Microbes and Health
ISSN: 2226-0 153 (Print) 2305-3542 (Online)
http://journal.bsvmph.org/

Microbes and Health, June 2017, 6 (1): 5-8
DOI: http://dx.doi.org/10.3329/mh.v6i1.34063

ORIGINAL RESEARCH

Phytochemical composition of *Anastatica hierochuntica* L., can it fight the toxigenic bacterial agents responsible for food poisoning?

Elhassan BENYAGOUB[1], Dalila RAZNI[1], Snoussi MOGHTET[2]

[1]Faculty of natural and life sciences, Department of Biology, Tahri Mohammed University of *Bechar* (08000), *Bechar*-Algeria,
[2]Institute of Sciences and Technology, Department of Geology and Universe, University Center of *Tindouf*, (37000), *Tindouf*-Algeria.

*Corresponding author's email: benyagoubelhassan@gmail.com

[**Received:** 18 April 2017, **Revised:** 31 May 2017, **Accepted:** 10 June 2017]

ABSTRACT

The present work aims to study the biological activity of *Anastatica hierochuntica* L., against four bacterial strains considered as toxigenic responsible for food-borne infection. The plant was collected from *Tindouf* region (Far Southwest of Algeria). In this study we performed a phytochemical screening and evaluation of antibacterial activity of three macerates of two vegetative parts (seeds and stems) by two methods (disk and wells diffusion method). The yield of aqueous, methanolic and etheric macerates of the seeds and stems were (5.1; 3.8), (5; 1.4) and (2; 0.95)% respectively. Also, it appears that macerates obtained were rich in bioactive phyto-constituents particularly the seed of the plant. They showed the presence of ten large chemical groups. The antibiotic resistance profile of the bacterial strains tested showed an increased resistance to several families of antibiotics. The evaluation of the antibacterial activity of the extracts showed that methanolic and aqueous macerates of the seed were more active against Gram positive bacteria. The methanolic macerate of the stems was less active. However, other macerates were ineffective. The results obtained show that the plant has an average antibacterial activity and that depends on extract concentration used.

Key Words: *Anastatica hierochuntica;* phytochemical screening; antibacterial activity; foodborne infection; *Tindouf* (Algeria)

Introduction

First ever estimates of the global burden of foodborne diseases caused by 31 agents (bacteria, virus, parasite, toxins and chemicals) states that each year as many as 600 million, or almost 1 in 10 people in the world, fall ill after consuming contaminated food and 420000 die as a result. Children under 5 years of age at particularity high risk, with 125000 children dying from foodborne diseases every year, where African and South-East Asian regions have the highest burden of this diseases (WHO, 2015).

Meanwhile, Plant biodiversity of the Sahara is characterized by the presence of medicinal plants having a great therapeutic potential against several diseases. For a long time, natural remedies, and especially medicinal plants were the principal recourse of medicine for our grandparents, despite the significant development of the pharmaceutical industry that allowed modern medicine to treat a large number of often fatal diseases (Bruneton, 1999).

The *Brassicaceaes* family, is among the ten families of the most economically important plants (Warwick, 1993). Indeed, many species are used as food plants, ornamentals, condiments, and others are considered as industrial sources of vegetable oils. According to Bellakhdar (Bellakhdar, 1997), *Brassicaceaes* are very used in traditional Moroccan medicine.

The species of *Anastatica hierochuntica* L., is known for its therapeutic properties as a hepato-protective plant, hypoglycemic and diuretic. It is used in traditional medicine for uterine bleeding and to facilitate the expulsion of dead fetus, to treat gastrointestinal disorders, depression, high blood pressure, indigestion, headache, fever, malaria, epilepsy, heart disease and infertility (Hegazy and Kabiel, 2007).

It's in this context that we were interested in phytochemical properties of the species *A. hierochuntica* L., and highlight the antibacterial activity of its some excerpts namely the methanolic, aqueous and etheric macerates against four bacterial strains responsible for poisoning and foodborne diseases.

Materials and methods

This study was conducted at the University of *Bechar* (Algeria), after preparing the plant as follows;

Harvesting plants

The plant studied was collected after being identified during the months of February-March 2015 in the far southwest of Algeria-*Tindouf* region (Algerian-Moroccan border). The dried plant was put into clean bags.

