

ANTIMICROBIAL STUDY OF TEPHROSIA NUBICA FLAVONES

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SUMMARY: *Tephrosia press. species (Leguminosae) used medicinally and as fish poison, are known to be a source of flavonoids. The antimicrobial activity of the prenylated flavones, semiglabin, pseudosemiglabrin, apollinine and lanceolatin A, which we have isolated from Tephrosia nubica herb, widely grown in Egypt, was studied. The results revealed that the four flavanoids exhibit an antifungal activity against Aspergillus niger, Penicillium funiculosum, Fusarium moniliform and Phoma spp.*

Key words: *Tephrosia nubica, prenylated flavones, antifungal activity.*

INTRODUCTION

Flavonoids are naturally occurring substances possessing several biological properties. An ever increasing number of pharmacologic effects have become known through the discovery of new plant flavonoids and through variations of chemical structure of flavones and related derivatives. Mention may be made of the spasmolytic, anti-anginal, anti-hepatotoxic, anti-inflammatory, anti-allergic, antimicrobial, antiviral, etc. effects of flavonoids (Garbor, 1986). The antimicrobial effect of some flavonoids were studied by several researcher. Shipp and Bailey, (1978) investigated the antifungal activity of the phytoalexins, phaseollin, phaseollidin, phaseollinisoflavan, kievitone, medicarpin and piastin against several fungi as *Alternaris brassiciola*, *Aspergillus niger*, *Botrytis cinera* and *Glomerella cingulata*. The flavonoids quercetin, kaemferol as well as the glycosides rutin and isoquercitrin possess an antifungal activity (Beschta *et al.*, 1984). Kramer *et al.*, (1984) concluded that the isoflavones and isoflavanones isolated from soybean and chickpea do not possess any remarkable activity, where as the isoflavans are comparatively good inhibitor of mycelial growth. The dihydrofuranoisoflavones isolated from Lupinus species, family Leguminosae showed an antifungal activity against *Aspergillus flavus* and *Botrytis cinerea* (Thara *et al.*, 1984).

The genus *Tephrosia* (Leguminosae, subfamily Papilionoideae) have led to the isolation and identification of numerous flavonoids. Some rotenoids (e.g. rotenone) from many *Tephrosia* species, possess pronounced insecticidal and fish poisoning properties (Ingham and Markham, 1982). Certain isoflavonoids particularly pterocarpan and isoflavans are also toxic to microorganisms. Lwande *et al.*, (1986) isolated a 6a-hydroxylated pterocarpan (hildecarpin) from the roots of *Tephrosia hidebrandtii* which exhibited an insect antifeedant activity and fungicidal property against *Cladosporium cucumerium*.

Therefore, it was found of interest to investigate the antimicrobial activity of the prenylated flavones, semiglabin, pseudosemiglabrin, apollinine and lanceolatin A, isolated from *Tephrosia nubica* herb widely grown in Egypt, as it has not been studied before.

MATERIALS AND METHODS

Materials

1. Sample of *Tephrosia nubica* (Boiss) Baker, herb, Leguminosae, collected from Gebal elba, Egypt and authenticated by Dr. Loutfy Boulou, Professor of Taxonomy at the National Research Centre, Dokki, Cairo-Egypt.

2.. Gram positive bacteria (*Bacillus subtilis*) and *Bacillus cereus* Gram negative bacteria (*Escherichia coli*) Yeast (*Saccharomyces cerevisia*) and Fungi (*Aspergillus niger*, *Penicillium funiculosum*, *Fusarium moniliform*) and (*Phoma* species), were used for microbiological study.

Methods

1. Extraction of flavonoids:

Semiglabin, pseudosemiglabrin, apollinine and lanceolatin A were isolated from the chloroformic extract of the defatted dried powdered herb material of *Tephrosia nubica* using Flash column chromatography and PTLC as described in details (Ammar and Jarvis,

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1986). The structures of all compounds were determined by physical and spectral analysis (m.p., T.L.C., uV, ir, ms and $^1\text{Hnmr}$) as well as comparison with published data (Waterman and Khalid, 1980).

2. Determination of the antimicrobial activity of *T. nubica* flavones against different microorganisms:

The cup-plate method (Abou-Zeid and Shehate, 1969) was adopted with slight modification using filter paper discs of 0.5 cm diameter. A solid medium containing the following ingredient (g/l). Peptone 6.0, Yeast extract 3.0 Meat extract 1.5, Glucose 1.0 and Agar agar 20.0. The medium was sterilized and divided while hot (40-50°C) in 15 ml portions among sterile petridishes of 9 cm diameter. One ml of cell suspension (2×10^8 cells/ml) of the test organism was spread all over the surface of the cold solid medium.

