

## Phytochemical Analysis and *In vitro* Antifungal Activities of Bioactive Fractions from Leaves of *Rhus coriaria* (SUMAC)

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*Rhus coriaria* Linn. (Anacardiaceae), commonly known as sumac, has been used as a spice, condiment, appetizer, and as a souring agent for centuries. A broad range of nutritionally and medicinally significant phytochemical components have been identified from various parts of sumac having antimicrobial, anti-inflammatory effects. However, little is known about the biological activity of constituents present in its leaves against fungal infections. The collected plant leaves were extracted with 80% acetone and fractionated into n-Hexane Fraction (HF), Dichloromethane Fraction (DCMF), Ethyl Acetate Fraction (EAF), and n-Butanol Fraction (BF). Various concentrations of these fractions and *Rhus coriaria* total extract (TE) were tested for their antifungal, phenolics, and flavonoids properties. The total phenolic and flavonoid contents were significantly higher in *Rhus coriaria* TE, which also resulted in higher antifungal activity as compared to other fractions. The MIC<sub>50</sub> values of TE and fractions were ranged from 39.3 to 500 µg/mL and as for the MFC<sub>50</sub> values were ranged from 125 to 1000 µg/mL. Among the tested fractions of *Rhus coriaria* leaves, DCMF, EAF, and BF showed higher content in phenolic and flavonoids along with promising antifungal activity against the tested organisms respectively. The presence of phenolic and flavonoid constituents recommended *Rhus coriaria* as a target for formulation of novel drugs against fungal infections with minimal side effects.

**Key words:** Antifungal activity, Polyphenol content, Sumac, *Rhus coriaria*, phytochemistry, antioxidant.

The versatility of harmful microorganisms in nature and unlimited human contact, gave the chance for major diseases among human kind. It was reported that pathogenic fungi produce toxic and carcinogenic substance which could be reached to human via food, drink, and in turn effect on human health (Peraica *et al.*, 1999; Samson *et al.*, 2002; Schuster *et al.*, 2004). Many serious human diseases such as aspergilloses, candidoses, coccidioidomycosis, mycetomas were reported as result of exposure to pathogenic related fungi species (Samson *et al.*, 2001; Nielsen & Heitman, 2007).

As result of increase in the number of resistant microbial strains against synthetic drugs, the research focused up on the potency of several constituents of some plants which has suitable conditions especially minimal side effects (Habtemarian *et al.*, 1993; Vermani & Garg, 2002).

Herbal medicine plays significant role in human health. Most of the world people depend on medical modulates of plant origin in their daily life (Owoabi *et al.*, 2007). This may be related to that plant can naturally produce biologically chemical compounds with promising vitality against the growth of large scale of microbes which be considered as a sole source of bioactive agents (Adesina *et al.*, 2000; Panico *et al.* 2005). Previously, the potency of several plant extracts such as Garlic (*Allium sativum*) against bacteria

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(Cavallito & Bailey, 1944), fungi (Adetumbi *et al.*, 1986) and viruses (Weber *et al.*, 1992).

Sumac (*Rhus coriaria* L., family Anacardiaceae) is a well-known, popular spice and has been utilized extensively for medicinal and other purposes. Its leaves have been used as a tanning agent for their high tannin content. The berries have diuretic properties, and are used in bowel complaints and for reducing fever. Other reports indicated that the extract of Sumac is used in traditional medicine as a medicinal herb for its antimicrobial and wound healing activity (Rayne & Mazza, 2007).

Previously, it was reported that Sumac leaves are good sources of phenolic acids and several flavonoids (Mavlyanov *et al.*, 1997; Zalacain *et al.*, 2003). These substances have gained interest and used frequently against many diseases as anti-inflammatory, antioxidant, antibacterial, fungicide, antiviral, and candidicide.

The objectives of the present study were to evaluate the antifungal profile of bioactive fractions from Sumac (*Rhus coriaria* L.) in order to provide a scientific basis for the traditional use of Sumac.

## MATERIALS AND METHODS

### Chemicals

All chemicals were purchased from sigma Aldrich Co (Milano, Italy). All chemicals and reagents used in this study were of analytical grade.

### Sumac (*Rhus Coriaria* L.) leaves

Leaves of Sumac (*R. coriaria* L.) were obtained from a commercial spice store (Othaim Markets) in Riyadh, Saudi Arabia.

### Test microorganisms:

The microorganisms used in this study consisted of eight different fungal species: *Candida albicans* ATCC 90028, *C. tropicalis* ATCC 750, *Cryptococcus neoformans* ATCC 66031, *Aspergillus niger* ATCC 322, *Penicillium chrysogenum* ATCC 5476, *Aspergillus flavus* ATCC 227, *Tricophyton rubrum* ATCC 2327 and *Candida krusei* ATCC 6258. Fungal strains were maintained on agar slant at 4°C and sub-cultured on a fresh appropriate agar plates 24 h prior to any antifungal activity. Sabouraud Glucose Agar was used for the activation of fungi. The Mueller Hinton Broth

(MHB) was used for the MIC and MFC determinations.

