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Short Communication

ANTIBACTERIAL, ANTIFUNGAL AND INSECTICIDAL ACTIVITIES OF THE BARK OF *MILLETTIA OVALIFOLIA*

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Abstract

The ethyl acetate fraction of the extracts of stem bark of *Millettia ovalifolia* was evaluated for their antibacterial, antifungal and insecticidal activities. The results obtained showed that ethyl acetate fraction showed no antibacterial activity. While in case of antifungal and insecticidal activities it showed 10% activity against *Microsporum canis* and 20% and 40% against *Rhyzopertha dominica* and *Callosbruchuanalis* respectively. All these results showed that ethyl acetate fraction of this plant have good insecticidal activity and moderate antifungal activity, which in turn revealed that this plant is of high medicinal importance.

Key words- *Millettia ovalifolia* bark, antibacterial, antifungal and insecticidal activities

INTRODUCTION

The genus *Millettia* belongs to family Leguminosae (Papilionaceae); consists of about 150 species distributed in the tropical and sub-tropical regions of the world. Only two species are present in Pakistan i.e. *M. extensa* and *M. ovalifolia*[1]. *M. ovalifolia* locally known as shewa (in pashto), villayati shisham (in Urdu), and rose wood (in

English) [2]. It is a tall ornamental deciduous tree, and the leaves are imparipinnately compound while the fruits are cylindrical pods 6.5 to 9 cm long. The genus *Millettia* is known for the medicinal importance due to a rich source of variety of compounds. The major class of compounds of this genus is flavonoids. The medicinal and pharmacological properties of genus *Millettia* is highlighted below: The species of genus *Millettia* such as *M. conrauai* is well known for its insecticidal, molluscicidal and pisticicidal activities[2]. Some of the species; *M. pachycarpa* inhibited the activities of murine retroviral reverse transcriptase and human DNA polymerases [3]. Compounds; rotenone and 3*α*-hydroxyrotenone isolated from *M. pervilleana* have shown inhibition of TPA-induced ornithine decarboxylase at the level of its m RNA expression and recommended as promising cancer chemo-preventive agents. The prenylated isoflavanone, pervilleanone from *M. pervilleana* showed anticancer activity[3], while *M. ovalifolia* contains hypotensive agents[4]. The flavonoids; chalcones isolated from *M. ovalifolia* showed antimalarial activitiy[5], and Isoflavonoids; griffonianone and maximaisoflavone isolated from *M. griffoniana* showed significant cytotoxicity[6]. *M. dura* contains 6α, 12α-didehydro-6-oxodeguelin has good insecticidal activity and played a key role as a phytotoxin inhibitor of ornithine decarboxylase[7]. *M. griffoniana* contains griffonianone C which showed a potent estrogenic activity[6], and Millepurone[8] has antitumour promoted reported from *M. atropurpurea*. Osajin isolated from *M. auriculata* has antioxidant activity [9] while *M. duchusnei* contains rotenones used as potent insecticides. In some parts of Africa especially in Cameroon the plants of genus *Millettia* are used by different communities as a potent inhibitor of intestinal parasites in children's as well as colic besides oral treatment for boils[10].

Material and methods

Experimental

Plant material

The bark of *M. o*valifolia was collected during the month of June 2008 from Pakistan Forest Institute Peshawar. The plant was identified and authenticated by Dr. Samin Jan, Associate Professor, Department of Botany, Islamia University, Peshawar, Pakistan. The voucher specimen (SJ-33) was deposited in the herbarium of Botany Department, Islamia University, Peshawar, Pakistan.

Extraction and isolation

The stem bark was shade dried and powdered (70 kg) were soaked in 5% aqueous methanol for one week (x3). The combined brownish extract was concentrated under reduce pressure by a vacuum rotary evaporator obtained brownish residue **F1** (5 kg) which was suspended in water and partitioned with *n*-hexane to get *n*-hexane fraction **FX** (1.2 kg). The *n*-hexane insoluble portion was acidified with HCl (pH 2) and subjected to further fractionation with ethyl acetate (x3), afforded ethyl acetate fraction **FX1** (1.0 kg), while remaining insoluble fraction was basified with ammonia (pH 8) and fractionated with chloroform; obtained chloroform fraction **FX2** (1.6 kg) and insoluble fraction **FX4** (0.8 kg) (**Scheme-26**). These fractions were studied for antifungal, antibacterial, phytotoxic and insecticidal activity. The chloroform fraction (500 g) was subjected to column chromatography using silica gel with *n*-hexane-chloroform increasing polarity to obtain several fractions, which were combined on the basis of TLC profile yielded six sub fractions (1-6). Fractions 3 (14.3 g), 4 (25 g) and 5 (19.7 g) were combined on the basis of TLC profile afforded major fraction which was subjected to chromatography using silica gel and *n*-hexane-chloroform in increasing polarity furnished 56 fractions.

