World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Evaluation of antifungal, insecticidal and phytotoxic activities of stem wood of *Millettia ovalifolia*

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Received: 03-09-2015 / Revised: 01-10-2015 / Accepted: 02-10-2015

ABSTRACT

In the current study, the stem wood of *Millettia ovalifolia* was evaluated for antifungal, insecticidal and phytotoxic activities. The results revealed that all the fractions showed no antibacterial activity against the tested bacterial strains. In case of antifungal activity, only ethyl acetate (EA) and chloroform (C) fractions exhibited minimum inhibitory concentration both at 500 µg/ml against *Microsporum canis*. The results of insecticidal activity reveled that ethyl acetate and chloroform fractions were effective against *Tribolium castaneum, Rhyzopertha dominica* and *Callos bruchuanalis.* The phytotoxicity data showed that all the fractions affected the growth of *lemna minor* at the concentration level of 500 µg/ml. The ethyl acetate (EA) and chloroform (C) fractions were found biologically active. These fractions should be further investigated for potential antifungal, insecticidal and phytotoxic compounds.

Keywords: *Millettia ovalifolia*; antifungal; antibacterial; insecticidal; phytotoxicity.

INTRODUCTION

The genus *Millettia* (family Papilionaceae) comprised of about 150 species dispersed in the tropical and subtropical regions of the world. Only two species are reported in Pakistan i.e., *Millettia extensa* and *Millettia* peguensis [1]. The genus *Millettia* have a large number of phytochemicals exhibiting various bioactivities. The secondary metabolites, reported from the genus include flavones, flavonones, flavans, flavanoles, prenylated isoflavones, chalcones, pongamol, lancelolatin, kanjone, ovalitenone, milletenone and pongaglabol [2]. The plants of the genus contain hypotensive agents [3]. Some species of *Millettia* genus display fish poisoning activity. Isoflavonoids such as griffonianone and maximaisoflavone are isolated from the species of this genus [4]. A new isoflavan-quinone namely laurentiquinone along with flavonol (laurentinol) and isoflavones i.e. glyricidin and calycosin are also reported from the genus [5]. The genus *Millettia* contains cis-jasmone, which can be used as activator for secondary metabolism of wheat seedlings [6]. Other compounds such as *O*-geranylisoliquiritigenin, isoliquiritigenin, barbigerone, jamaicin and maximaisoflavone-G are also isolated from various species of the genus [7].

Millettia ovalifolia is traditionally used for treatment of pain, fever, rheumatism, diabetes, and a number of other infectious diseases. Earlier study [8] conducted on the plant, indicated the presence of flavonoid which have anti-malarial activity. In the current study, various fractions of stem wood of *Millettia ovalifolia* were assessed for antifungal, antibacterial, insecticidal and phytotoxic activities.

MATERIAL AND METHODS

Collection of the plant material: The stem wood of *Millettia ovalifolia* was collected during the month of June, 2008 from Pakistan forest institute (PFI), Peshawar. The plant was identified by Mr. Samin Jan, Associate Professor, Department of Botany, Islamia University, Peshawar, Khyber Pakhtunkhwa province, Pakistan. A voucher specimen (No.SJ-33) was retained in the herbarium of Botany Department, Islamia University, Peshawar, Pakistan.

Extraction: The powdered stem wood (20 kg) was soaked (cold extraction) in water-methanol (1:19) for one week (thrice). The crude water-methanol extract obtained was filtered and concentrated at reduce pressure using rotary evaporator at temperature of 50 ºC, afforded a crude semi solid

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mass of (1.8 kg) fraction and labeled as F1. The fraction was then suspended in water and partitioned with solvents of increasing polarity starting from *n*-hexane, which afforded *n*-hexane fraction (*n*-H 400 g). The remaining water fraction was partitioned with ethyl acetate and subsequently with chloroform which afforded ethyl acetate (EA 380 g) and chloroform (C 430 g) fractions, respectively. The remaining water fraction was filtered to separate water insoluble part (R 240 g) from water soluble portion (W 180 g).