Qualitative phytochemical screening

About tri-phytochemical study, three extractions were conducted according to the protocol developed by Emad (2014). The crude extracts were obtained by successive extractions with solvents of increasing polarity. In this order, petroleum ether, methanol and distilled water were used. Preparing extracts allowed performing a qualitative phytochemical screening of two vegetative parts (seeds and stems).

Table 1 : Phytochemical screening of *A.hierochuntica* L.
Comp.: Components, **NC**: Negative control (petroleum ether, methanol and distilled water), **Alc. Bases**: base alkaloids, **Com.** : Coumarins, **Sté.Trit**: Sterols and triterpenes, **Quin. libres**: free Quinones, **Alc. sels**: salts alkaloids, **Flav.** : Flavonoids, **Terp.** : Terpanoides, **Anth.** : Anthracénosides, **Hété. Ster**: sterol heterosides

Comp	Alc. bases	Com	Sté. Trit	Quin libres	Alc sels	Flav	Terp	Anth	Hétér Stér.	Tan	Sap	F. Ac
Seeds	-	+	+	-	+	+	+	-	+	+	-	+
Stems	-	+	+	+	-	-	+	-	+	+	-	+
NC	-	-	-	-	-	-	-	-	-	-	-	-

triterpenic, **Tan.** : Tannins, **Sap.** : Saponins, **Ac. G**: Fatty acid, **(-)**: Negative test, **(+)**: Positive test.

Extraction processes

The aqueous, methanolic and etheric macerates of the plant studied were obtained by maceration with the method described below;

Preparation of macerates

A test sample 10 g of the dried plant was mixed with 100 ml of distilled water. The mixture is stirred for 24 hours. After filtration through a filter paper, the filtrate is evaporated (rota vapor), till it dried under reduced pressure at 100°C in order to obtain the aqueous macerates residue.

Table 2: Values of inhibition zone diameters and inhibition percentages (I%) of antibiotics against the bacterial strains responsible for food-borne Infection.

Bacterial strains / *ATB*	*E. coli*			*S. aureus*			*S. typhimurium*			*L. monocytogenes*		
	D (mm)	**I (%)**	**P.ATB**	**D (mm)**	**I (%)**	**P.ATB**	**D (mm)**	**I (%)**	**P.ATB**	**D (mm)**	**I (%)**	**P.ATB**
Fosfomycin	-	-	-	9	10	**R**	-	-	-	-	-	-
Chloramphenicol	22	24,44	S	-	-	-	28	31,11	S	27	30	S
Cefazolin	6	6,66	**R**	-	-	-	6	6,66	**R**	-	-	-
Amox- CA	24	26,66	S	-	-	-	25	27,77	S	-	-	-
Oxacillin	-	-	-	6	6,66	**R**	-	-	-	-	-	-
Penicillin	-	-	-	6	6,66	**R**	-	-	-	6	6,66	**R**
Vancomycin	-	-	-	6	6,66	**R**	-	-	-	-	-	-
Gentamicin	30	33,33	S	25	27,77	S	24	26,66	S	-	-	-
Erythromicin	-	-	-	6	6.66	**R**	-	-	-	6	6,66	**R**
Tetracyclin	-	-	-	-	-	-	-	-	-	24	26,66	S
Clindamycine	-	-	-	6	6,66	**R**	-	-	-	-	-	-
Cefoxitin	24	26,66	S	-	-	-	6	66.66	**R**	-	-	-
Cefotaxime	6	6,66	**R**	-	-	-	10	11,11	S	-	-	-
Ofloxacin	22	24,44	S	-	-	-	18	20	S	-	-	-
Imipenem	32	35,55	S	-	-	-	35	38,88	S	-	-	-
Ceftazidime	-	-	-	-	-	-	-	-	-	-	-	-
Amikacin	-	-	-	21	23,33	S	-	-	-	-	-	-
Fusidic acid	-	-	-	6	6,66	**R**	-	-	-	-	-	-
Ampicillin	19	21,11	S	-	-	-	32	35,55	S	-	-	-

(D): Inhibition zone diameter (mm), **I (%)**: Inhibition percentage, **(P.ATB)**: antibiotic resistance profile, **Amox-CA**: Amoxicillin- clavulanic acid, **(S)**: Sensitive, **(R)**: Resistant, **(I)**: Intermediate, **ATB**: antibiotics, ***E. coli***: *Escherichia coli*, ***S. aureus***: *Staphylococcus aureus*, ***S. typhimurium***: *Salmonella typhimurium*, ***L. monocytogenes***: *Listeria monocytogenes*

However, for the methanol and etheric macerates, a test sample 5g of dried plant was mixed with 85ml of methanol and diethyl ether, respectively. The mixture is stirred for 24 hours. After filtration, the filtrate is evaporated (rota vapor) till it dried under reduced pressure at 65 and 35°C respectively.