### Extraction and fractionation:

Fifty grams (50 g) of dried plant powder was extracted in a Soxhlet apparatus using 500 mL of 80% aqueous acetone. Crude extracts were maintained at +4 °C until use (Thakare, 2004). For aqueous extracts fractionations, sequential liquid-liquid extraction with n-hexane, dichloromethane, ethyl acetate and n-butanol was performed. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain n-Hexane Fraction (n-HF), Dichloromethane Fraction (DCMF), Ethyl Acetate Fraction (EAF) and n-Butanol Fraction (n-BF). The different fractions were freeze-dried by Telstar Cryodos 50 freeze-dryer. The fraction residues were packed in waterproof plastic flasks and stored at 4°C until use.

### Polyphenols determination:

#### Total phenolic and flavonoid content

Total polyphenols were determined by Folin-Ciocalteu method as described previously (Lamien-Meda *et al.*, 2008). The results were obtained from gallic acid calibrated curve and expressed as mg of Gallic Acid Equivalents (GAE)/ 100 mg of fractions. The total flavonoids were estimated according to the Dowd method as adapted by Lamien-Meda *et al.* (2008). The amounts of flavonoids in plant fractions were expressed as mg of Quercetin Equivalents (QE) / 100 mg of fractions from the calibrated curve.

### In vitro antifungal activity: Preparation of inocula

Inoculum of each fungal strain will be subjected for growing on nutrient agar (Muller Hinton broth) at 35°C for 72 h. After growth, the fungal strains were suspended in a saline solution (0.9%, w/v) NaCl and adjusted to a turbidity of 0.5 Mac Farland standard ( $5 \times 10^5$  CFU/mL) as previously reported (Konaté *et al.*, 2012).

### Preparation of fraction substances

The fractions of plant extract were dissolved in 10% aqueous Dimethylsulfoxide (DMSO) at a final concentration of 1000  $\mu$ g/mL. The stock solutions were subjected to sterilization by filtration process using 0.22  $\mu$ m sterilizing Millipore express filter. Standard of Fluconazole (100 mg/ml) was used as a positive control.

### Minimum Inhibitory Concentration (MIC) assay

The MIC of the phytoconstituents and

reference antibiotics was determined by serial broth microdilution method as reported previously (Konaté *et al.* 2012). The fungal strains were subjected for growth in clear microtitre plates of round bottom 96-well. The plates were sealed with parafilm and left for growth maintenance at 37°C for 48 hours. MIC was defined as the first well with no visible growth after incubation. The experiment was carried out in duplicates (each experiment was done in triplicates).

#### Minimum Fungicidal Concentration (MFC)

From the wells which showed no visible growth in MIC test, only 50  $\mu$ L were re-inoculated in fresh wells containing Sabouraud dextrose broth and incubated at 37°C for 48. MFC represented as the lowest concentration resulting in no growth on subculture (Hafidh, *et al.*, 2011).

## RESULTS

#### Polyphenol content

The total phenolics content per 100 mg of sumac extract and fractions ranged from 48.7 $\pm$ 3.5 to 15.3 $\pm$ 1.30 mg GAE. The highest content of total phenolics was detected in TE, with 48.7 $\pm$ 3.5, following by DCMF with 36.1 $\pm$ 1.12 mg GAE, EAF with 29.16 $\pm$ 0.19 mg GAE, and, BF with 24.8 $\pm$ 1.5 mg GAE. The lowest total phenolics were obtained in HF with, respectively 15.3 $\pm$ 1.30 mg GAE. The total flavonoids content per 100 mg of Sumac extract and fractions ranged from 13.5 $\pm$ 2.7 to 4.80 $\pm$ 0.31 mg QE. The highest content of total flavonoids in

sumac was recorded in TE with 13.5 $\pm$ 2.7, followed by DCMF with 11.4 $\pm$ 1.14 mg QE, EAF with 9.46 $\pm$ 0.83 mg QE, and BF with 8.7 $\pm$ 1.4 mg QE. The lowest total flavonoids were obtained in HF with, respectively 3.70 $\pm$ 0.28 mg QE. The results are recorded in the (Fig. 1).

