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Antimicrobial activity of 7,3′,4′-trihydroxyflavonol isolated from *Acacia nilotica* var. *ad stringens*

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Acacia species (Mimosaceae) is widely distributed in tropical and subtropical countries and has a variety of ethnomedicinal uses. There is inadequate laboratory investigation to identify bioactive compounds and therapeutic effect of *Acacia nilotica* var. *ad stringens*. This research has been conducted to extract, isolate and identify major compounds from heartwood of *Acacia nilotica* var. *ad stringens* and to test them against representative bacteria. Powdered air-dried heartwood of *A. nilotica* var. *ad stringens* has been extracted with methanol/water, 4:1 and the extract has been then purified using chromatographic techniques (column and paper chromatography). A pure flavonoid compound has been isolated and the structure has been elucidated based on extensive spectroscopic analysis procedures (IR, UV, ^{1}H NMR, and mass spectrometery). The isolated compound has then been evaluated for antimicrobial potential against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*) using cup-plate agar diffusion method. The spectroscopic analysis of the isolated compound has led to its identification as 7,3′,4′-trihydroxyflavonol. The compound shows varying antimicrobial responses with high potency against Gram-negative human pathogens. The Gram-positive bacteria which are inhibited are *Bacillus cereus*, *Corynebacterium* sp., *Enterococcus faecalis*, *Staphylococcus aureus* , and *Streptococcus agalactiae.* The Gram-negative bacteria are *Acinetobacterbaumannii* , *Acinetobacter* sp. , *Escherichia coli*, *Pseudomonas aeruginosa* and yeasts are *Candida albicans* and *Cryptococcus neoformans.* The present study has demonstrated that 7,3′,4′-trihydroxyflavonol is an effective antimicrobial compound. If applied in suitable pharmaceutical formulations it could be valuable for treating various bacterial infections or introduced as adjunct treatment along with standard agents.

Keywords: Ethnomedicine, *Acacia*species, flavonols, spectroscopic analysis, *in vitro* antimicrobial activity

Acacia nilotica is the most significant genus of family Leguminosae first described by Linnaeus in 1773 [1]. This plant is therapeutically used as anti-cancer, antiscorbutic, astringent, anti-oxidant, natriuretic, antispasmodic, diuretic, for intestinal pains and diarrhea, as nerve stimulant, for colds, congestion, coughs, dysentery, and fever[2]. Pods are not necklace-like, with margins straight or serrated and rarely narrowly constricted between the seeds, if constricted then only occasionally along with the pod. Branches and pods are densely pubescent to tomentose especially when young. Pods often rather wide (1.3-2.2cm) with distinctly and often irregularly crenate margins. It has occurrence in Algeria, Burkina Faso, Cameroon, Cape Verde, Chad, French Guiana, Gambia, Ghana, Guinea Bissau, India, Iran, Ivory Coast, Libya, Mali, Niger, Nigeria, Oman, Pakistan, Senegal, Somalia, Sudan, and Togo[3, 4]. It is used as an effective medicine in the treatment of malaria, sore throat (aerial part) and toothache (bark)[5].

The powdered bark of the plant with a little salt is used for treating acute diarrhea[6]. The gum or bark is used for cancer of ear, eye, or testicles, induration of the liver, spleen and excess flesh. Bark, leaves, and young pods are strongly astringent due to tannins and are chewed in Senegal as antiscorbutic. The bark decoction is drunk for controlling intestinal pains and diarrhea. Other preparations are used for coughs, toothache, ophthalmia, and syphilitic ulcers. In Lebanon, the resin is mixed with orange-flower and used as an infusion for typhoid convalescence[4,7]. Egyptian Nubians believe that diabetics may eat unlimited carbohydrates as long as they also consume powdered pods[7]. Extracts of the

plant were found to be inhibitory to at least four species of pathogenic[8] microbes.

