Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 9 (5): 234-240, 2015
ISSN 1819-3455 / DOI: 10.3923/rjmp.2015.234.240

Evaluation of Antibacterial Activity of *Artemisia vulgaris* Extracts

A. Addo-Mensah, G. Garcia, I.A. Maldonado, E. Anaya, G. Cadena and L.G. Lee
Department of Biology and Chemistry, Texas A and M International University, Laredo, Texas, 78041, USA

Corresponding Author: Alfred Addo-Mensah, Department of Biology and Chemistry, Texas A and M International University, Laredo, Texas, 78041, USA

ABSTRACT

Plants have been and still are the main source of a variety of drugs. Due to this trend, there is a need to investigate plants and determine if they possess any medicinal property. In this study, medicinal compounds in the stem, root and shoot of *Artemisia vulgaris* were extracted sequentially via Soxhlet extract using petroleum ether, acetone and 90% ethanol on water respectively. Antimicrobial activity of these extracts was tested against *Staphylococcus aureus* (SA), Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* (BS) using the disc diffusion method. Whereas acetone extract exhibited high antimicrobial activity (5 mg mL^{-1} against *B. subtilis,* 25 mg mL^{-1} against MRSA and 5 mg mL^{-1} against *S. aureus*), the ethanol extract showed very moderate activity (25 mg mL^{-1} against *B. subtilis* 50 mg mL^{-1} against *S. aureus* and no activity against MRSA*).* Diminished or lack of antibacterial activity from the ethanol extract reported in this study conflicts previous studies which report higher antimicrobial activity in ethanolic extracts. Findings in this study therefore suggest that the active antimicrobial agents in *A. vulgaris* can be extracted better acetone than with ethanol.

Key words: Antimicrobial, *Artemisia vulgaris*, *Staphylococcus aureus* and *Bacillus subtilis*

INTRODUCTION

Natural products are compounds found in nature whose consumption has physiological effects in organisms. Many natural products' properties are exploited in the treatment of some illnesses, for pain alleviation and as supplements to stimulate good health, so they have been used in traditional medicine and home remedies since the times of ancient civilizations, dating to the Middle Paleolithic era (Fabricant and Farnsworth, 2001; Cowan, 1999).

Today, there is much interest in studying natural products and their derivatives in search of options for disease treatment and other medical applications. Natural products are especially notorious as anticancer and anti-infective agents. Over 60% of approved and proposed drug candidates are natural products or derived from them (Zhang and Demain, 2007; Cowan, 1999). Interest in research about natural products in drug applications is fueled by the subsequent reasons. For instance, natural products already contain components that have the desirable effects of a commercial drug, sometimes with limitations. Therefore, economical savings could result from eliminating unnecessary and extensive chemical modification of natural products in order to create commercial drugs (Zhang and Demain, 2007). In other cases, simpler analogs can be synthesized based on the pharmacophore of the active compounds. Additionally, some of these products are found in abundance, so finding their exploitable properties for medicinal purposes is desirable. This knowledge affects traditional or folk medicine, to which people with very limited access to drugs

and health care often resort to. Consequently, isolating the active compounds helps regulate the consumed dosage and prevent some side effects that may be caused by other compounds found in the natural source (Cordell and Colvard, 2012).

The species found in the *Artemisia* genus in the Asteraceae family vary in both characteristics and the type of climate in which they prosper, but some species share traits in common (Hawke, 2003; Navarro *et al*., 1996). Several plants of this genus have been used for their diverse anti-inflammatory, anticancer, antiviral, antifungal and antibacterial properties (Mucciarelli and Maffei, 2002; Einollah *et al*., 2012). Their pharmacological activity and potential applications make the *Artemisia* genus a current subject of interest (Abad *et al*., 2012; Kordali *et al*., 2005). *Artemisia dranculus* is employed as a digestion-aiding drink for hiccups, nausea, gout, toothaches and is also an antibacterial agent (Deans and Simpson, 2003). The oil of *A. fragrans* has appreciable antibacterial activity in the presence of Gram-positive bacteria (Einollah *et al*., 2012). *Artemisia herba-alba*'s properties exhibit anti-parasitic and antibacterial behavior that foments its use for parasitic infections (Proksch, 2003). *Artemisia santonicum* and *A. spicigera* have shown antibacterial effects on several bacteria, including *Staphylococcus aureus* (Kordali *et al*., 2005). *Artemisia annua* is well-known for its application in the treatment of malaria, a disease of parasitic sources and its essential oil shows strong antifungal and antibacterial activity (Ramezani *et al*., 2005). Some of the observed features in the *Artemisia* genus are linked with the secondary metabolites its species produce, including sesquiterpene lactones (Abad *et al*., 2012). They possess various pharmacological properties of antimicrobial, anti-inflammatory, antiviral, antibacterial and antifungal nature (Chen *et al*., 1989). *Artemisia vulgaris* is among the species that synthesizes sesquiterpene lactones, called vulgarin (Correa-Ferreira *et al*., 2014).

