The Pharmaceutical and Chemical Journal, 2019, 6(6):32-37

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Isolation, Partial Characterization of a Dihydrochalcone from Sudanese *Salvadore persica* (L.) and Antimicrobial Activity of Fractions

Abdel Karim, M.[1], Nisrin, A.[1], Omelhassan Talha Rahama[2]

[1]Sudan University of Science and Technology, Faculty of Science
[2]Nile Valley University, Faculty of Education

Abstract *Salvadore persica* is an evergreen shrub in the family Salvadoraceae. Among global Muslim community, *Salvadore persica* is commonly used for oral health hygiene. Some biologically active molecules like alkaloids, flavonoids, tannins have been isolated and identified from this plant**.** Phytochemical screening of *Salvadore persica* roots revealed the presence of flavonoids, saponins, coumarins, alkaloids, tannins, sterols and triterpenes. From the root ethanolic extract a dihydrochalcone has been isolated and partially characterized on the basis of its spectral data. Different fractions of *Salvadore persica* root have been assessed for antimicrobial activity. The chloroform fraction showed moderate activity against *Pseudomonas aeroginosa*. The ethanol and chloroform fractions exhibited partial activity against *Staphylococcus aureus.*

Keywords Isolation, Dihydrochalcone, *Salvadore persica* (L.), Antimicrobial Activity

Introduction

Salvadore persica is an evergreen shrub in the family Salvadoraceae. Among global Muslim community, *Salvadore persica* is commonly used for oral health hygiene [1]. Some biologically active molecules like alkaloids, flavonoids, tannins have been isolated and identified from this plant [2-4].

Aqueous extract of *Salvadore persica* contains, among others, flavonoids, tannins, saponins, alkaloids, cyanogenic glycosides and vitamin C [5-9]. Some of these constituents possess significant *in vitro* and *in vivo* antimicrobial activity [10-16]. The antiplasmodial potential of *Salvadore persica* has been reported [17]. It has been shown that *Salvadore persica* possesses antipyretic, analgesic, antiinflammatory, and diuretic effects [18]. The plant is traditionally used agains piles, scabies, leucoderma, gonorrhea, hook worms, rheumatism, cough and asthma [5, 19]. Seed oil is a natural remedy for skin infections and joint pain [20]. Fruit juice is said to be a general tonic [20-21].

Materials and Methods

Plant Material

Roots of *Salvadore persica* were collected from the premises Khartoum state- Sudan. The plant was authenticated by The Medicinal and Aromatic Plants Research Institute-Sudan. The plant material was shade-dried at room temperature and finally powdered.

Microorganisms

Organisms used for the antimicrobial assay are:

Gram +ve: *Bacillus subtilis and Staphylococcus aureus;* Gram –ve *Escherichia coli, Pseudomonas aeruginosa* and

the fungal strain *Candida albicans*

Media for microbial culture

-Mueller Hinton agar – for bacteria

-Sabouraud dextrose agar (Oxid, England) – for fungus

Equipments

A Shimadzu UV spectrophotometer (model UV 240) was used for UV measurements. The ^{1}H NMR spectrum was obtained on a Joel- Nuclear Magnetic Resonance (NMR) spectrophotometer, (Brucker AC-250) operating at 500 MHz.

Methods

Extraction and Isolation of Flavonoids

Powdered plant material (1.5 Kg) was macerated with 95% ethanol for 72h at room temperature. The solvent was removed under reduced pressure and the dried extract of *Salvadore persica* was applied as narrow strips on glass plates (20x20 cm) and run in 50% acetic acid. After the usual workup, a chromatographically pure flavonoid (compound I) was isolated.

Antimicrobial Activity

The antimicrobial activity was evaluated using the cup plate agar diffusion assay. Briefly, holes (6 mm in diameter) were made in the seeded agar. Aliquots of test sample (100 mg/ml) were added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and then incubated at 37^{o}C for 24 h-for bacteria – and for three days at 25^{o}C for fungi. The assay was performed in duplicate and the resulting inhibition zones were measured in (mm) and averaged.

Results and Discussion

Phytochemical Screening

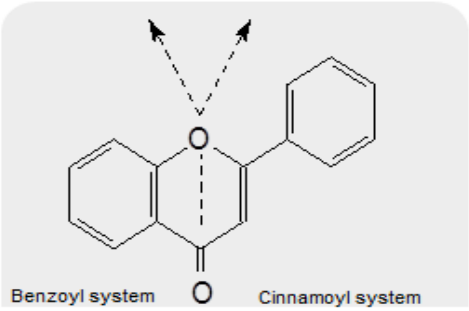
Salvadore persica root was screened for major secondary metabolites and the results are displayed in table 1.