Fungal and bacterial strains: Six fungal and six bacterial strains were selected for antimicrobial assays [9-11]. The bacterial strains used in the study were *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Shigella flexner (clinical isolate), Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* ATCC 19430. The fungal strains chosen for the study were *Trichophyton longifusis* (*clinical Isolate*), *Candida albicans* ATCC 2091, *Aspergillus flavus* ATCC 32611, *Microsporum canis* ATCC 11622, *Fusarium solani* 11712, *Aspergillus flavus* and *Candida glabrata* ATCC 90030. All these strains were maintained on agar slants at 4 ºC. The slants were allowed to activate at 37 ºC for 24 hours on nutrient agar.

Antibacterial activity: Antimicrobial tests were conducted using plate-hole diffusion method [12] by making use of a cell suspension of 1.5 x 10^{-5}CFU/mL, keeping in view Mcfarland turbidity standard No.0.5. The suspension concentration was standardized by adjusting the optical density to 0.1 at 600 nm on Shimadzu UV visible spectrometer. Holes with 6 mm diameter were allowed to bore on Mueller-Hinton agar plate (8 mm thick) and were filled with 150 µL of extract fractions (1mg/mL) or standard drug (s) in DMSO. The plates were then allowed to incubate at 37 ºC for 24 hours. The extent of antimicrobial activity was determined by measuring the diameter of zone of inhibition around the hole. The bioassay was repeated thrice and then the mean inhibition diameter was calculated. Iimipenem was used as standard antibiotic.

Antifungal activity: Agar tube dilution method [13] was used to assess the antifungal activity of various crude fractions of stem wood of *Millettia ovalifolia*. All the test samples were dissolved in sterile DMSO (1000 µg/mL) and appropriate test solutions (750, 500 and 250 µg/mL) were prepared. Sabourad dextrose agar was prepared and cooled to 50 ºC. The test samples were mixed with the non-solidified agar. The test tubes were then allowed to solidify at room temperature in slanting position. Afterward each tube was inoculated with a 4 mm diameter piece of inoculums from seven days old culture of fungi and incubated at 28 ºC±1 for seven days. Amphotericin B and miconazole were used as standard antibiotics. The antifungal activity was recorded as minimum inhibitory concentration (MIC) for the test sample with no visible growth. The activity was expressed as µg/ml.

Insecticidal activity: Insecticidal bioassay was carried out by direct contact application of the test compounds by using filter paper [14-15]. In this experiment, 3 mL of all fractions (1mg/mL) were applied to the filter papers having 90 mm diameter. After drying, each filter paper was allowed to place in individual petri dish along 10 adults of each of *Tribolium castaneum*, *Callosobruchus analis* and *Rhyzopartha dominica*. Afterward, the plates were incubated at temperature of 27 °C for a total duration of 24 hours with 50% relative humidity in growth chamber. Permethrin (239.5 µg/cm^2) was used as a reference insecticide in this experiment, while acetone was used as negative control. The insects were kept to stand without food for 24 hours after which the mortality number was calculated.

Phytotoxicity assay: In this investigation, the crude extract was tested against *lemna minor* [16-19]. Stock solutions (20 mg/mL) of various extracts were diluted to obtain a final concentration of 500, 50 and 5 µg/mL, respectively. Each flask was then mixed to a 20 mL medium sized 10 plants, each one containing rosette of three fronds. Paraquat (0.015 µg/mL) was used as a standard growth inhibitor in this experiment. All flasks were allowed to keep in growth cabinet for seven days. Afterward, the inhibition percentage was calculated with reference to the negative control. IC_{50} values were determined by calculating through finny computer program.

RESULTS AND DISCUSSION

All fractions of the stem wood of *Millettia ovalifolia* exhibited no antibacterial activity against *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Shigella flexner (clinical isolate), Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* ATCC 19430, as mentioned in **Table-1**.