1 mg and 10 mg of each of the tested compounds was dissolved in 1 ml of chloroform. An amount of 0.1 ml of the tested solution was placed on the filter paper discs and the solvent was left to evaporate. The saturated discs were placed carefully on the surface of the inoculated solid medium, and the Petri-dishes were incubated at 5°C for 1-2 hours to permit good diffusion and the transferred to a incubator of 35°C for 10-16 hours. Then examined and the results were recorded by measuring the inhibition zones caused by the various compound on the test microorganisms.

RESULTS AND DISCUSSION

Four 7-oxygenated -8- prenyl flavones, semiglabrin (1) pseudosemiglabrin (2), apollinine (3) and lanceolatin A (4) were isolated from *Tephrosia nubica* herb (Figure 1). The antimicrobial screening of the four compounds revealed a marked antifungal activity. The effect proved to be potent against *Aspergillus niger*, *Penicillium funiculosum*, *Fusarium moniliform* and *Phoma* species in a concentration of 10 mg/ml. Dilute concentra-

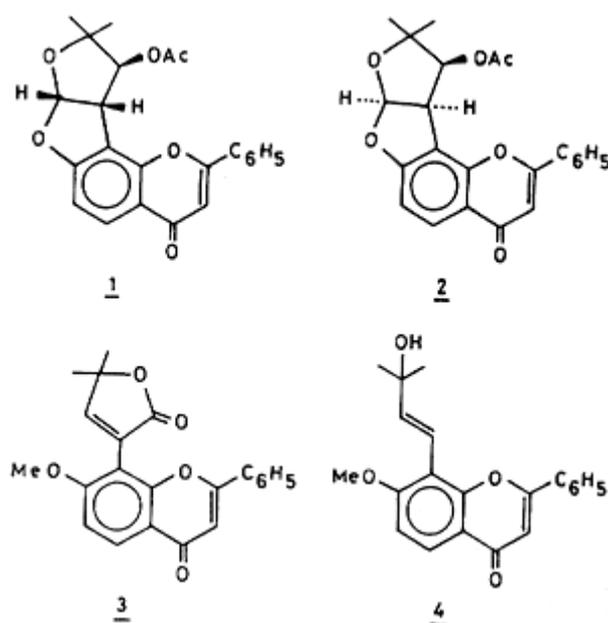


Figure 1.

tions of each the four compounds (1 mg/ml) showed no inhibitory effect on mycelial growth, while a mixture of them in the same concentration proved to be effective, while Gram positive and Gram negative bacteria and yeast were not affected by these compounds in all concentrations.

Regarding the substitution pattern within the tested compounds, we observe the methoxy group at the 7 position in (3) and (4) which possess a positive effect on fungal inhibition, the compounds (1) and (2) without a methoxy group at the 7 position shows also an activity against these fungi. It seems that the fungicidal property of the prenylated flavones is specific to individual fungi, substances and their concentration and no absolute generalization is possible in this context. Kramer *et al.*, (1984) came to the same conclusion in their investigation with differently substituted isoflavones.

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REFERENCES

1. Abou-Zeid AA, YM Shehata: A simple technique for assaying antibiotics using methylene blue as an indicator. *Indian J of Pharmacy* 31: (72-75), 1969.
2. Ammar NM, Jarvis BB: Major flavonoids of *Tephrosia nubica*. *Journal of Nat Prod* 49:719, 1986.
3. Beschia M, Leonte A, Oancea I: Phenolic compounds with biological activity. *Bull Univ Galati Faso* 6:23,27, 1984.
4. Ga'bor M: Anti-inflammatory and anti-allergic properties of flavonoids. *Plant flavonoids in biology and medicine. Biochemical Pharmacological and Structure Activity Relationships*, Alan R, 471-480, Liss Inc. 1986.
5. Ingham JL, Markham R: Tephrocarpin, A pterocarpan phytoalexin from *Tephrosia Bidwilli* and a structural proposal for acanthocarpan. *Phytochemistry* 21:2969-2972, 1982.
6. Kramer RP, Hindorf H, Jha HC, Kallage J, Zilliken F : Antifungal activity of soybean and chickpea isoflavones and their reduced derivatives. *Phytochemistry* 23:2203-2205, 1984.
7. Lwande W, Hassanali A, Njoroge PW: A new 6a-hydroxypterocarpan with insect antifeedant and antifungal properties from the roots of *Tephrosia hildebrandtii*. *Insert Sci Its Appl* 6:537-541, 1985.
8. Shipp RA, Bailey JA: The fungitoxicity of isoflavonoids phytoalexins measured using different types of bioassay. *Physiol. Plant Pathol* 11:101-102, 1978.
9. Thara S, Ingham J, Nakahara S, Mizutani J, Harborne JB: Antifungal isoflavones in *Lupinus*. *Phytochemistry* 23:1889-900, 1984.
10. Waterman PG, Khalid SA: The major flavonoids of the seed of *T. Apollinea* *Phytochemistry* 19:909-915, 1980.