The obtained residues are weighted to calculate the yield of aqueous, methanolic and etheric macerates (Majhenic *et al.,* 2007).

Bacterial strains

Four reference strains: *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 25923; *Listeria monocytogenes* ATCC 19115 and *Salmonella typhimurium* ATCC 27924 were collected from the Pasteur Institute of Algiers and used to evaluate the antibacterial activities of the plant extracts.

Antibacterial resistance

The bacterial colonies which were isolated from young cultures on nutrient agar medium (Fluka, India) incubated at 37°C for 18 to 24 hours are transferred into tubes containing sterile physiological saline (0.9% NaCl) to prepare bacterial suspensions with similar turbidity of 0.5 McFarland. Then, the bacterial suspension, prepared beforehand, was seeded using a sterile swab over the entire surface of a Mueller-Hinton agar medium (Himedia, India).

The study of antibiotic resistance profile of the bacterial strains against antibiotics was performed by the diffusion method on MH agar medium using loaded discs of antibiotics as recommended by NCCLS (National Committee for Clinical Laboratory Standards) (NCCLS, 2002).

The results of antibiotic resistance were interpreted according to the reference table prepared by antibiogram committee of the French microbiology society (Soussy, 2005).

The antibacterial activity of the extracts was determined by the disk diffusion method and well diffusion method on agar medium (Sacchetti *et al*., 2005; Celiktas *et al*., 2007).

Results and Discussion

Qualitative phytochemical screening

The phytochemicals tests results of the plant exhausted by water, methanol and diethyl ether, are summarized in Table 1. The phytochemical screening performed showed the presence of ten major chemical groups in parts seeds and stems as following; tannins, coumarins, fatty acids, reducing compounds, sterols or triterpenes, sterol heterosides triterpenic, flavonoids, free quinones, terpanoides and alkaloids salts. These tests showed the presence of flavonoids and alkaloids salts but only in the seeds. As well as the absence of saponin, anthracenosides, starch, emodols, anthocyanosides and alkaloids bases in the two parts of the plant studied. These results are corroborated by the work of Daur (2012), which showed that all parts of the A.hierochuntica L., have been proved rich in total phenolic content. This representation of nearly all chemical families could justify the multiple use of A. hierochuntica. Also, these results were similar to those given by Bouhadjera et al., (2005) which has been shown the presence of almost all secondary metabolites of Brassicaceae family such as alkaloids salts, flavonoids, saponins, tannins, anthocyanins, sterols and steroids with little differences. This difference in composition was probably due to various conditions including the environment, genotype, geographical origin, harvest time, location, temperature and drying time (Svoboda and Hampson, 1999; Lis-Balchin and Hart, 1999).

Antibiotic resistance profile of the bacterium used in this study:

The antimicrobial resistance profile results of the bacterial strains against antibiotics performed by the diffusion method on Mueller-Hinton agar medium are reported in Table 2. The antibiotic resistances profile of the bacterial strains tested showed an increased resistance to ampicillin, trimethoprim-sulfamethoxazole, cefazolin and cefoxitin for *E. coli*. While *S. aureus* and *L. monocytogenes* showed resistance to vancomycin, clindamycin, erythromicin, penicillin and oxacillin. The antibiotic resistance results are in agreement with our works series (Benyagoub *et al*., 2014), about toxigenic bacterial strains responsible for food-borne infection antibiotic resistance is relatively high for specific molecules, in particular: amoxicillin-clavulanic acid, cefazolin, penicillin, vancomycin, erythromycin, cefoxitine and tetracycline.

Antibacterial properties of the plant extracts; Agar diffusion method (Vincent Technique and wells method)

Faced to the problems of bacterial resistance to synthetic antibiotics, many researches have been conducted on the antimicrobial activity of natural products and plant extracts. The results of antibacterial test performed by the agar diffusion method, which are measured by measuring the inhibition zones around the discs, are shown in Table 3 and 4. The results showed that the methanolic macerate of the seeds presented an antibacterial effect only against the Gram-positive bacterial strains; *S. aureus* and *L. monocytogenes*, with no inhibition against *E. coli* and *S. typhimurium*. In addition, the aqueous macerate of the seeds presented an antibacterial effect on *S. aureus*; In parallel, no inhibition was observed against *E. coli*, *S. typhimurium* and *L. monocytogenes* for the aqueous and etheric macerates. For the stems macerates, the results show that the methanolic macerate presented only an antibacterial effect on *L. monocytogenes* with inhibition zones 10; 8 and 7 mm corresponding to the concentrations 235; 117.5 and 56mg/ml for the methanolic macerate. Other macerates were revealed ineffective and have not shown any effect on the bacterial strains tested.