#### Antifungal activity

*R. coriaria* extract and fractions possessed a good antifungal activity in 100 mg/ml concentrations. Flavonoids and phenols extracts of *R. coriaria* showed a significant inhibitory activity of a greater extent as compared to fluconazole (Table 1). As for MIC<sub>50</sub> and MFC<sub>50</sub> of extract and fractions, result varied according to microorganism (Table 2). The MIC values of fractions were ranged from 39.3 to 500  $\mu$ g/mL and as for the MFC values were ranged from 125 to 1000  $\mu$ g/mL (Table 3). The data showed remarkable variation in biological activities among fractions of *R. coriaria* extract towards fungal strains

## DISCUSSION

The use of herbal plants as remedies in folk medicine and its promising effect in different human diseases makes it as a main target for most drug designing scientists to synthesize new drugs of plant origin with minimal side effects (Alam *et al.*, 2009; Hussaini *et al.*, 2014), this may be related to increase in the rate of infection and development of disease worldwide which in turn devote the drug designers to screen and validate new biological

**Table 1.** Antifungal activity of extract and active constituents of Sumac (*R. coriaria*) at concentration 100 mg / ml.

Constituents	Inhibition zones (mm)						
	<i>C. al.</i>	<i>C. tro.</i>	<i>C. neo.</i>	<i>A. n.</i>	<i>A. f.</i>	<i>P. ch.</i>	<i>T. r.</i>
Total extract	25	24	24.8	24	32.5	27.8	25.7
EHA	22	22	25	23	23	24	21
HF	25	23	21	23	22	23	23
DCMF	26	25	23	24	27	30	24
EAF	23	21	20	29.2	19	21	23
BF	25	23	22	23	23	28	23
Antibiotic	22	21	19	25	23	24	22

*C. al.*: *Candida albicans*; *C. tro.*: *C. tropicalis*; *C. neo.*: *Cryptococcus neoformans*; *A. n.*: *Aspergillus niger*; *P. c.*: *Penicillium chrysogenum*; *A. f.*: *Aspergillus flavus*; *T. r.*: *Tricophyton rubrum*; *C. kr.*: *Candida krusei*; The results are the means of number of the colonies  $\pm$  standard deviations. Standard of Fluconazole (100 mg /ml) was used as a positive control. The antibiotic Inhibition zones (Resistant 12mm or less: Sensitive 19 mm or more). EHA: Ethylhexyl Acetate; HF: Hexane Fraction; DCMF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanol Fraction.

constituents of plant origin (WHO, 2000).

Although there are many drugs of plant origin were discovered and clinically established as antimicrobial, more flowering plants with

recommended medical actions still need to be discovered worldwide (Madureira, 2008). So, intensive research protocols were performed lastly on plants of medical actions as source of human

**Table 2.** Minimum Inhibitory Concentration (MIC) of extract and fractions from *Sumac (RhusCoriaria)*

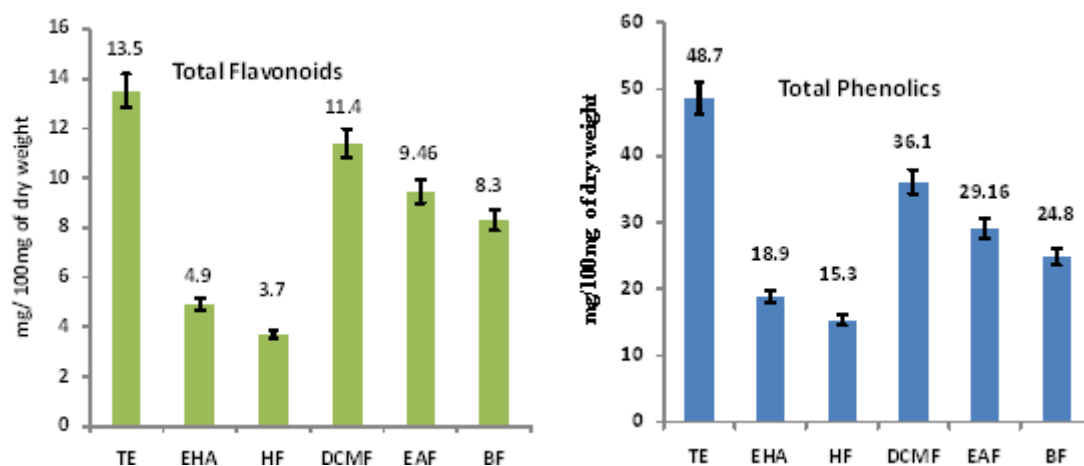
	<i>C. al.</i>	<i>C. tro.</i>	<i>C. neo.</i>	<i>A. n.</i>	<i>A. f.</i>	<i>P. ch.</i>	<i>T. r.</i>	<i>C. kr.</i>
Total extract	35.7	65.8	125	68.7	42.3	125	125	125
EHA	500	250	250	250	250	250	250	250
HF	250	250	500	250	250	250	500	250
DCMF	65.7	125	125	65.7	39.3	125	125	125
EAF	39.3	125	68.9	65.7	65.7	125	125	65.7
BF	65.7	125	125	65.7	65.7	125	125	125

*C. al.*: *Candida albicans*; *C. tro.*: *C. tropicalis*; *C. neo.*: *Cryptococcus neoformans*; *A. n.*: *Aspergillusniger*; *P. c.*: *Penicilliumchrysogenum*; *A. f.*: *Aspergillusflavus*; *T. r.*: *Tricophytonrubrum*; *C. kr.*: *Candida krusei*; The results are the means of number of the colonies  $\pm$  standard deviations.