Artemisia vulgaris, commonly called western mugwort, moxa and estafiate, is popular in infusion drinks in Mexican culture, employed traditionally to treat indigestion, asthma, sprains and wounds, as well as an expectorant, decongestant, anthelmintic and emmenagogue (Valsaraj *et al*., 1997; Correa-Ferreira *et al*., 2014). Its effectiveness for treating asthma and hyperactive guts has been attributed to the presence of a competitive histamine receptor antagonist on the smooth muscle in ileum and trachea. The observed polysaccharides in infusion extracts from aerial parts of *A. vulgaris* are inulin-type fructans, which have prebiotic properties and improve resistance against intestinal pathogens (Correa-Ferreira *et al*., 2014). There are precautions with the dosage. It is recommended to ingest 2-3 cups of decoctions *A. vulgaris* since it increases the blood flow in the pelvic region, this can lead to uterine contractions that can proceed to miscarriage. When administered in large quantities however, *A. vulgaris* is toxic and can cause epileptic episodes and bleeding problems because it also acts as an anticoagulant (Natividad *et al*., 2011). Prior antibacterial studies on *A. vulgaris* have revealed the potential of this plant. Chen *et al*. (1989) reported Minimum Inhibitory Concentration (MIC) of aqueous leaf extract of the plant as 7.8 mg mL^{-1}. Sequential extraction of the plant using a range of solvents from non-polar to polar has not been reported. This study reports the antimicrobial activity of extracts prepared by sequential Soxhlet extraction using petroleum ether, acetone and 10% water in ethanol.

MATERIALS AND METHODS

Sample collection and identification: Complete organisms of *Artemisia vulgaris*, collected and donated from a private garden in southern Laredo, Texas, were taken to Mr. Willis E. Gentry, a United States Department of Agriculture Identifier, to confirm the correct species was gathered. The study was conducted from February 2014-May 2014 as part of an undergraduate Medicinal Chemistry class at Texas A and M International University, Laredo, Texas.

Table 1: Summary of procedure

Day	Procedure
1	Plant samples were defatted with 300 mL of petroleum ether at 60-80°C
2	Petroleum ether extract was collected
	Second solvent, 200 mL of acetone, was added to the extraction at the same temperature range
3	Acetone extract was collected
	Third solvent mixture, 180 mL of ethanol and 20 mL of water, was added to the extraction at the same temperature range
4	Ethanol/water extract was collected

Extraction process: The extraction method from Ynalvez *et al*. (2012) was adopted with few modifications. The plants were sundried for five days over aluminum foil and then introduced in small pieces into a laboratory oven at 60°C for a day. Once dried, the leaves, stems and roots were partially crushed utilizing a mortar and pestle. The pieces were enveloped in cheesecloth (in place of a thimble) and introduced into a Soxhlet apparatus. The solvents chosen were the following: petroleum ether, acetone and 90% ethanol in water. Each solvent was used for continuous extraction until the solvent in the arm of the extractor was clear (Table 1).

The solvents of the acetone and ethanol/water extracts (petroleum ether extract was discarded) were evaporated using a Heidolph rotary evaporator at a temperature no higher than 60°C. A Branston sonicator was employed to remove the residues from the flasks by adding about 35 mL of distilled water. The remaining liquid from the evaporation procedure, were displaced to two 50 mL VWR centrifuge tubes and frozen in a -80°C freezer. Then the tubes were uncapped and covered with kimwipes secured with rubber bands to then be lyophilized in a LABCONCO FreeZone 2.5 freeze dryer until all the water was removed and each tube had powder contents. Concentrations of 5, 10, 25, 50 and 100 mg mL^{-1} were prepared for each organic sample.

Microorganism used: Antimicrobial studies were carried out using gram positive bacteria *Staphylococcus aureus* (Presque Isle Cultures 4651), Methicillin Resistant *Staphylococcus aureus* (Presque Isle Cultures 4656) and *Bacillus subtilis* (Presque Isle Cultures 420). These organisms were chosen based on their availability.