The results of the antibacterial test demonstrate low efficiency of the three macerates of *A. hierochuntica* L., against the bacterial strains tested. These results are consistent with the work of Al-Fatimi *et al.*, (2007); Mohamed *et al*., (2010) which have not found any antibacterial activity against the same bacterial species we tested. The aqueous and methanolic macerates of the seeds having an average antibacterial especially against *S. aureus* and *L. monocytogenes*. While the methanolic macerate of the stems was only active on *L. monocytogenes*. According to the work of Rahmoun *et al*., (2014) who tested the antimicrobial activity of hydro-methanolic and chloroformic extracts of *A. hierochuntica*, gave a mediocre activity against *Acenitobacter baumanii, Citrobacter freundii, Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis* and *S. typhimurium*. This activity is attributed to phenolic compounds which have a large spectrum of activity against Gram negative bacteria. However, they did not show any interesting activity against Gram positive bacteria we tested on. The Sitosterol and the Sigmasterol, are the phytosterols the most encountered in higher plants, they represent more than 80% of existing sterols. The Brassicasterol found mainly in the *Brassicaceae* family is represented by a rate of 10% (Gaignaut *et al*., 1989). The antimicrobial tests showed that the steroids' extracts of leaves and fruits prevent the growth of *Pseudomonas sp* and show a broad spectrum antifungal (Bouhadjera *et al*., 2005).

Table 3: Percentage inhibition of different macerates seeds part on bacterial strains responsible for food borne infection.

Macerates \ Bacterial strains		*E. coli*	*S. typhimurium*	*S. aureus*	*L. monocytogenes*
MM	235mg/ml	-	-	12	8
	117.5mg/ml	-	-	10	7
	56mg/ml	-	-	9	9
AM	190mg/ml	-	-	8	-
	95mg/ml	-	-	9	-
	45mg/ml	-	-	8	-
EM	100mg/ml	-	-	-	-

AM: aqueous macerate, **MM**: methanol macerate, **EM**: etheric macerate, ***E. coli***: *Escherichia coli*, ***S. aureus***: *Staphylococcus aureus*, ***S. typhimurium***: *Salmonella typhimurium*, ***L. monocytogenes***: *Listeria monocytogenes*, **(-)**: no inhibition [Only the diameter of disc (6mm)].

Several studies have highlighted the high sensitivity of Gram-positive bacteria to bioactive compounds compared to Gram-negative bacteria (Koné *et al*., 2004; Hayouni *et al.*, 2007; Turkmen *et al.*, 2007; Shan *et al*., 2007; Falleh *et al.*, 2008). This can be attributed to the difference in the outer layers of Gram negative and Gram positive bacteria. Gram-negative bacteria and apart of the cell membrane, have an outer membrane which is composed of phospholipids, lipo-polysaccharides and proteins. This membrane is impermeable to most molecules. Nevertheless, the presence of porins in this layer will allow the free diffusion of molecules (Marzouk *et al*., 2006).

Table 4: Percentage inhibition of different macerates stems part on bacterial strains responsible for food borne infection.

Macerates \ Bacterial strains		*E. coli*	*S. typhimurium*	*S. aureus*	*L. monocytogenes*
MM	235mg/ml	-	-	10	-
	117.5mg/ml	-	-	8	-
	56mg/ml	-	-	7	-
MA	190mg/ml	-	-	-	-
	95mg/ml	-	-	-	-
	45mg/ml	-	-	-	-
ME	100mg/ml	-	-	-	-

AM: aqueous macerate, **MM**: methanol macerate, **EM**: etheric macerate, ***E. coli***: *Escherichia coli*, ***S. aureus***: *Staphylococcus aureus*, ***S. typhimurium***: *Salmonella typhimurium*, ***L. monocytogenes***: *Listeria monocytogenes*, **(-)**: no inhibition [Only the diameter of disc (6mm)].