Table 1: Phytochemicals of *Salvadore persica* root

Secondary metabolite	Occurrence
Saponins	+
Coumarins	+
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Terpenes	+

Identification of compound I

Flavonoids usually show in the UV two absorption bands; band I and II. Band I is due to the absorption of the cinnamoyl system, while band II originates from the benzoyl system. Flavones, flavonols, chalcones and aurones give band I and II, due to conjugation between the carbonyl function and the aromatic B ring, while flavanones, isoflavones, dihydroflavonols and dihydrochalcones give only band II in the range: 230-290nm. These classes of flavonoids lack conjugation between the B ring and the carbonyl function.



From the roots of *Salvador persica* a flavonoid-compound I- has been isolated in a chromatographically pure form by silica gel TLC. Compound I absorbs in the UV at λ_{max} 271nm. Such absorption is characteristic of flavanones, isoflavones, dihydroflavonols and dihydrochalcones. Isoflavones possess a spectrum characterized by a shoulder in the range 300-340nm and such shoulder was not detected in the UV spectrum (Fig. 1). Dihydrflavonols are distinguished by a 3-OH function which was not detected via the sodium methoxide spectrum (a bathochromic shift was not observed in the spectrum) - see Fig. 2. Flavanones and dihydrochalcones are distinguished via their ^{1}HNMR spectra. No multiplets characteristic of C-2 and C-3 protons of flavanones appeared in the ^{1}HNMR spectrum (Fig. 5) of compound I (such resonances usually appear at δ2.80 and 5.20 ppm). These findings suggest that the isolated flavonoid is a dihydrochalcone.

Some UV shift reagents are usually used in the chemistry of flavonoids for the specific detection of some hydroxylation on the nucleus of flavonoids. These are: sodium acetate (diagnostic of 7-OH); aluminium chloride (diagnostic of catechols and 3-, 5-OH functions) and boric acid which is diagnostic of catechols. These reagents afford bathochromic shift diagnostic of certain hydroxylation pattern on the skeleton of flavonoids.

The sodium acetate spectrum (Fig. 3) of compound I failed to reveal any bathochromic shift indicating absence of a 7-OH function. Also the aluminium chloride (Fig. 4) spectrum failed to reveal any bathochromic shift and thus suggesting absence of 3-, 5-OH groups as well as catechol moieties.