Inoculation: Antimicrobial analysis was carried out using cultured Methicillin Resistant *Staphylococcus aureus*, *Staphylococcus aureus* and *Bacillus subtilis*. One colony was transferred to a test tube containing 2 mL of LB broth; the process was repeated eight more times in addition to two negative controls. The tubes were left in a water bath shaker set to 110 rpm for 16-18 h at 37°C. Turbidity of the broth was adjusted to the value of absorbance 0.132±0.005 at 625 nm on a spectrophotometer (Bausch and Lomb Spectronic 20). Hundrad microliter of the newly adjusted inoculum was added to 10 previously prepared Mueller-Hinton agar plates, distributing the inoculum evenly utilizing sterile L-shaped rods.

Biological assay: The disc diffusion method was employed to determine antibacterial inhibition of the plant extracts (Bonev *et al*., 2008; Ynalvez *et al*., 2012). Each 6 mm filter paper disc was infused with 20 µL of each plant extract (ethanol and acetone) and extract concentrations along with DMSO (negative control) and ready bought penicillin discs (positive control). After incubation for 18-20 h at 38°C, the zones of inhibition were measured in mm. The tests were repeated five times and were set up in sextuplicate.

RESULTS

Preliminary screening of the antimicrobial properties for the ethanol and the acetone extracts of *A. vulgaris* were tested using DMSO as negative control and Penicillin as positive controls. The negative control showed no inhibition, which indicates that any inhibition observed in the bioassay can be attributed to the penicillin (positive control) or the antibacterial properties exhibited by active component of the extracts. Antimicrobial activity of petroleum extract was not done because the sample size was not enough for analysis. Result of the bioassays is presented in the Fig. 1(a-b).

Acetone extract

***Bacillus subtilis*:** *Artemisia vulgaris* extracts prepared from acetone showed most activity against *B. subtilis*. Result of antimicrobial activity of acetone extract is represented in the graph below. The result shows acetone extract had a positive correlation ($R^2 = 0.998$) against *B. subtilis* as evidenced by an increase in the Zone Of Inhibition (ZOI) as the concentration of acetone extract was increased (Fig. 2). The acetone extracts had well-defined zones of inhibition.

It took 5 mg mL^{-1} of acetone extract just like *B. subtilis* to inhibit the growth of *S. aureus*. The inhibition zones however, were not as large when compared to inhibition zones of *B. subtilis* and it had a much weaker correlation coefficient ($R^2 = 0.840$).

Methicillin resistant *Staphylococcus aureus*: The activity of *A. vulgaris* acetone extract against MRSA was observed at 25 mg mL^{-1}, whereas the activity of the ethanol extract was not observed in any of the concentrations.

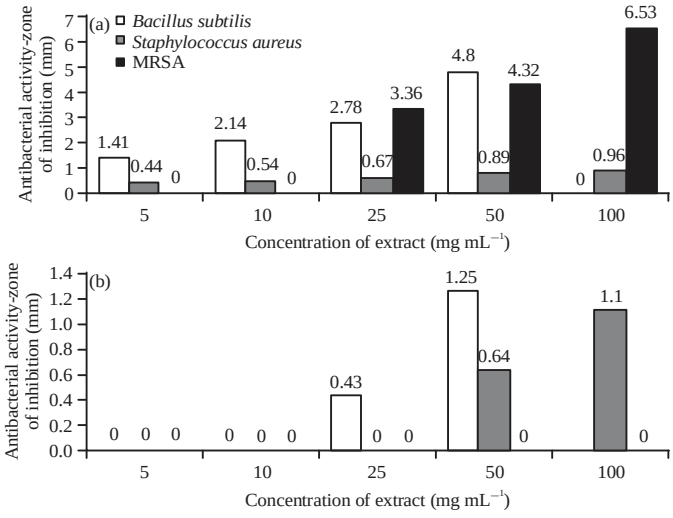

Fig. 1(a-b): Antibacterial activities of *Artemisia vulgaris* extracts against *Bacillus subtilis*, *Staphylococcus aureus* and MRSA, (a) AcBS: Acetone extracts against *Bacillus subtilis*, AcSa: Acetone extracts against *Staphylococcus aureus*, AcMRSA: Acetone extracts against MRSA and (b) EtBs: Ethanol extracts against *Bacillus subtilis*, EtSa: Ethanol extracts against *Staphylococcus aureus* and EtMRSA: Ethanol extracts against MRSA