Flavonoids are natural phenolic compounds which appear as secondary metabolites of the plant[9], they are found in many plant tissues, which present inside of the cells or on the surfaces of different plant organs. The flavonoids may be modified by hydroxylation, methylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to the carbon atom of the flavonoid skeleton. Also, alkyl groups (or phenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core[10]. Flavonoids occur both in the (Free State) and as glycosides; their chemical structure is based on a C15 skeleton consisting of two benzene rings connected by a three-carbon chain, that is, C6-C3-C6. The three-carbon on chain is generally closed to form a heterocyclic ring (the C-ring)[11]. The position of the substitution also affects the properties. The flavonols containing two *ortho* or *para* hydroxyl in the 2-phenyl ring have anti-oxidant properties, while free hydroxyl groups at the 5,7-positions have a pro-oxidant effect. Apigenin and genkwanin, which are present in the Chinese, drug "Yuen-Hua" are believed to have diuretic and anthelmintic properties[12].

Some flavonoids like myricetin and kaempfero-l3-glucoside have an anti-HIV-I potency at a non-toxic concentration[13]. Some of the minor flavonoids have very interesting activities. They have anti-microbial, anti-fungal and cytotoxic properties[10]. Anthocyanin, peonin, and isorhamnetin of the algae *Chlamydomonas* are highly potent sex-determining hormone[8]. The health effects of flavonoids have long been recognized for anti-oxidant, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anti-carcinogenic properties[14]. Isoflavones, the bioactive ingredient in leguminous vegetables, not only cause a small reduction in blood cholesterol but also reduce blood pressure, arterial dimensions, and oxidative stress[15].

Results and Discussion

Identification of the isolated compound

The IR spectrum of a compound (Figure S1) (KBr disc) spectrum was showed ν 3520(OH), 1608 (C=O), 1569, 1528, and 1476 (C=C, Ar), 1116 (C-O), 854, 808, 770 cm^{-1} (C-H, Ar, bending).

The isolated compound gave UV absorption characteristic flavonol revealed λmax (MeOH) 248, 363 nm (Figure S2A). Addition NaOMe to a methanolic solution gave a 41 nm (Figure S2B) bathochromic shift in a band I without a decrease in intensity and this is diagnostic of a free 4`-OH. Sodium acetate spectrum (Figure S2C) gave a 17 nm band II bathochromic shift which is indicating a 7-OH function. The boric acid spectrum (Figure S2D) revealed an 18 nm bathochromic shift in band 1, indicating the presence of a B ring catechol moiety. When $AlCl_3$ was added to a methanolic solution, a band I shifted bathochromically by 67 nm (Figure S2E). This indicates a 3- or 5-OH or an ortho-dihydroxyl system. The spectrum was acid-stable (Figure S2F) this indicating of a 3-OH function.

^{1}H-NMR showed doublet at δ 7.8ppm (1H) characteristic of C_5- proton (Figure S3). The doublet at δ 6.8 ppm (2H) is characteristic of C_6- and C_8-protons. The doublet at δ 7.6 ppm (2H) is account for $C_{2`}$- and $C_{6`}$-protons while the doublet at δ 7.4 ppm (1H) was assigned for $C_{5`}$- proton.

Mass spectrum of the isolated compound shown at base peak *m/z* 286 (Figure S4). According to the above cumulative data, the following structure suggested that the compound is 7,3′,4′-trihydroxyflavonol (Figure 1).

Antimicrobial activity

The antimicrobial activity of the tested compound was examined against selected gram positive-bacteria and gram-negative bacteria in comparison to reference antibiotics (Figure 2). Results revealed that the tested compound had different antimicrobial responses (Table I). The gram-positive bacteria which were inhibited were *Bacillus cereus*, *Corynebacterium* sp., *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus agalactiae*. The gram-negative bacteria were *Acinetobacter baumannii*, *Acinetobacter* sp., *Escherichia coli*,