The antibacterial activity of *A. hierochuntica* is attributed not only to the phenolic compounds grouped into several classes; phenols, phenolic acids, flavonoids, anthocyanins, tannins, coumarins and quinones (Arimboor *et al*., 2008), which are exploited in phytotherapy, having a vasculo-protectrices, antispasmodic and antioxidants properties (Shon *et al*., 2004; Macheix *et al.*, 2005), but also to the alkaloids by their different physiological effects, which these compounds are mainly represented by tropanic, imidazole and indolic in the *Brassicaceae* family (Berghioua *et al*., 2009). These are substances that possess various activities: anticancer (Charpentier *et al.*, 2008), local anesthetic and antimicrobial activity (Waller and Novacki, 1978).

The flavonoids have a very large and diverse antibacterial activity. Indeed, they attack a lot of bacteria with different intensity depending on the microorganism and the ecosystem in which it is located; the flavonoids are able to inhibit the growth of various types of Gram positive and Gram negative bacteria namely *S. aureus*, *E. coli*, *P. mirabilis* and *Enterococcus faecalis*. Also, terpenoids have strong activity against *S. aureus*, and also low activity against Gram negative bacteria (Bouhadjera *et al.*, 2005). It should also be noted that methanol can extract bioactive compounds; anthocyanins, terpenoids, saponins, tannins, xanthoxyllines, flavones, polyphenols) more than other solvents used (Cowan, 1999). It argues that the inhibitory effect of methanolic macerate observed as well as in the work of Mohamed *et al.*, (2010) on the same species.

Conclusion

The bioactive phytochemicals detected and the antibacterial activities of *A.hierochuntica* L., against toxigenic bacterial strains responsible for food born infection support their medicinal properties which are used in traditional medicine. The modest results of the antibacterial activity of methanolic and aqueous macerates of the seeds reflects the need for further investigation regarding fractionation and purification of bioactive compounds, as well as the mode of action in microbial cells.

Acknowledgments

My heartfelt thanks are addressed to *D. Razni* PhD Student at Tahri Mohammed University of Bechar for her contribution to the writing and the discussion of the manuscript, also to *S. Moghtet* for the collection of the plant '*A. hierochuntica*' and finally for the pedagogical laboratory of biology and chemistry team members at Tahri Mohammed University of *Bechar*-Algeria.

Conflict of interest

The authors have declared no conflict of interest

References

Al-Fatimi, M., Wurster, M., Schröder, G., and Lindequist, U. (2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol, 111(3): 657-666.

Arimboor, R., Kumar, KS., and Arumughan, C. (2008). Simultaneous estimation of phenolic acids in sea buckthorn (*Hippophae rhamnoides*) using RP-HPLC with DAD. J Pharm Biomed Anal, 47(1): 31-38.

Bellakhdar, J. (1997). Medecine arabe ancienne et savoir populaire, Ibrispress : 213-224.

Berghioua, A., Cheriti, A., Belboukhari, N., and Marouf, A. (2009). Aperçu Ethnopharmacologique et chimique des *Brassicaceaes*. U Bechar Ann, 5 : 13-25.

Bouhadjera, K., Kebir, T., Baba-Ahmed, A., and Bendahou, M. (2005). Anti-microbial activity of the sterols and steroids extracted from the Algerian *Oudneya Africana* R.Br. Pak J Biol Sci, 8(6): 834-838. http://dx.doi.org/10.3923/pjbs.2005.834.838.

Bruneton, J. (1999). Pharmacognosie, phytochimie, plantes médicinales. Techniques et Documentation. 3ème Ed. Paris : Lavoisier, p. 199-204.

Charpentier, MJE., Van Horn, RC., Altmann, J., and Al-berts, SC. (2008). Paternal effects on offspring fitness in a multimale primate society. Proc Natl Acad Sci, 105: 1988-1992.

Celiktas, OY., Hames Kocabas, EE., Bedir, E., Verdar Sucan, O., and Baser, KHC. (2007). Antimicrobial activities of methanolic extract and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chem, 100: 553-559.

Cowan, MM. (1999). Plant product as antimicrobial agents. Clin Microbiol Rev, 12(4): 564-582.

Benyagoub, E., Nabbou, N., Sirat, M., and Dahlis, Z. (2014). Propriétés antibactériennes et constituants phytochimiques des extraits de la lavande de la région de Tlemcen et leur effet sur quelques espèces bactériennes responsables d'infection alimentaire. Rev Bioressources, 4(2):18-28.

