Antifungal, Antibacterial, Phytotoxic and Cytotoxic Potential of Casticin Isolated from *Vitex agnus-castus*

[1]AZIZUDDIN*, [1]IFFAT MAHMOOD, [2]MUHAMMAD IQBAL CHOUDHARY AND [2]KHALID MOHAMMED KHAN
[1]*Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi-75300, Pakistan.*
[2]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*
azizpobox1@yahoo.com

(Received on 17th January 2012, accepted in revised form 7th July 2012)

Summary: The secondary metabolite, casticin (**1**) was isolated from a medicinal plant, *Vitex agnus-castus*, which is widely used in traditional medicines against various diseases. The structure of the compound **1** was identified with the help of modern spectroscopic techniques. The compound **1** was screened for its antifungal, antibacterial, phytotoxic and cytotoxic activities. Casticin (**1**) was found to have moderate antifungal activity against *Trichophyton schoenleinii*, *Aspergillus niger*, *Trichophyton simii* and *Fusarium oxysporum var. lycopersici* (tomato) whereas no antibacterial and phytotoxic activities were found against the tested bacteria and *Lemna aequinoctialis welv*, respectively. Preliminary cytotoxicity tests were done with the casticin (**1**) using the larvae of the brine shrimp, *Artemia salina*. Compound **1** however found to be relatively non-toxic.

Introduction

Vitex agnus-castus Linn. (Verbenaceae) is a well known medicinal plant, which is abundantly found in Pakistan [1]. It is locally known as “Hub-el-faked” and “Sumbhalu-ke-bij”. It is traditionally used as an emmenagogue, sedative, anaphrodisiac and galactagogue [2]. Ethanolic extract of the *Vitex agnus-castus* is used as a homeopathic drug (agnus castus) for the treatment of impotence and disorders related to the central nervous system [3]. The plant is also used for the treatment of mastopathy, premenstrual syndrome and luteae insufficiency [4]. Flowers of the plant are effective in diarrhoea and liver affections, whereas powder of its green parts is used as internal antihemorrhagic agent [4].

Previous phytochemical studies on this plant have resulted in the isolation of several flavonoids including casticin (**1**) and its immunomodulatory properties were also studied [5]. In order to find some new natural sources of pesticides from botanical origin, we screened this secondary metabolite, casticin (**1**) isolated from *Vitex agnus-castus* (Fig. 1) by measuring their antibacterial, antifungal, phytotoxic and cytotoxic potential.

Results and Discussion

In Vitro Antifungal Bioassay

Casticin (**1**) was tested against *Trichophyton schoenleinii*, *Candida albicans*, *Pseudallescheria boydii*, *Aspergillus niger*, *Microsporum canis*, *Trichophyton simii*, *Fusarium oxysporum var. lycopersici and Fusarium solani var. lycopersici.* It showed moderate *in vitro* fungicidal activity against pathogenic fungi including *Trichophyton schoenleinii*, *Aspergillus niger*, *Trichophyton simii and Fusarium oxysporum var. lycopersici* as shown in Table-1.

Antibacterial Activity

Casticin (**1**) was screened against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeroginos*, *Shigella boydii*, *Staphylococcus pyogenes* and *Salmonella typhi* strains (Table-2). It is not found active against any human pathogen bacterial strain as compared to amoxicillin and ampicillin as reference drugs.

Phytotoxic Activity

The herbicidal potentials of the casticin (**1**) was evaluated against *Lemna aequinoctialis welv.* and the results are shown in Table-3. The results obtained indicated that casticin (**1**) obtained from *Vitex agnus-castus* does not have any phytotoxicity at the tested concentrations (500, 50 and 5 µg/mL).

Cytotoxicity

In the cytotoxic investigations of casticin (**1**), it did not show considerable brine-shrimp cytotoxicity. This indicates the safe nature of this compound and thus provides non-toxic and environmentally friendly alternate sources of fungicide to the synthetic ones.

*To whom all correspondence should be addressed.

Table-1: *In vitro* fungicidal bioassay of casticin (1).

S. No.	Fungi tested	Clinical implications	Linear growth (mm)		Inhibition (%)	Reference drug	Inhibition (%)
			Sample	Control			
1	*Trichophyton schoenleinii* (human pathogen)	Cutaneous mycoses scaring of the scalp; permanent alopecia	40.0	80.0	50.0	Miconazole Ketoconazole	100 100
2	*Candida albicans* (human pathogen)	Opportunistic mycoses candidosis; infection of lungs, vagina, ear, bones, heart and thrush	40.0	45.0	11.1	Miconazole Ketoconazole	100 100
3	*Pseudallescheria boydii* (human pathogen)	CNS infection; brain abscesses; spinal pachymeningitis; chronic meningitis; intraventricular device-related ventriculitis; cranial epidural abscess and acute meningitis	50.0	70.0	28.5	Miconazole Ketoconazole	100 100
4	*Aspergillus niger* (human pathogen)	black mold on certain fruits and vegetables	25.0	70.0	64.0	Amphotericin -B	100
5	*Microsporum canis* (animal pathogen)	Cutaneous mycoses, ringworm infection of hair and skin in dogs and cats	50.0	80.0	37.0	Miconazole Ketoconazole	100 100
6	*Trichophyton simii* (animal pathogen)	Infections in chickens, dogs and man	30.0	70.0	57.1	Miconazole Ketoconazole	100 100
7	*Fusarium oxysporum var. lycopersici* (tomato) (plant pathogen)	Wilt diseases of a great variety of plants; abnormal bone development in animals	30.0	70.0	57.1	Benlate	100
8	*Fusarium solani var. lycopersici* (tomato) (plant pathogen)	Seed borne pathogen rootrot; siemcankers associated with wounds; damping off seeding; destruction of spawn imbeds of cultivated mushrooms and pea crop	40.0	70.0	42.8	Benlate	100

