

BIOASSAY SCREENING OF SOME IRANIAN MEDICINAL PLANTS BY BIOAUTOGRAPHIC METHOD

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ABSTRACT

The methanolic and dichloromethane of three medicinal plants viz., *Pterocarya fraxinifolia*, (Juglandaceae), *Ilex spinigera* (Aquifoliaceae), *Pyrus boissieriana* (Rosaceae), were tested for their antibacterial activity against *Bacillus subtilis*, antifungal activity against *Candida albicans* and *Cladosporium cucumericum*, as well as for their larvicidal activity against *Aedes aegypti*. A bioautographic assay on TLC plates has been used for this screening. The methanolic extract of *P. fraxinifolia* was found to be the most effective extract against *C. cucumericum*, *C. albicans* and *Bacillus subtilis*. Two fractions of the *P. fraxinifolia* were the major constituents for these activities. Both fractions had naphthoquinone structure.

Key-words: Medicinal plants, bactericidal, fungicidal and larvicidal activities

INTRODUCTION

Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutic, natural products still remain as one of the best reservoirs of new structural types. Farnsworth claims that 119 characterized drugs are still obtained commercially from higher plants and that 74% were found from ethnobotanical information (Farnsworth, 1990).

For the purpose of the present study, benchtop bioassays have been performed on three Iranian medicinal plants. These include tests for fungicidal, antibacterial, and larvicidal activities.

There is a growing interest in antifungal compounds as the occurrence of systemic mycoses associated with immunodeficiency diseases (such as AIDs) and the use of immunosuppressors is continually increasing. The routine screening includes assays using *Cladosporium cucumericum* and *Candida albicans* as test organisms (Diallo *et al.*, 2001).

Investigations confirm that higher plants used as antiinfective phytomedicine may serve as a valuable source for novel antibiotic. In this study, antibacterial activity was carried out using *Bacillus subtilis* (Gram + ve bacterium), that is non-pathogenic to humans (Lahalison *et al.*, 1991).

The potency of extract in the destruction of vector of tropical disease was also assessed. The capacity of extract to kill larvae from *Aedes aegypti*, which spreads yellow fever and danguue fever, was measured. *Aedes spp.* mosquitoes are well known vector for transmitting arboviruses (Manson-bahr and Apted, 1982; Keller *et al.*, 2002).

By means of the above-mentioned bioassays, 6 crude extracts from 3 Iranian medicinal plants viz., *Pterocarya fraxinifolia* (Juglandaceae), *Ilex spinigera* (Aquifoliaceae), *Pyrus boissieriana* (Rosaceae), collected in north of Iran, were screened. *P. fraxinifolia* is used for fishing in north of Iran. *I. spinigera* is important for its purine alkaloids; *P. boissieriana* can be used as good natural source of arbutin.

MATERIALS AND METHODS

Plant materials:

Leaves of the *P. fraxinifolia*, *I. spinigera*, *P. boissieriana*, were collected in September 2002 in Mazandaran forest, Iran, and identified by the Department of Pharmacognosy, Mazandaran University of Medical Sciences. Voucher specimens have been deposited in the herbarium of the department of Pharmacognosy.

Preparation of extract:

The collected plant materials were air dried and ground in liquid nitrogen. The materials were that macerated successively, first with dichloromethane (DCM) using 110g *P. fraxinifolia* for 1000 ml, 500g *I. spinigera* for 3000ml and 404g *P. boissieriana* for 2000ml solvent, and then with methanol (MeOH) in the same way. Maceration was

performed 3 times for each solvent during 24 h at room temperature and under constant agitation. the DCM, MeOH extracts were evaporated to dryness under reduced pressure at 40°C and then lyophilized.

Antibacterial assays:

The extracts were tested for antifungal and antibacterial activity by bioautography on thin-layer chromatography (TLC) (Rahalison *et al.*, 1991; Hamburger *et al.*, 1987; Hams and Fuchs, 1970). Each extract (100 µg) was applied on Al-back silica gel 60F254 (Merck) TLC plates (*C. cucumericum*) or glass-backed silica gel 60F254 TLC plates (*C. albicans* and *B. subtilis*). The plates were developed with the following solvent system: ethyl acetate- hexane (1:1) for DCM extracts and chloroform-methanol-water (65:35:5) for MeOH extracts. After elution, the chromatograms were dried for complete removal of solvents and sprayed with a conidial suspension of *C. cucumericum*. After 3 days incubation at room temperature in polystyrene boxes with a moist atmosphere, active compounds appeared as clear zones against a dark background.

For *C. albicans*, yeast inoculums (approximately 10 * cell/ml) in malt agar were distributed over the chromatograms. After solidification of the medium as a thin layer (approximately 1 mm layer thickness), the plates were incubated overnight at 30°C and then sprayed with methylthiazolyl-tetrazolium chloride (MTT). Active compounds appeared as clear spots against a purple-colored background. Nistatin was used as a reference compound.