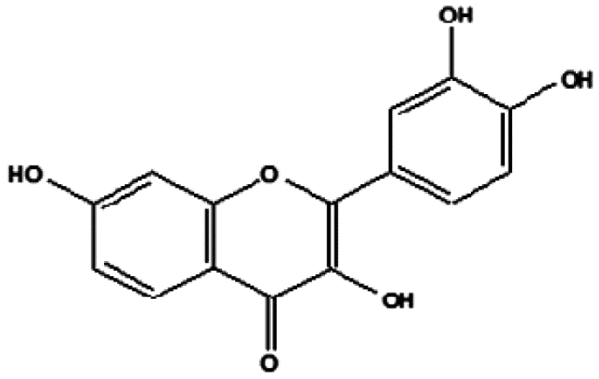

Figure 1 — Chemical structure depiction of the 7,3′,4′-trihydroxy flavonol

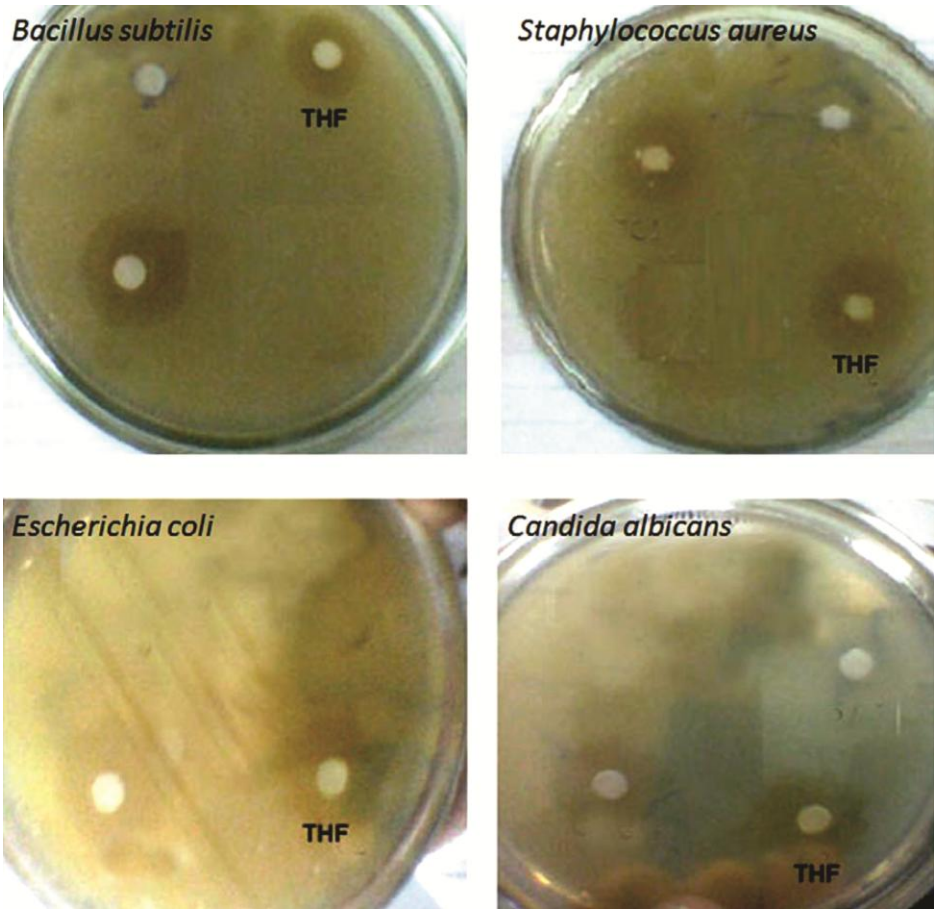

Figure 2 — Antimicrobial activities of7,3′,4′-trihydroxyflavonol (THF) isolated from *Acacia Nilotica* var. *ad stringens* (inhibition zone diameter mm/mg sample). Abbreviations:1, 1 mg/ mL; 1, 10 mg/ mL; 3, 100 mg/ mL.

Pseudomonas aeruginosa. The inhibited yeasts were *Candida albicans* and *Cryptococcus neoformans.*

In conclusion, the compound, 7,3′,4′-trihydroxy flavonol, was extracted and isolated from *A. nilotica* var. *ad stringes* (collected from Kordofan area, western Sudan). The isolated compound was extracted with methanol and purified using the TLC technique, the structure of the isolated compound was studied and identified using UV-Vis, IR, ^{1}H NMR, and mass spectra. The biological activity was studied and showed good results.