Concentration of casticin (**1**): 200µg/mL of SDA.

Table-2: *In vitro* bactericidal bioassay of casticin (**1**).

S. No.	Bacteria tested	Clinical implications	Zone of inhibition (mm)	Reference drug	Zone of inhibition (mm)
1	*Shigella boydii*	Inflammation of GIT; bacterial dysentry	NA	Amoxicillin $(H_2O)_3$ Ampicillin $(H_2O)_3$	7.0 8.0
2	*Escherichia coli*	Infections of wounds and urinary tract; inflammations of peritoneum and GIT; dysentery; septicaemia; neonatal meningitis	NA	Amoxicillin $(H_2O)_3$ Ampicillin $(H_2O)_3$	10.0 9.0
3	*Klebsiella pneumoniae*	Infections of respiratory and urinary tract; suppurative infections in sinuses and middle ear; septicemia	NA	Amoxicillin $(H_2O)_3$ Ampicillin $(H_2O)_3$	10.0 7.0
4	*Salmonella typhi*	Typhoid fever; salmonella food poisoning; localized infection: pyelonephritis, endocarditis, salpingitis, and chronic osteomyelitis	NA	Amoxicillin $(H_2O)_3$ Ampicillin $(H_2O)_3$	8.0 7.0
5	*Pseudomonas aeroginosa*	Infections of wounds, urinary tract and eyes; septicemia	NA	Amoxicillin $(H_2O)_3$ Ampicillin $(H_2O)_3$	8.0 7.0
6	*Staphylococcus pyogenes*	Acute rheumatic fever; scarlet fever; sore throat; septic wound; impetigo; inflammations of post- glomerulonephrone (kidney); tonsils and middle ear; puerperal sepsis, erythema nodosum	NA	Amoxicillin $(H_2O)_3$ Ampicillin $(H_2O)_3$	11.0 8.0
7	*Staphylococcus aureus*	Food poisoning; scalded skin syndrome; toxic shock syndrome; infections of upper respiratory tract and wounds; abscesses and endocarditis	NA	Amoxicillin $(H_2O)_3$ Ampicillin $(H_2O)_3$	10.0 10.0

Concentration of casticin (**1**): 200µg/100µL of DMSO.
NA: Stands for no activity.

Table-3: *In vitro Lemna welv.* phytotoxic bioassay of casticin (**1**).

S. Nos.	Concentrations	No. of fronds		Growth regulation (%)	Reference inhibitor	Growth regulation (%)
		Sample	Control			
1	500 µg/mL	86.0	86.0	00		
2	50 µg/mL	89.0	73.0	-19.17	Paraquat	100
3	5 µg/mL	95.0	78.0	-21.79		

Experimental

In Vitro Antifungal Bioassay

Antifungal activity was performed using the agar tube dilution method [6-9]. The compound **1** (1.5 mg) dissolved in 1 mL of sterile dimethylsulphoxide (DMSO) served as stock solution. SDA (sabouraud dextrose agar) medium (4 mL) was added into screw-capped tubes and autoclaved at 121 °C for 15 min and then cooled to 50 °C. The non-solidified SDA media was poisoned with 66.6 µL of the stock solution to give 200 µg compound/ mL of SDA. Tubes were then allowed to solidify in slanting position at room temperature for overnight. Each tube was inoculated with 4 mm diameter piece of the inoculum removed from a 7 days old culture of fungi. For non-mycelia growth, an agar surface streak was employed. Inhibition of fungal growth was observed after 7 days of incubation at 28±1 °C. Negative and positive control experiments were also carried out with DMSO and reference antifungal drugs (miconazole, ketoconazole, amphotericin-B and benlate), respectively.