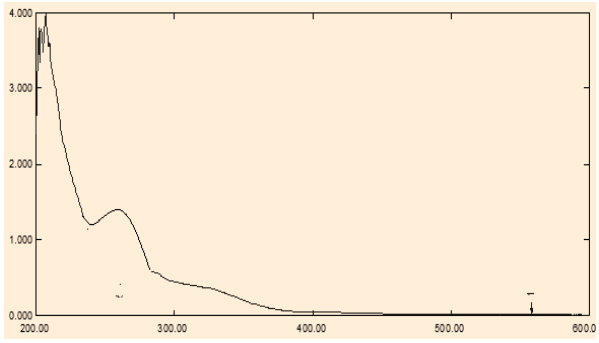


Figure 1: UV spectrum of compound I

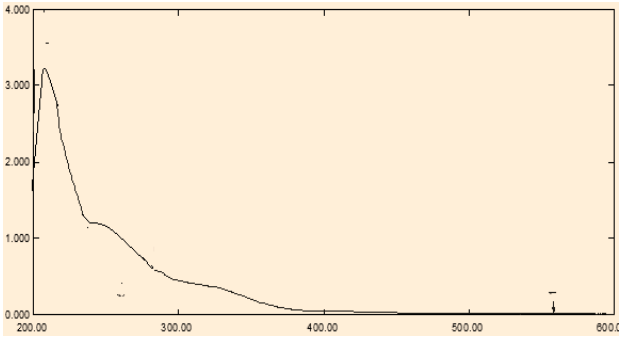


Figure 2 : Sodium methoxide spectrum of compound I

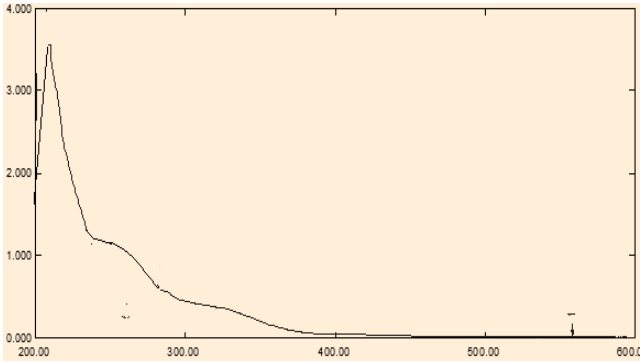


Figure 3: Sodium acetate spectrum of compound I

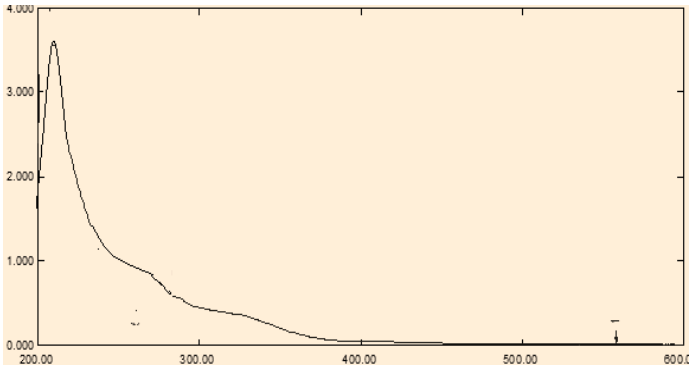


Figure 4: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig. 5) showed a resonance at δ 1.78ppm assigned for an acetyl group. The multiplet at δ3.04-3.60ppm is due to sugar protons (this sugar was not identified in this study). The signal at δ4.10ppm was attributed to two methoxyl groups. The aromatic protons resonated at δ7.34 ppm.

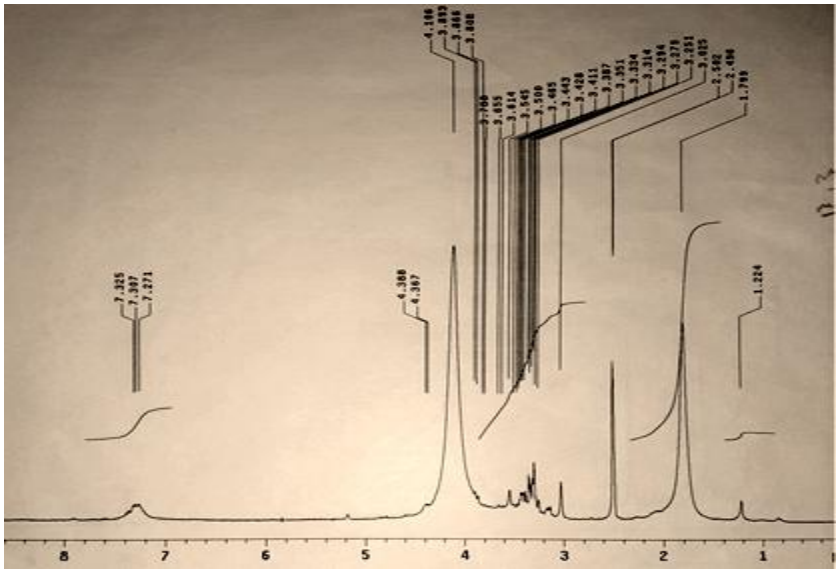
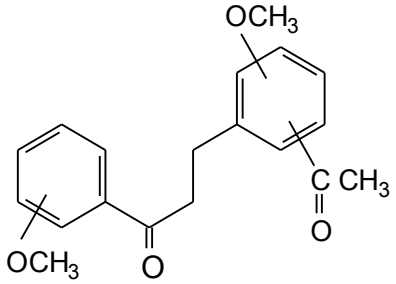


Figure 5: ^{1}H NMR spectrum of compound I

On the basis of the above argument, the following partial structure was proposed for the aglycone of compound I:



Aglycone of compound I

Antimicrobial Assay

Different fractions of *Salvadore persica* root have been screened for antimicrobial activity against five standard human pathogens. The results are depicted in Table 2. Tables 3 and 4 display the antimicrobial activity of standard drugs used as positive control.

The chloroform fraction showed moderate activity against *Pseudomonas aeroginosa.* The ethanol and chloroform fractions exhibited partial activity against *Staphylococcus aureus.*

Table 2: Inhibition zones of fractions

Fraction (100mg/ml)	Sa	Bs	Ec	Ps	Ca
Ethanolic	10	--	---	---	--
n-Butanol	--	--	--	--	--
Ethyl acetate	--	--	--	--	--
Chloroform	10	--	--	14	--

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

Table 3: Antibacterial activity of standard chemotherapeutic agents

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4: Antifungal activity of standard chemotherapeutic agent

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

References

[1]. Sher H, Al-Yemeni MN, Yahya SM, Arif HS., Ethnomedicinal and ecological evaluation of *Salvadora persica* L: A threatened medicinal plant in Arabian Peninsula. *J Med Plants Res.,* 2010, 4: 1209-1215.