Anti-bacterial activity

The microorganisms; *B. subtilis ATCC 6633, E. coli ATCC 25922, Straptodirimus, S. flexenari (clinical isolate), S. aureus ATCC 25923, P. aeruginosa ATCC 27853 and S. typhi ATCC19430* were used for evaluation of antibacterial activity. The organisms were stored in muller hantin agar in the refrigerator at 4^0C prior to subculture. Antibacterial testing was carried out on the already developed agar well diffusion method to study the potency of the ethyl acetate fraction of bark extract of *M. ovalifolia*. Broth media were prepared and the test organisms were transferred to the broth media from agar plate and were grown at 37^0C for 24 hours. After 24 hours 25 ml of MHA were poured into each petri plate and cooled in sterile condition. The fresh culture was prepared from day old culture, after solidification of MHA in plate, 0.6 ml of fresh culture of test organism were poured on to MHA. Wells of 6 mm diameter were digged in to the medium by using sterile borer and 22 mg of ethyl acetate fraction of the bark extract of *M. ovalifolia* were used against each organism. DMSO and standard antibiotic (Imipenum) were added into other

wells. The plates were kept in sterilized inoculation chambers for 1 hour to facilitate diffusion of the antimicrobial agent into the medium. The plates were then incubated at 37 ^{0}C for 24 hours and the diameters of the zone of inhibition of microbial growth were measured in millimeters.[11]

Antifungal activity

The microorganisms; *T. longifusis, C. albicans ATCC 2091, A. flavus ATCC 32611, M. canis ATCC 11622, F. solani 11712* and *C. glaberata ATCC 2091* were used for antifungal assay. All these strains were maintained on agar slant at 4^{0}C, the slant was allowed to activate at a temperature of 37 ^{0}C for duration of 3-4 days on nutrient agar (NA), for fungi, before any screening is made. The crude ethyl acetate extract fraction was dissolved in DMSO (24 mg/ml) and sterile medium (5 ml) was placed in a test tube and inoculated with the sample solution (400μg/ml) which was then kept in a slanting position at room temperature for overnight. The tubes were inoculated by a piece of fungus (4 mm diameter) from seven day old culture. The samples were then incubated for 7 days at 28^{0}C and the fungal strain starts growth on the slant. The growth inhibition was observed and percentage growth inhibition was determined by calculating with reference to the positive control by applying the formula

$$\% \text{ Inhibition} = \frac{100 - \text{linear growth and test (mm)}}{\text{linear growth in control (mm)}} \times 100$$

Miconazole was used as standard drug a positive control.[12-13]

Results and discussion

1. Anti-bacterial activity of the crude fraction

Ethyl acetate soluble fraction of *M. o*valifolia stems bark showed no activity against selected bacterial strains (**Table-1**).

Table-1: Antibacterial activity of ethyl acetate extract fraction of *M. o*valifolia stems bark

Micro organisms	Gram	F1	DMSO (-)	Imipenum 10 μg/Disc (+)
E. coli	-	-	-	35
B. subtilis	+	-	-	36
S. flexenari	-	-	-	36
S. aureus	+	-	-	43
S. aeruginosa	-	-	-	32
S. typhi	-	-	-	40

Key words: Well size 6 mm, F1 = EtOAc

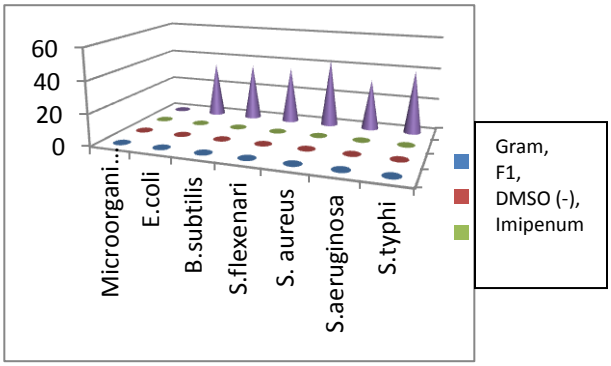

Figure-1: Antibacterial activity of ethyl acetate extract fraction of *M. o*valifolia stems bark