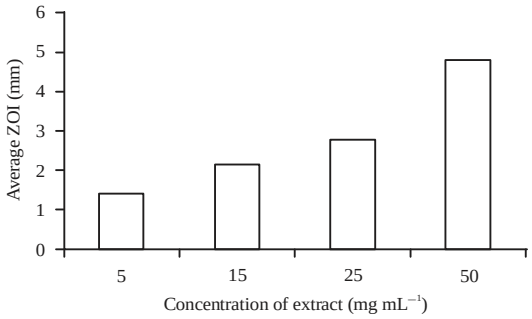

Fig. 2: Antibacterial activities of *Artemisia vulgaris* acetone extract against *Bacillus subtilis* and *Staphylococcus aureus*

Ethanol extract: Unlike acetone extract, the ethanol extract was not very active against all strains tested. Antimicrobial inhibition was observed at 25 mg L^{-1} against *B. subtilis* and 50 mg mL^{-1} against *S. aureus* and no inhibition against MRSA.

DISCUSSION

The results of both bioassays indicate that the acetone extracts have a greater activity against both strains tested bacteria than ethanol extract. Various reasons can explain why such behavior occurred between the two extracts. Sequential extraction with solvents of increasing polarity allows different solvents to extract different compounds in the plant material. In this case, the active component was extracted more efficiently using acetone than ethanol, thus the zones of inhibition of acetone were more well-defined because there is a higher concentration of the active component in the acetone extract. This indicates that acetone is able to extract more compounds that are active antibacterials than the ethanol extract. Previous studies on acetone extracts of plants revealed greater antibacterial activity of less polar extracts compared to more polar solvents (Doughari and Okafor, 2008; Eloff *et al*., 2008). This can explain the higher activity observed in this current study. Furthermore, different parts of the plant contain different active components (Cowan, 1999). In this study, the leaves stem and roots of the *A. vulgaris* plant were all crushed together and placed in the Soxhlet apparatus. This can result in either enhanced antimicrobial activity (as a result of active compounds from other parts) or reduced antimicrobial activity (as a result of dilution effects from other compounds from other parts). The slightly higher antimicrobial activity against *S. aureus* in this study (5 mg mL^{-1}) compared to previous studies (6.25, 10 mg mL^{-1}) can be inferred that other parts of *A. vulgaris* contain compounds that exhibit antibacterial activity.

Valsaraj *et al*. (1997) reported ethanolic extract of leaf of *A. vulgaris* having antibacterial activity against *Staphylococcus aureus* of 6.25 mg mL^{-1} which was quite different from what was reported in this current study. This current study involved whole plant as opposed to leaf. Extra compounds extracted from the other parts of the plant (stem and root) enhanced the concentration of the active components and hence increased the potency of the extract. When the antibacterial activity of the ethanolic extract in this study against *S. aureus* (25 mg mL^{-1}) is compared to that reported in literature (~10 mg mL^{-1}), it can be inferred that the active antibacterial component was extracted during the extraction with acetone, hence the observed great antibacterial activity with acetone extract (Chen *et al*., 1989).

CONCLUSION

Overall, the suspected antibacterial activity of *A. vulgaris* was clearly determined to be attributed to secondary compounds in the acetone extract. The MIC for acetone extract against *B. subtilis* was 5 mg mL^{-1} and against *S. aureus* was 5 mg mL^{-1}. While the ethanol extract was 25 mg mL^{-1} against *B. subtilis*, 50 mg mL^{-1} against *S. aureus* and no activity against MRSA. This justifies the use of *A. vulgaris* in traditional medicine for microbial infections. Because the plant's extracts were not purified before the bioassay, it is not known which compound(s) in the plant and in which part of it, exhibit the shown antibacterial properties. As such, purification of the acetone and ethanol extracts may give better results and thus, offer better insight to the behavior of the active compound(s) of *A. vulgaris*. Further research may give the lead compound which can be modified later to optimize its activity against bacterial infections.

ACKNOWLEDGMENTS

The authors express their appreciation to Kassandra Compean, Elester Williams, Idalia Lim and Dr. Ruby Ynalvez for helping them with the bioassays. The Labconco freeze-dry system was acquired through NSF-MRI grant award (NSF DBI 0959395) to Ruby Ynalvez.