[2]. Abdillahi HS, Stafford GI, Finnie JF, Staden JV., Ethnobotany, phytochemistry and pharmacology of Podocarpus sensus latissimo (S.I). *South Afr J Bot*., 2010, 76: 1-24.

[3]. Garboui SS, Borg-Karlson AK, Palsson K., Tick repellent properties of three Libyan plants. *J Med Entomol.,* 2009, 46: 1415-1419.

[4]. Kamil M, Jayaraj AF, Ahmed F, Gunasekhar C, Samuel S., Pharmacognostic and phytochemical studies on *Salvadora persica*. *J Pharm Pharmacol.,* 1999, 227: 51-58.

[5]. Alali F, Al-Lafi T., GC-MS analysis and bioactivity testing of the volatile oil from the leaves of the toothbrush tree *Salvadora persica* L. *Nat Prod Res*., 2003, 17: 189-194.

[6]. Almas K., The effect of *Salvadora persica* extract (miswak) and chlorhexidine gluconate on human dentin: A SEM study. *J Contemp Dent Prac.,* 2002, 3: 27-35.

[7]. Almas K, Skaug N, Ahmad I., An *in vitro* antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses. *Int J Dent Hyg*., 2005, 3: 18-24.

[8]. Rajesh V, Suresh P, Anil B, Brijesh K, Priyanka P., *Salvadora persica* L (Tooth Brush Tree): A Review. *J PR*, 2009, 2: 1809-1812.

[9]. Noumi E, Snoussi M, Trabelsi N, Hajlaoui H, Ksouri R., Antibacterial, anticandidal and antioxidant activities of *Salvadora persica* and *Juglans regia* L. extracts. *J Med Plant Res*., 2011, 5: 4138-4146.

[10]. Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A., Antifungal properties of *Salvadora persica* and *Juglans regia* L. extracts against oral Candida strains. *Eur J Clin Microbiol Infect Dis*., 2010, 29: 81-88.

[11]. Kamel MS, Ohtani K, Assaf MH., Lignan glycosides from stems of *Salvadora persica*. *Phytochem*., 1992, 31: 2469-2471.

[12]. Darout I, Christy A, Skaug N., Identification and quantification of some potentially antimicrobial anionic components in miswak extract. *Indian J Pharmacol*., 2000, 32: 11-14.

[13]. Abd-Rahman HF, Skaug N, Whyatt A, Francis GW., Volatile compounds in crude *Salvadora persica* extracts. *Pharmaceut Biol*., 2003, 41: 399- 404.

[14]. Sher H, Nasser Al-yemeni M, Leonard Wijaya, Ethnobotanical and antibacterial potential of *Salvadora persica* l: A well-known medicinal plant in Arab and Unani system of medicine. *J Med Plant Res.*, 2011, 5: 1224-1229.

[15]. Al-Sabawi AK, Al-Sheikh-Abdal AKK, Taha MY J., The antimicrobial activity of *Salvadora persica* solution (Miswak-Siwak) as root canal irrigant (A comparative study). *Univ Sharjah J Pure Appl Sci.*, 2007, 4: 69-91.

[16]. Chelli-Chentouf N, Tir Touil Meddah A, Mullié C, Aoues A, Meddah B., *In vitro* and *in vivo* antimicrobial activity of Algerian Hoggar *Salvadora persica* L. extracts against microbial strains from children's oral cavity. *J Ethnopharmaco*l., 2012, 144: 57-66.

[17]. Ali H, Konig GM, Khalid SA, Wright AD, Kaminnsky R., Evaluation of selected Sudanese medicinal plants for their *in vitro* activity against hemoflagellates, selected bacteria, HIV and tyrosine kinase inhibitory, and for cytotoxicity. *J Ethnopharmacol.*, 2002, 83: 219-228.

[18]. Galletti GC, Chiavari G, Duale Kahie Y., Pyrolysis/gaschromatography/ ion-trap mass spectrometry of the ''tooth brush'' tree (*Salvadora persica* L.), *Rapid Commun Mass Spectr*, 1993, 7: 651-655.

[19]. Khalessi AM, Pack ARC, Thomson WM, Tompkins GR, An in vivo study of the plaque control efficacy of Persica TM: a commercially available herbal mouthwash containing extracts of *Salvadora persica*, *Int Dent J*, 2004, 54: 279-283.

[20]. Ahmed S, Soaad E, Essawy E, Mohamed EI, Ewald S., Preliminary phytochemical and propagation trial with *Salvadora persica*. L, *Agric For Res.*, 2008, 1: 135-138.

[21]. Darmani H, Al-Hiyasat AS, Elbetieha AM, Alkofahi A., The effect of an extract of *Salvadora persica* (meswak, chewing stick) on fertility of male and female mice. *Phtomed*., 2003, 10: 63-65.