The *n*-Hexane (*n*-H), ethyl acetate (EA), chloroform (C), water (W) and residue (R) fractions were investigated for their antifungal bioassay against *Trichophyton longifusis*, *Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata*. All these fractions showed no bioactivity (**Table-2**) against selected fungal strains except ethyl acetate (EA)

and chloroform (C) fractions, which showed minimum inhibitory concentrations both at 500 µg/ml against *Microsporum canis*.

Different fractions of the stem wood of *Millettia ovalifolia* (**Table-3**) were investigated against various insects viz. *Tribolium castaneum, Rhyzopertha dominica* and *Callosobruchus analis*. Results were determined as percentage mortality, with reference to the positive and negative controls. The *n*-hexane, water and residue fractions showed no activity against all the three insects, while chloroform (C) and ethyl acetate fractions (EA) were found effective. Choloroform fraction showed 22, 25 and 21% mortality against *Tribolium castaneum, Rhyzopertha dominica and Callosbruchu analis*, respectively. The ethyl acetate fraction exhibited 22, 18 and 25% mortality against *Tribolium castaneum, Rhyzopertha dominica and Callosbruchu analis*, respectively.

Results of phytotoxicity bioassay of various fractions of the *Millettia ovalifolia* stem wood are shown in the (**Table-4**). The *n*-hexane (*n*-H), ethyl acetate (EA) chloroform (C), water (W) and residue (R) fractions showed good phytotoxicity at 500 µg/ml concentration and comparatively low activity at 50 µg/ml concentration. Only residue and *n*-hexane fractions showed some activity at the concentration level of 5 µg/ml.

Earlier literature on various species of the genus *Millettia* revealed a variety of biological activities. The root bark of *Millettia pervilleana* showed significant cytotoxic and anticancer activity [20]. *Millettia laurentii* contain millaurine A, which acts as hypotensive agent [3]. A study on *Millettia duchesnei* showed anticancer and antimicrobial activities [21]. The root bark of *Millettia* revealed the presence of isoflavonoids having antitumor activity [22]. The heartwood of *Millettia laurentii* displayed various bioactivities [6]. The dichloromethane extract of the stem bark of *Millettia usaramensis* subspecies *usaramensis* showed activity against *Plasmodium falciparum* [23]. The root and stem barks of *Millettia griffoniana* contained bioactive isoflavones, which exhibited estrogenic activity [24]. The phytochemical examination of the extract of *Millettia ovalifolia* bark yielded a novel flavonoid namely 7-(4-methoxyphenyl)-9H-furo[2,3-f] chromen-9-one, which displayed significant inhibition of cytosolic form of bovine carbonic anhydrase-II. The flavonoid can be used as a new pharmacophore to treat cystic fibrosis, glaucoma, epilepsy, leaukomia and other disorders such as neurology etc. [25]. Flavonoids and chalcones, isolated from *Millettia ovalifolia* showed anti-malarial activity [26].

CONCLUSION

The current study revealed that the stem wood extract fractions of *Millettia ovalifolia* have promising antifungal, insecticidal and phytotoxic activity. The plant is traditionally used for the treatment of various diseases. This data supports the conclusion that extensive research should be conducted to isolate phytochemicals responsible for antifungal, insecticidal and phytotoxic activities.

ACKNOWLEDGMENTS

Higher education commission (HEC) of Pakistan is highly acknowledges for providing financial support to complete this project.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest regarding publishing of this manuscript.