Lemna Welv. Phytotoxic Bioassay

This test was performed according to the modified protocol of McLaughlin *et al* [10]. The test compounds were incorporated with sterilized E-medium at different concentrations, i.e. 5, 50, and 500 µg/mL in methanol. Sterilized conical flasks were inoculated with compounds of the desired concentration prepared from a stock solution and allowed to evaporate overnight. Each flask was inoculated with sterilized E-medium (20 mL) and 10 plants of *Lemna aequinocitalis welv* each containing a Roselle of three fronds. Other flasks were supplemented with methanol serving as a negative control and reference inhibitor, i.e. parquat serving as a positive control. Treatments were replicated three times and the flasks incubated at 30 °C in a Fisons Fi-Totran 600H growth cabinet for seven days, 9000 lux light intensity, 56±10 rh (relative humidity), and 12 h day length. Growth of *Lemna aequinocitalis* in the compound-containing flask was determined by counting the number of fronds per dose and growth inhibition in percentage calculated with reference to the negative control with the help of the following formula.

$$\text{Growth regulation (\%)} = 100 - \frac{\text{Number of fronds in test flasks}}{\text{Number of fronds in negative control}} \times 100$$

Antibacterial Activity

Antibacterial activity was performed using agar well diffusion method. For antibacterial screening, 3 mg of sample was taken and dissolved in 3 mL of DMSO (1mg/mL). Molten nutrient agar (45 mL) was poured on the sterile *petri* plates and allowed to solidify. Bacterial lawn was made on these nutrient agar plates by dispensing 7 mL sterile soft agar containing 100 mL of cultures of the test organisms. Wells were drugged with a 5 mm sterile metallic borer at appropriate distance, then 100 µL of sample was poured into each well, and the plates were incubated at 37 °C for 24 hours. The results in terms of zones of inhibition were recorded. The drugs, amoxicillin and ampicillin were used as positive control, while DMSO was used as a negative control.

Cytotoxicity Test

Cytotoxicity activity was performed using brine-shrimp cytotoxic protocols. The extracts were tested using initial concentrations of 10, 100 and 1000 µg/mL in vials containing 5 mL of brine and 30 shrimps in each of three replicates, using the method of McLaughlin [11]. Survivors were counted after 24 h. The data were processed using a Finney program on a simple computer and LD_{50} values were obtained.

Conclusion

The present study has identified that casticin (**1**) (Fig. 1) isolated from of *Vitex agnus-castus* is among one of the significant antifungal secondary metabolites. Therefore, it could be a candidate for the commercial botanical fungicide formulation in order to increase the crop production.

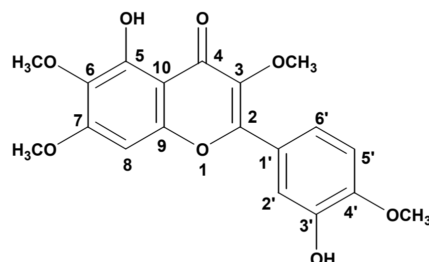

Fig. 1: Structure of casticin (**1**) isolated from *Vitex agnus castus*.

Acknowledgements

Dr. Iffat Mahmood and Dr. Azizuddin gratefully acknowledge the enabling role of the Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal, Karachi, Pakistan, and appreciate its financial support through "Mini Research Project-2012".

References

1. Y. R. Chadha, *The Wealth of India*, Council of Scientific and Industrial Research, New Delhi, **10**, 520 (1976).
2. J. Bruneton, Pharmacy Phytochemistry, *Medicinal Plants*, Intercept Limited, Andover, England, p. 602 (1993).
3. W. Schwabe, *Homeopathic Repetitorin*, Dr. William Schwabe GMBH and Co, Karlsruhe, Germany, p. 17 (1987).
4. H. Jarry, S. Leonhardt, C. Garkow and W. Wutlke, *Experimental and Clinical Endocrinology*, **102**, 448 (1994).
5. M. A. Mesaik, Azizuddin, S. Murad, K. M. Khan, R. B. Tareen, A. Ahmed, Atta-ur-Rahman and M. I. Choudhary, *Phytotherapy Research*, **23**, 1516 (2009).
6. G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherstone, *Biochemical Pharmacology*, **7**, 88 (1961).
7. C. Brass, J. Z. Shainhouse and D. A. Stevens, *Antimicrobial Agents and Chemotherapy*, **15**, 763 (1979).
8. S. Q. Yu, H. W. Holloway, T. Utsuki, A. Brossi and N. H. Greig, *Journal of Medicinal Chemistry*, **42**, 1855 (1999).
9. V. Tougu, *Current Medicinal Chemistry*, **1**, 155 (2001).
10. J. L. McLaughlin, C. J. Chang and D. L. Smith, In: Atta-ur-Rahman (Ed.), Studies in Natural Produt Chemistry, Bench-Top Bioassays for the Discovery of the Products: *Structure and Chemistry*, Elsevier Science Publishers B.V, Netherlands, Part B, **9**, 383 (1991).
11. J. L. McLaughlin, *Brine shrimp and crown gall tumours*: Simple bioassays for the discovery of plant anti-tumour agents. Proceeding of the NIH Workshop, Bioassays for Discovery of Anti-tumour and Anti-viral Agents from Natural Sources, Bethesda, MD, October, 22 (1988).