Bacillus subtilis strain ATCC 6633 obtained from sigma was used for antibacterial bioassay. The plates were prepared as described for *Candida albicans*. Also, as for *C. albicans*, the plates were incubated overnight at 30°C in humid atmosphere, and then sprayed with MTT. Active compounds could be detected as white spots on the purple formazan background. The same amounts were spotted on the TLC plates. Chloramphenicol was used as positive control.

Antioxidant assay:

These test were performed on thin-layer chromatography (Tako *et al.*, 1994; Cuendet *et al.*, 1997). The plant extracts (100 mg) were applied on Al-backed silica gel 60F254 plates (Merck). For antioxidant assay, a solution of 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH) in MeOH was sprayed on the developed TLC (Cuendet *et al.*, 1997). After about 30 minutes, the active compounds were seen as clear spots against a purple background.

Larvicidal assays.

For larvicidal testing, DCM, MeOH extracts were solubilized in dimethyl sulfoxide (DMSO) at 50mg/ml (for final test solution of 500mg/L). DMSO solutions (100ml) were added to 9.9 ml of distilled water (final DMSO concentrations 1%) in graduate test tube. *Aedes aegypti* eggs were incubated for 24 h in river water. Eggs of *A. aegypti* were provided by the tropical institute in Basel, Switzerland. Approximately 20 second-instar larvae were placed in the test solutions. The tubes were incubated in darkness at 26-28°C for 24 h. larvae lethality was observed under laboratory light. All samples were measured in duplicate. Rotenone (sigma) was used as a reference compound (Marston *et al.*, 1993; Cepleanu *et al.*, 1994).

RESULTS AND DISCUSSION

Plants contain chemical constituents and are a valuable source of new and biologically active molecules. For their investigations, it is important to have the necessary tools at hand. These include suitable biological assays and chemical screening methods. Bioassays should be as simple as possible and attempts should be made to have access to a large number of different tests so that many biological properties can be screened. Existing assays, however, are often not reliably predictive for clinical efficiency and care should be taken when interpreting the results

The bioassay work here involves antifungal, antibacterial, larvicidal and antioxidant/radical scavenging (the chemical assay) activities.

Table I shows the Iranian names of the plants with their families and botanical names, the place of collection, the part used, the dry weight, the type of extract for testing, volume of solvent for extraction and weight of extract.

Table 1. Three Iranian medicinal plants for biological and antioxidant activities

Iranian name	Family	Botanical name	Collectet at	Part used	Dry weight (g)	Type of extract	Vollume of extract (ml)	Weight of extract (g)
Larg	Juglandaceae	<i>Pterocarya fraxinifolia</i>	Neka	Leave	110	DCM MeOH	1000*3 1000*3	2.91 29.57
Verktali	Aquifoliceae	<i>Ilex spinigera</i>	Behshar	Leave	500	DCM MeOH	3000*3 3000*3	30.87 63.39
Telka	Rosaceae	<i>Pyrus boissieriana</i>	Sari	Leave	404	DCM MeOH	2000*3 2000*3	17.88 89.05

Table 2 shows the CH₂Cl₂ and MeOH extracts from *P. fraxinifolia* (Juglandaceae), *I. spinigera* (Aquifoliaceae), *P. boissieriana* (Rosaceae) were preliminarily screened for their antifungal, antibacterial, antioxidant (radical scavenging) and larvicidal activities.

Table 2. Antifungal, Antibacterial, Antioxidant, and larvicidal activities of three Iranian Medicinal Plant Extracts.

Sample	Assay	Antifungal		Antibacterial (<i>Bacillus subtilis</i>)	Antioxidant	Larvicidal (<i>Aedes aegypti</i>)
		Anticladados- Anticandida porium				
<i>Pterocarya fraxinifolia</i>	MeOH ext.	+++	+++	+++	+++	+
	DCM ext.	-	-	+++	++	-
<i>Ilex spinigera</i>	MeOH ext	-	-	-	+	-
	DCM ext.	-	-	+	+	++
<i>Pyrus boissieriana</i>	MeOH ext.	+	+	-	++	+
	DCM ext.	-	-	++	+	-

Antimicrobial activity was most consistently detected in the species *P. fraxinifolia*, especially, against *C. cucurmericum*, *C. albicans*, and *B. subtilis*. Notable, *P. fraxinifolia* exhibits more activity against yeast and bacterium, while the others exhibit less effect against the microorganisms used in this study. This indicated that antifungal and antibacterial activities in *P. fraxinifolia* might be associated with different compounds.

In the DPPH assay, all the extracts were active as radical scavengers (Table 2). MeOH extract of *P. fraxinifolia* was extremely active as antioxidant.

I. spinigera DCM extract, *P. fraxinifolia* MeOH extract and *P. boissieriana* MeOH extract had toxic properties against the larvae of the yellow fever transmitting mosquito *Aedes aegypti* as 50 ppm. In this test, DCM extract of *I. spinigera* was extremely active as larvicide.

The above mentioned biological activities of these plants have not previously been reported. The results of this survey thus show that these plant extracts are potentially a rich source of bioactive compounds and worthy of further study.

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