Experimental Section

Plant materials

The heartwood of *A. nilotica* var. *ad stringens* was collected from Elobied, North Kordofan, Sudan. The plant was authenticated by the Department of Botany, University of Khartoum, Sudan.

Extraction of flavonoids

Powdered air-dried heartwood (1 kg) of *A. nilotica* var. *ad stringens* was extracted with 80% methanol

Table I — Antimicrobial activities of the 7,3′,4′-trihydroxyflavonol(THF) isolated from *Acacia nilotica* var. *ad stringens* (inhibition zone diameter mm/mg sample)

Microorganism	Source / Code	Inhibition zone diameter (mm / mg sample)
Gram-positive bacteria		
Bacillus cereus	Laboratory isolates	15
Corynebacteriumdiphtheriae	ATCC 13812	10
Enterococcusfaecalis	ATCC 19433^{T}	12
Staphylococcus aureus	ATCC 25923	26
Staphylococcus aureus	Clinical isolate	18
Streptococcus agalactiae	ATCC 13813^{T}	15
Gram-negative bacteria		
Acinetobacterbaumannii	ATCC 19606^{T}	19
*Acinetobacter*sp.	Clinical sample	20
Escherichia coli	ATCC 25922	22
E. coli	Clinical sample 4662	20
Pseudomonas aeruginosa	ATCC 27853	22
Yeasts		
Candida albicans	ATCC 10231	15
Cryptococcus neoformans	Clinical isolate; VY 4662	16

(5L) at ambient temperature for 72 hours. The solvent was removed in vacuum to give a crude product.

Isolation method

The methanol extracts of *A. nilotica* var. *ad stringens* were separately slurred with water, mixed with a small amount of polyamide and applied on top of the polyamide (800g) column (100×5cm). Stepwise gradient elution was carried out using a solvent system of decreasing polarity starting with 100% water and ending with 100% methanol. Fractions of 250 ml were collected and investigated by PC (Whatman No. 1) using three solvent systems: BAW, 15% AcOH and distilled water. Chromatograms were visualized under UV light before and after exposure to ammonia vapor. Similar fractions were combined and concentrated to dryness under reduced pressure to obtain main fractions. The detected compound was isolated by subsequent PPC from column fraction using different solvent systems (BAW, 15%AcOH). The isolated compound was further purified by re-chromatography on Sephadex LH-20 column using MeOH/H_2O, 4: 1.

Preparation of bacterial suspensions

One ml aliquots of 24-hours growth culture of the test organisms were aseptically distributed on to agar slopes and incubated at 37°C, for 24 h. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 108 – 104 colony forming units per ml. The suspension was stored in the refrigerator at 4ºC until used. The average number of the viable organism per ml of the saline suspension was determined through the surface viable counting technique. Serial dilution of the stock suspension was made in sterile saline in tubes and on drop volumes (0-20 ml) of the appropriate dilution were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature to dry and then incubated at 37ºC for 24 h.

Antimicrobial assay

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobial activity of Mannich bases and their reaction products with *o*-phenylenediamine. Two ml of the standardized bacterial stock suspension was mixed with 200 ml of sterile molten nutrient agar which was maintained at 45ºC in the water bath. Aliquots (20 ml) of the incubated nutrient agar were distributed into sterile Petri dishes; the agar was left to settle in each of these plates which were divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No.4). Each of the holes was designed for one compound. Separate Petri dishes were designed for standard antibacterial chemotherapeutic (ampicillin and gentamycin) agents.

The agar discs were removed, a hamates cup was filled with 0.1 ml sample of each compound using adjustable volume microtiter pipette and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37ºC for 48 h.

The above procedure was repeated for different concentrations of the isolated compounds and the standard antimicrobial chemotherapeutic agents. After incubation, the diameters of resultant growth inhabitation zones were measured.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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