Daur, I. (2012). Chemical properties of the medicinal herb Kaff Maryam (*Anastatica hierochuntica* L.) and its relation to folk medicine use. Afr J Microbiol Res, 6: 5048-5051.

Emad, MA. (2014). Antimicrobial properties and phytochemical constituents of the methanol extracts of *Euphorbia retusa* Forssk., and *Euphorbia terracina* L. from Saudi Arabia. South Asian J Exp Biol, 4(2): 48-53.

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., and Abdelly, C. (2008). Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. C R Biol, 331(5):372-9. http://dx.doi.org/10.1016/j.crvi.2008.02.008.

Gaignaut, JC., Bitdet, D., Perronet, J., and Gaillard, M. (1989). "Stérols et stéroïdes", Partie I, Paris, pp : 11-31.

Hayouni, EA., Abedrabba, M., Bouix, M., and Hamdi, M. (2007). The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem, 105, 267–273.

Hegazy, A., and Kabiel, H. (2007). Significance of microhabitat heterogeneity in the spatial pattern and size-class structure of *Anastatica hierochuntica* L. Acta Oecol, 31: 332-342.

Koné, WM., Atindehou, KK., Terreaux, C., Hostettmann, K., Traoré, D., and Dosso, M. (2004). Traditional medicine in north Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. J Ethnopharmacol, 93(1): 43-49.

Lis-Balchin, M., and Hart, S. (1999). Studies on the mode of action of the essential oil of Lavender (*Lavandula angustifolia*). Phytotherapy Res, 13: 540-542.

Macheix, JJ., Fleuriet, A., and Jay-Allemand, C. (2005). Les composés phénoliques des végétaux: Un exemple de métabolites secondaires d'importance économique. Presses polytechniques et universitaires romandes. Collection Biologie, 192 p.

Majhenic, L., Skerget, M., and Knez, Z. (2007). Antioxidant and antimicrobial activity of Guarana seed extracts. Food Chem, 104: 1258-1268.

Marzouk, Z., Neffati, A., Marzouk, B., Charaif, I., Khemisse, F., Chekirchedira, L., and Boukef, K. (2006). Chemical composition and antibacterial and antimutagenic activity of Tunisian *Rosmarinus officinalis* L. oil from Kasrine. J Food Agric Environ, 4(3-4): 61-65.

Mohamed, AA., Khalil, AA., and El-Beltagi, HES. (2010). Antioxidant and antimicrobial properties of kaffmaryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). Grasas Aceites, *61(1): 67-75*. http://dx.doi.org/10.3989/gya.064509.

NCCLS. (2002). Performance standards for antimicrobial disk susceptibility tests. Approved standard M02-A7. National Committee for Clinical Laboratory Standards, Villanova. (Etats-Unis) PA. USA.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Mansredini, S., Radice, M., and Irimi, R. (2005). Comparative evaluation of 11 essentials oils of different origin as functional antioxydants, antiradicals and antimicrobial in food. Food Chem, 91: 621-632.

Shan, B., Cai, YZ., Brooks, JD., and Corke, H. (2007). The *In vitro* antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol, 117(10):112-119.

Shon, MY., Choi, SD., Kohng, GG., Nam, SH., and Sung, NJ., (2004). Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red Onion. Food Chem Toxicol, 42:659-666.

Soussy, JC. Communiqué (2005). Comité de l'antibiogramme de la Société Française de Microbiologie.

Svoboda, KP., and Hampson, JB. (1999). Bioactivity of essential oils of selected temperate aromatic plants: antibacterial, antioxidant, anti-inflammatory and other related pharmacological activities. Plant Biology Department, SAC Auchincruive, Ayr, Scotland, UK., KA6 5HW.

Turkmen, N., Velioglu, YS., Sari, F., and Polat, G. (2007). Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. Molecules, *12(3)*:484-496. http://dx.doi.org/10.3390/12030484

Waller, GR., and Novacki, EK. (1978). Alkaloid biology and metabolism in plants. Plenum Press, New York.

Warwick, SI. (1993). Guide to wild germplasm of *Brassica* and Allied crops. Part IV. Wild species in the trible *Brassicaceae* (Cruciferae) as sources of agronomic traits. Centre for Land and Biological Ressources Research, Research Branch, Agriculture Canada, pp: 1-19.

World Health Organization. (2015). WHO's first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. News release (media center), Geneva, Switzerland.