Table-1: Antibacterial activities of stem wood extract fractions of *Millettia ovalifolia*

Bacterial Species	**Zone of Inhibition (mm)**					
	Imipenem (Standard drug)	***n*-H**	**EA**	**C**	**W**	**R**
Escherichia coli	35	-	-	-	-	-
Bacillus subtilis	36	-	-	-	-	-
Shigella flexner	36	-	-	-	-	-
Staphylococcus aureus	43	-	-	-	-	-
Pseudomonas aeruginosa	32	-	-	-	-	-
Salmonella typhi	40	-	-	-	-	-

Key word: *n*-H = *n*-Hexane, EA = EtOAc, C = Chloroform, W = Water, R = Residue

Table-2: Antifungal activities of stem wood extract fractions of *Millettia ovalifolia*

Fungal species	**Minimum Inhibitory Concentration (µg/mL)**						
	Standard		***n*-H**	**EA**	**C**	**W**	**R**
Trichophyton longifusis	Miconazole	70	-	-	-	-	-
Candida albicans		110.8	-	-	-	-	-
Microsporum canis		98.4	-	500	500	-	-
Fusarium solani		73.10	-	-	-	-	-
Candida glabrata		110.8	-	-	-	-	-
Aspergillus flavus	Amphotericin B	20	-	-	-	-	-

Key word: *n*-H = *n*-Hexane, EA = EtOAc, C = Chloroform, W = Water, R = Residue

Table-3: **Insecticidal activity of the stem wood extract fractions of *Millettia ovalifolia***

Insect	% Mortality						
	Permethrin (+ve control)	-ve control	*n*-H	EA	C	W	R
Tribolium castaneum	100	0	0	22	22	0	0
Rhyzopertha dominica	100	0	0	18	25	0	0
Callosobruchus analis	100	0	0	25	21	0	0

Key word: *n*-H = *n*-Hexane, EA = EtOAc, C = Chloroform, W = Water, R = Residue

Table-4: Phytotoxic activities of stem wood extract fractions of *Millettia ovalifolia*

Con. of	% Growth Regulation						
Sample (µg/ml)	Paraquat (standard drug)	-ve Control	*n*-H	EA	C	W	R
500	100	0	83.3	43.3	33.3	50	86.6
50	100	0	16.6	16.6	26.6	-	13.3
5	100	0	6.6	-	-	-	3.3

Key word: *n*-H = *n*-Hexane, EA = EtOAc, C = Chloroform, W = Water, R = Residue

REFERENCES

1. Ali SI. Flora of Pakistan, Shamim Printing Press, Karachi. 1977; (13)100: 51-52.
2. Sritularak B, Likhitwitayawuid K, Conrad JR and Kraus W. Falvonoids from the roots of *Millettia erythrocalyx*. Phytochemistry 2002; 61(8): 943–947.
3. Ngamga A, Fanso F, Tane A and Fomum ZT. Millaurine A, a new guanidine alkaloid from seeds of *Millettia laurentii*. Fitoterapia 2007; 78 (6): 276–277.
4. Abiy Y, Solomon J and Hellen AOR. Anti-plasmodial activities and X-ray crystal structures of rotenoids from *Millettia usaramensis*. Phytochemistry 2003; 64(3): *773*–779.
5. Yankep E, Mbafor JT, Fomuma ZT, Steinbeck C, Messanga BB, Nyasse B, Budzikiewicz H, Lenz C and Schmickler H. Further isoflavonoid metabolites from *Millettia griffoniana* (Bail). Phytochemistry 2001; 56 (4): 363-368.
6. Pierre K, Samuel NY, Fanso F, Nkengfacka GF and Zacharias TF. An isoflavone-quinone and a flavanol from *Millettia laurentii*. Phytochemistry 1999; 51(6): 829-832.
7. Moraes MCB, Birkett MA, Ruth W, Smart LE, Martin JL, Pye BJ, Bromilow R and Picket JA. Graphical contents list. Phytochemistry 2008; 69(1): 1-7.
8. Yenesew A, Jacob MO and Waterman PG. Rotenoids, isoflavones and chalcones from the stem bark of *Millettia usaramensis*. Phytochemistry 1998; 47(2): 295-300.
9. Jan AK, Shah MR, Anis I and Marwat IK. *In vitro* antifungal and antibacterial activities of extracts of *Galium tricornutum* subsp. *Longipedunculatum*. J. Enzyme Inhib Med Chem 2009; 24 (7): 192-196.
10. Nisar M, Tariq SA, Marwat IK, Shah MR and Khan IA. Antibacterial, antifungal, insecticidal, cytotoxicity and phytotoxicity studies on *Indigofera gerardiana*. J Enzyme Inhib Med Chem 2009; 24(4): 224-229.
11. Shakirullah M, Ahmad H, Shah MR, Ahmad I, Ishaq M, Khan N, Badshah A and Khan I. Antimicrobial activities of Conyzolide and Conyzoflavone from *Conyza canadensis*. J Enzyme Inhib Med Chem 2011; 26(4): 468-471.
12. Berghe VA and Vlietinck AJ. Screening methods for antibacterial and antiviral agents from higher plants. Method Plant Biochem 1991; 6(3): 47-68.
13. Rahman AU, Choudhary MI and Thomsen WJ. Bioassay Techniques for Drug Development. Harwood Academic Publisher, Singapore. 2005; pp 20-21.
14. Rashid R, Farah M and Mirza MN. Biological screening of *Salvia cabulica*. Pak J Bot 2009; 41(3): 1453-1462.