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Antifungal activity

Ethyl acetate fractions of stem bark of *M. o*valifolia was also investigated for antifungal bioassay against the selected fungal strains; *T. longifusis, C. albicans, A. flavus, M. canis, F. solani, C. glaberata* (**Table-2**). The ethyl acetate (F1) fraction showed 10 % bioactivity against *M. canis.*

Table-2: Antifungal activity of the extract fractions *M. ovalifolia* stem bark

Fungal species	Standard (μg/ml)	F1
Trichophyton longifusis	Miconazole 70.08	-
Candida albicans	Miconazole 110.8	-
Aspergilus flavus	Miconazole 20	-
Microsporum canis	Miconazole 98.4	10
Fusarium Solani	Miconazole 73.10	-
Candida glaberata.	Miconazole 110.8	-

Key words: F1 =EtOAc

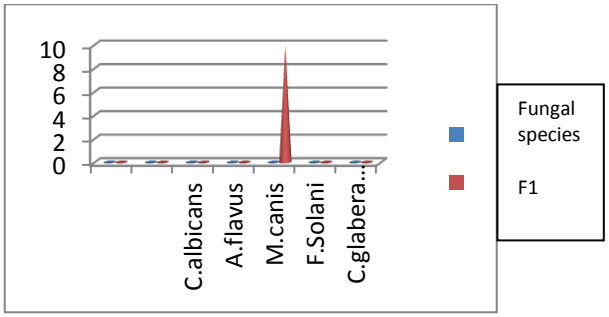

Figure-2: Antifungal activity of ethyl acetate extract fraction *M. ovalifolia* stem bark

Insecticidal activity

Ethyl acetate fraction (F1), (**Table-3**; **Figure-3**) was evaluated for insecticidal activity against the selected insects; *T. castaneum, R. dominica* and *C. bruchuanalis.* The results are summarized in table-3 which clearly indicated that the ethyl acetate fraction has 20 % and 40 % activity against *R. dominica* and *C. bruchuanalis.*

Table- 3: Insecticidal activity of the extract fractions of *M. ovalifolia* stem bark.

Insect	NOs	F1
T. castaneum	100	-
R. dominica	100	20 %
C. huanalis	100	40 %

Key words: F1=EtOAc,

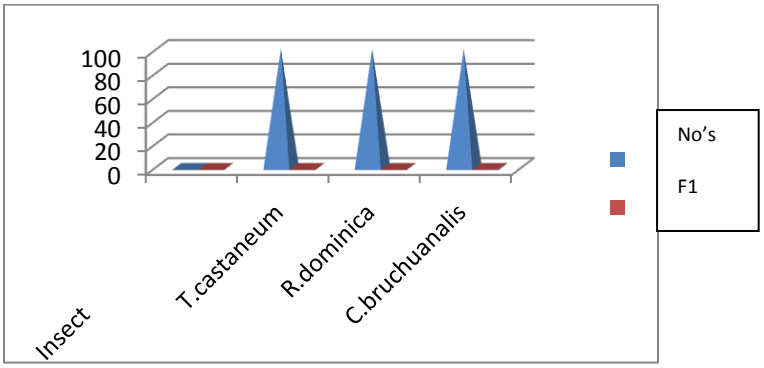

Figure- 3: Insecticidal activity of ethyl acetate extract fraction of *M. ovalifolia* stems bark.

CASE STUDY

In the present study, an exertion was made to scientifically authenticate bioassay of the ethyl acetate fraction of stem bark of medicinal plant known as *M. ovalifolia* to further elaborate its medicinal importance. The results obtained are given both in tabular and graphical form which reveals that it contain variety of therapeutic agents which further need to explore phytochemicaly.

CONCLUSIONS

From the results above it is clear that the medicinal plant *M. ovalifolia* possess high medicinal importance as clear from the bioassay results and in the current study. Further scientific approach is needed to explore phytochemicaly this plant.

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