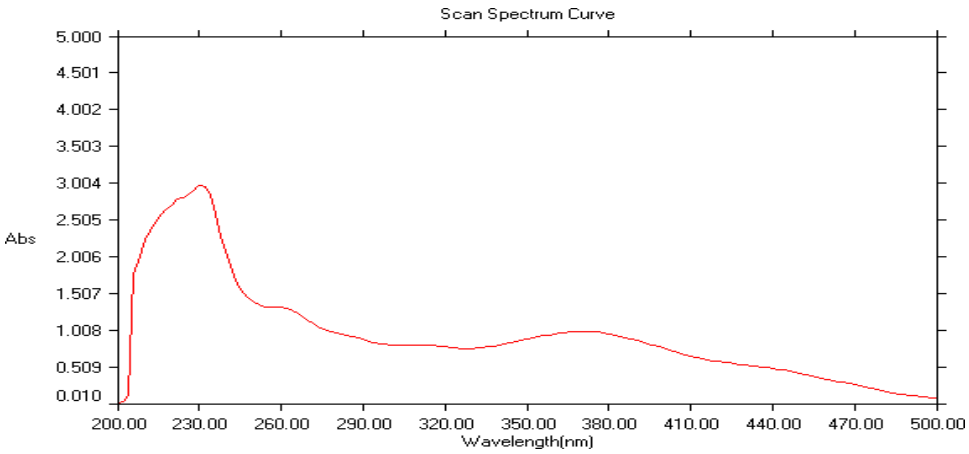


Fig.7: Boric acid UV spectrum of the compound.

The ^{1}HNMR spectrum of the compound (Fig.8) showed $^{\delta}$ ppm: multiplet (6.0 – 8.0) assigned for aromatic protons of ring a and B; multiplet (3.0 – 5.5) assigned for a sugar moiety (not identified in this study); (0.8 – 2.5) minor signals possibly from aliphatic parts, solvent, or impurities.

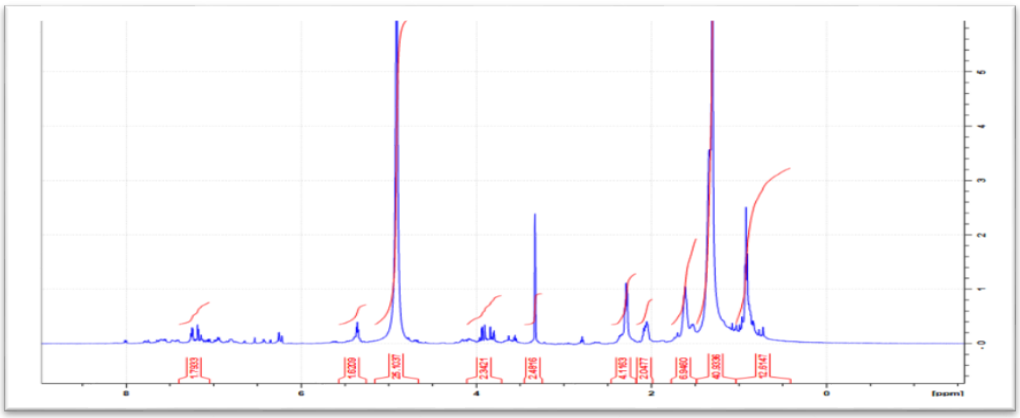
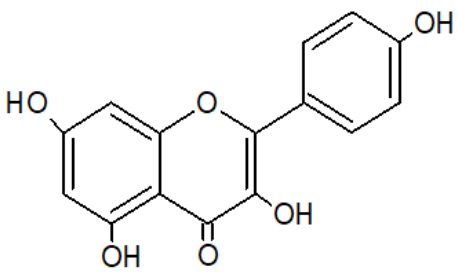


Fig. 8: ^{1}HNMR spectrum of the compound.

On the basis of the above spectral data, the following partial structure was proposed for aglycone of the compound.



Aglycone of the compound.

Antimicrobial activity

The crude ethanol extract of Acacia tortilis stem was screened for its antimicrobial activity against five standard microorganism. The results are depicted in (Table 2). Results were interpreted in the following conventional terms: (< 9mm : inactive; 9-12mm: partially active; 13- 18mm: active; >18mm: very active).

Table 1: Test organisms.

No	Micro organism	Type	Source
1	Bacillus subtillus	G +ve	ATCC 2836
2	Staphylococcus aureus	G +ve	ATCC 29213
3	Pseudomonas aeroginosa	G +ve	NCTC 27853
4	Escherichia coli	G +ve	ATCC 25922
5	Candida albicans	G +ve	ATCC 7596

NCTC. National collecntion of type culture, Colindale, England ATCC. American type culture collection, Maryland, USA.

Table 2: Inhibition zones (mm/mg sample)

Candida albicans Sample	EC	Ps	Sa	Bs	Ca
Ethanol extract(100mg/ml)	18	16	18	20	15
Ampicilin (40mg/ml)	-	-	35	22	-
(20mg/ml)	-	-	29	20	-
(10mg/ml)	-	-	19	17	-
Gentamycin (40mg/ml)	25	24	25	28	-
(20mg/ml)	19	18	22	24	-
(10mg/ml)	16	15	16	16	-
Clotrimazole (30mg/ml)	-	-	-	-	37
(15mg/ml)	-	-	-	-	29
(7.5mg/ml)	-	-	-	-	26

Sa: Staphylococcus aureus Ca: Candida albicans

Ec: Escherichia coli

Ps: Pseudomonas aeroginosa Bs: Bacillus subtillus

Ca: Candida albicans

The ethanol extract exhibited very good activity against Bacillus subtillus and good activity against Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia Coli and Candida albicans.

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