**Table 3.** Minimum Fungicidal Concentration (MFC) of extract and fractions from Sumac (*RhusCoriaria*)

	<i>C. al.</i>	<i>C. tro.</i>	<i>C. neo.</i>	<i>A. n.</i>	<i>A. f.</i>	<i>P. ch.</i>	<i>T. r.</i>	<i>C. kr.</i>
Total extract	250	125	250	125	125	125	250	250
EHA	1000	500	500	500	500	1000	1000	500
HF	1000	1000	1000	500	500	1000	1000	1000
DCMF	125	250	250	250	250	500	250	250
EAF	250	500	500	125	125	500	250	250
BF	500	250	500	500	500	500	250	500

*C. al.*: *Candida albicans*; *C. tro.*: *C. tropicalis*; *C. neo.*: *Cryptococcus neoformans*; *A. n.*: *Aspergillusniger*; *P. c.*: *Penicilliumchrysogenum*; *A. f.*: *Aspergillusflavus*; *T. r.*: *Tricophytonrubrum*; *C. kr.*: *Candida krusei*; The results are the means of number of the colonies  $\pm$  standard deviations.



**Fig. 1.** Total phenolics and flavonoid contents of extract and fractions from Sumac (*RhusCoriaria*).TE: total extract; EHA: Ethylhexyl Acetate; HF: Hexane Fraction; DCMF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanol Fraction.

disease management (Aiyelaagbe, 2001; Woldemichael *et al.*, 2003; Fatima *et al.*, 2014), one of these protocols is investigation the inhibition activity of whole extracts or chemical fractions of natural plants against different microbes (Nasar-Abbas & Halkman, 2004; Shah *et al.*, 2014). Previous studies, revealed the presence of naturally occurring polyphenol compounds, saponosides, coumarins, steroids and triterpenies in most plant materials using phytochemical assays (Nacoulma, 1996), these compound were shown to have antimicrobial activities (El-Astal *et al.*, 2005).

In this present study, acetone 80% was used as solvent. In effect, according certain studies, acetone 80% is the best extraction solvent to extract the different secondary metabolites. Moreover, acetone extracts content more phenolic compounds than the other solvents (Sun & Ho, 2005). The results obtained in this study confirmed this statement. Our results showed that *R. coriaria* presented the highest amount of polyphenol content. The abundance of our extracts in polyphenols may explain the utility of *R. coriaria* in the treatment of infectious diseases (Jai & Hosmani, 2003; Sudharameshwari & Radhika, 2007). The data obtained indicate that, the antimicrobial activity of *R. coriaria* extracts mainly depends on the presence of phenols and glycosides such as anthocyanins, hydrolysable tannins, and gallic acid (the main phenolic acid in *R. coriaria*) (Kosar *et al.*, 2007). Traditional using of this spice may help in protecting from several microbial diseases spontaneously and may aid in control of bacterial or fungal growth in foods. The antimicrobial activity of *R. coriaria* extract on food pathogens has been demonstrated in many in vitro studies (Nasar-Abbas *et al.*, 2004; Duman-Ayd *et al.*, 2008). As a matter of fact, secondary metabolites, such as flavonoids, are important antimicrobial activity (Scalbert, 1991; Hussaini *et al.*, 2014).

The presence of biologically active compounds such as flavonoids, phenolic or terpenoids naturally in *R. coriaria* and related medical plants were shown to be the main source of plant defensive mechanism against microbes and insect damage, pharmacological activities which is the reason to be used as antimicrobial agents for human (Cowan, 1999; Nostro *et al.*, 2000; Dastagir *et al.*, 2014).

In the same manner, the presence of flavones and flavanones constituents provides *R. coriaria* plant a priority to be used as food preservative against pathogenic fungi (Lattanzio *et al.*, 2008). The inhibition mechanism of these compounds occurred via suppression of enzymes actions and cellular membrane functions of pathogenic fungi. Thus, in this study, the biological activity of *R. coriaria* fractions against the studied fungal strains was related to the presence of active phenolic, and flavonoids constituents as previously reported (Mariita *et al.*, 2011).

## CONCLUSION

The presence of phenolic and flavonoid constituents recommended *Rhus coriaria* as a new target for formulation of novel drugs against fungal infections, and could be used as a natural preservative in food industries against microbes

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