REFERENCES

Abad, M.J., L.M. Bedoya, L. Apaza and P. Bermejo, 2012. The *Artemisia* L. Genus: A Review of Bioactive Sesquiterpene Lactones. In: Studies in Natural Products Chemistry, Atta-Ur-Rahman (Ed.). Vol. 7, Elsevier, London, UK., ISBN-13: 9780444595157, pp: 43-65.

Bonev, B., J. Hooper and J. Parisot, 2008. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J. Antimicrob. Chemother., 61: 1295-1301.

Chen, C.P., C.C. Lin and N. Tsuneo, 1989. Screening of Taiwanese crude drugs for antibacterial activity against *Streptococcus mutans*. J. Ethnopharmacol., 27: 285-295.

Cordell, G.A. and M.D. Colvard, 2012. Natural products and traditional medicine: Turning on a paradigm. J. Nat. Prod., 75: 514-525.

Correa-Ferreira, M.L., G.R. Noleto and C.L.O. Petkowicz, 2014. *Artemisia absinthium* and *Artemisia vulgaris*: A comparative study of infusion polysaccharides. Carbohydr. Polym., 102: 738-745.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Deans, S.G. and E.J.M. Simpson, 2003. Artemisia Dranculus. In: Medicinal and Aromatic Plants-Industrial Profiles, Wright, C.W. (Ed.)., Vol. 18, Taylor and Francis Inc., London, UK., pp: 75-80.

Doughari, J.H. and N.B. Okafor, 2008. Antibacterial activity of *Senna siamae* leaf extracts on *Salmonella typhi*. Afr. J. Microbiol. Res., 2: 42-46.

Einollah, V., J. Behboud, D.S. Jalil and N.B. Vahid, 2012. Evaluating antibacterial activity from essential oil of *Artemisia fragrans* Willd. in North-Western of Iran. Afr. J. Microbiol. Res., 6: 834-837.

Eloff, J.N., D.R. Katerere and L.J. McGaw, 2008. The biological activity and chemistry of the southern African Combretaceae. J. Ethnopharmacol., 119: 686-699.

Fabricant, D.S. and N.R. Farnsworth, 2001. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect., 109: 69-75.

Hawke, R., 2003. Plant evaluation notes: Garden-worthy Artemisia. Chicago Botanic Garden. https://www.chicagobotanic.org/downloads/planteval_notes/no19_artemisias.pdf.

Kordali, S., A. Cakir, A. Mavi, H. Kilic and A. Yildirim, 2005. Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish Artemisia species. J. Agric. Food Chem., 53: 1408-1416.

Mucciarelli, M. and M. Maffei, 2002. Introduction to the Genus. In: Artemisia (Medicinal and Aromatic Plants: Industrial Profiles), Wright, C.W. (Ed.). Taylor and Francis, London, UK., ISBN-13: 978-0415272124, pp: 1-42.

Natividad, G.M., K.J. Broadley, B. Kariuki, E.J. Kidd, W.R. Ford and C. Simons, 2011. Actions of *Artemisia vulgaris* extracts and isolated sesquiterpene lactones against receptors mediating contraction of guinea pig ileum and trachea. J. Ethnopharmacol., 137: 808-816.

Navarro, V., M.L. Villarreal, G. Rojas and X. Lozoya, 1996. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. J. Ethnopharmacol., 53: 143-147.

Proksch, P., 2003. Artemisia Herba-Alba. In: Medicinal and Aromatic Plants-Industrial Profiles, Wright, C.W. (Ed.)., Vol. 18, Taylor and Francis Inc., London, UK., pp: 81-86.

Ramezani, M., J. Behravan and A. Yazdinezhad, 2005. Chemical composition and antimicrobial activity of the volatile oil of *Artemisia khorassanica* from Iran. Pharmaceut. Biol., 42: 599-602.

Valsaraj, R., P. Pushpangadan, U.W. Smitt, A. Adsersen and U. Nyman, 1997. Antimicrobial screening of selected medicinal plants from India. J. Ethnopharmacol., 58: 75-83.

Ynalvez, R.A., C. Cardenas, J.K. Addo, G.E. Adukpo, B.A. Dadson and A. Addo-Mensah, 2012. Evaluation of the antimicrobial activity of *Zanthoxylum zanthoxyloides* root bark extracts. Res. J. Med. Plant, 6: 149-159.

Zhang, L. and A.L. Demain, 2007. Natural Products: Drug Discovery and Therapeutic Medicine. Springer, New York, USA., ISBN-13: 9781592599769, Pages: 386.