15. Rehman AU, Choudhary MI and William JT. Bioassay techniques for drug development. Harward academic Publisher, Singapore. 2001; 8(4): 67-68.
16. Rehman AU. Studies in Natural Product Chemistry. Elsevier Science Publishers, Netherlands. 1991; 9: 383-384.
17. Finny DJ. Probit Analysis. 3rd edition, Cambridge University Press, Cambridge, 1971; 333.
18. Hideji IY, Oshida A, Inatomi IH and Adachi T. Phenolic plant growth inhibitors from the flowers of *Cucurbita pepo*. Phytochemistry 1982; 21(8): 1935-1937.
19. Lewis MA. Use of freshwater plants for phytotoxicity testing: a review. Environ Pollut 1995; 87(3): 319-336.
20. Giovanna P, Philippe R, Elena F, Marcello N and Corrado G. Prenylated isoflavonoids from *Millettia pervilleana*. Phytochemistry 2003; 63: 471–474.
21. Ngandeu FO, Bezabih M, Dieudonne N, Alembert T, Tchinda B, NgadjuiT, Berhanu M, Abegaz DH and Francois T. Rotenoid derivatives and other constituents of the twigs of *Millettia duchesnei*. Phytochemistry 2008; 69: 258–263.
22. Emmanuel Y, Mbafor J, Tanee F, Christoph S, Bernard BM, Barthelemy N, Budzikiewicz H,Christof L and Hans SF. Isoflavonoid metabolites from *Millettia grifoniana* (Bail). Phytochemistry 2001; 56: 363-368.
23. Abiy Y, Solomon D, Jacob O, Midiwo HA, Oketch R, John L, Rex P, Matthias H, Martin G, Peter, HA, Julia W, Pamela L and Norman C. Anti-plasmodial activities and X-ray crystal structures of rotenoids from *Millettia usaramensis* subspecies *usaramensis*. Phytochemistry 2003; 64: 773–779.
24. Ketcha WG, Njamenb E, Yankepc M, Tagatsing F, Tanee Z, Fomumc J, Wobera S, Starckea O and Zieraua G. Estrogenic properties of isoflavones derived from *Millettia griffoniana*. Phytomedicine 2006; 13: 139–145.
25. Rahman TU, Khattak KF, Liaqat W, Zaman K and Musharraf SG. Characterization of one novel flavone and four new source compounds from the bark of *Millettia ovalifolia* and in-vitro inhibition of carbonic anhydrase-II by the novel flavonoid. Rec Nat Prod 2015; 9(4): 553-560.
26. Yenesew A, Derese S, Midiwo JO, Oketch-Rabah HA, Lisgarten J and Palmer R. Anti-plasmodial activities and x-ray crystal structures of rotenoids from *Millettia usaramensis* subspecies *usaramensis*. Phytochemistry 2003; 64: 773–779.