academicJournals

Vol. 9(2), pp. 130-134, 14 January, 2015
DOI: 10.5897/AJMR2014.7223
Article Number: EBB4C3650145
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

*Full Length Research Paper*

# Antimicrobial activity of extracts from an endemic *Salvia cilicica* Boiss. and Kotschy

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Received 28 October, 2014; Accepted 22 December, 2014

**The antimicrobial activities of the different aerial and root extracts of *Salvia cilicica* were determined aiming to evaluate whether, it can be used in phytotherapy as an antimicrobial agent. In this study, the antimicrobial activity of roots and aerial parts of *S. cilicica* extracts was evaluated using micro dilution and disc diffusion methods against Gram positive and Gram negative reference standard microorganisms and yeast *Candida albicans*. All of the extracts, with the exception of ethanol extract, showed antimicrobial activity by using minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) tests. The roots-petroleum ether and acetone extracts showed the highest antimicrobial activity (MIC's ranges 20-313 µg/ml) against Gram positive bacteria. The root-acetone extract showed higher antimicrobial activity against Gram negative bacteria in comparison to other extracts. The most remarkable result of the antimicrobial activities is that, except for the ethanol extract, the *S. cilicica* had a good inhibitory effect (MIC's ranges 20-313 µg/ml) against *Bacillus cereus* and *Salmonella cholerasuis*, and was noticed to be more active in paper disc diffusion test against Gram positive than Gram negative bacteria. The roots petroleum ether and acetone extracts exhibited activity against *C. albicans*. In conclusion, *S. cilicica* had a potential therapeutic value supporting its traditional usage in folk medicine.**

**Key words:** *Salvia cilicica*, Gram positive, Gram negative, yeast, antimicrobial, endemic.

## INTRODUCTION

The genus *Salvia* L. (Lamiaceae) comprises about 900 species world-wide, while it is presented with 89 species and 93 taxons in Turkey, approximately half of which is endemic. The genus has been distributed extensively in 3 regions of the world: 500 species in Central and South America, 200 species in western Asia, and 100 species in eastern Asia (Davis, 1988a; 1988b; Walker and Sytsma, 2007). Anatolia is the major gene center in Asia. *Salvia* species are used in folk medicine for the treatment of a variety of diseases, including infectious diseases. The species of *Salvia*, known as ''adacayi'' in Anatolia, are used as antiseptics, stimulants, diuretics and for wound healing in Turkish folk medicine and for herbal teas (Baytop, 1999; Demirci et al., 2003; Tepe et al., 2005).

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Since the antimicrobial and antioxidant activities of these species, especially *Salvia officinalis*, were determined, similar studies on these species increased gradually all over the world. These studies suggest that the hydroxyl-cinnamic acid analogs, flavonoids and diterpenoids contribute to the biological activities of the *Salvia* species (Deans and Simpson, 2000). The essential oil and various extracts of *Salvia tomentosa* Miller showed a moderate activity against *Staphylococcus aureus (S. aureus), Streptoccocus pneumoniae, Moraxella catarrhalis, Bacillus cereus (B. cereus), Acinetobacter lwoffii, Clostridium perfringens, Mycobacterium smegmatis* and *Candida albicans* (*C. albicans*) with water insoluble extracts (Tepe et al., 2005).

In a previous study, the extracts of the aerial parts of 16 *Salvia* species were tested against five isolates (*B. cereus, S. aureus*, *K. pneumoniae, E. coli)* and *M. tuberculosis)*. The extracts of the majority of species exhibited moderate to good antibacterial activity with minimum inhibitory concentration (MIC) values ranging from 0.03 to 8.00 mg/ml, while the highest activity elicited was that against *M. tuberculosis* ( MIC<0.50 mg/ml) with *S. radula, S. verbenaca* and *S. dolomitica* (Kamatou et al., 2007).

Askun et al. (2009) tested the antibacterial activity of *Salvia fruticosa* Mill., *Salvia tomentosa* Mill., *Sideritis albiflora* Hub.-Mor. (endemic), *Sideritis leptoclada* O. Schwarz & P.H. Davis, (endemic), and *Origanum onites* L. against *Staphylococcus aureus*, *Staphylococcus epidermidis (S. epidermidis)*, *Enterococcus faecalis (E. faecalis)*, *B. cereus*, *E. coli*, *Salmonella typhimurium (S. typhimurium)*, *Enterobacter aerogenes (E. aerogenes)*, and *K. pneumoniae*. The best antibacterial activity (MIC 640 µg/ml) was that shown against *S. typhimurium* and *E. aerogenes* by *S. fruticosa, E. coli,* and *S. typhimurium, E. aerogenes* by *S. tomentosa; S. typhimurium,* and *E. aerogenes* by *S. leptoclada* and *S. typhimurium, E. aerogenes* and *S. epidermidis* by *O. onites,* respectively.

In another study on the essentials oil, ethyl acetate and ether extracts of *S. urmiensis* Bunge, a high antimicrobial activity was observed with the ethyl acetate extract against *B. subtilis, C. albicans* and with the ether extract against *K. pneumoniae and Saccharomyces cerevisiae* (Farjam, 2012).

*Salvia cilicica* Boiss. and Kotschy (SC), an endemic species, has only a limited number of studies in the literature regarding its chemical composition and biological activity. In our previous study, we have presented the antileishmanial, antioxidant and cytotoxic activities. We also dealt with the isolation and structure elucidation of the terpenoid compounds from the root extracts of SC, which is utilized in traditional medicine (Tan et al., 2002).

Antibiotic resistance is a long-evolved trait in prokaryotes. In Europe, 25, 000 people die every year from drug-resistant infections. In 2009, there were 440, 000 new cases of multidrug resistant (MDR) tuberculosis in 69 countries. These figures, and rising resistance levels observed in the global surveillance programmes, show that antibiotic resistance has reached a critical point, as human and economic cost escalate. Antibiotic resistance is an emerging global healthcare threat and today's armory of antibiotics is limited. For some pathogens, the choice of available drugs is now greatly reduced. Several factors such as (i) increasing mortality from infections caused by resistant strains, (ii) the strong link between resistant pathogens and increasing levels of hospital-acquired infections, and (iii) the escalating healthcare costs have placed antibiotic resistance at the top of the healthcare agenda (ECDC/EMEA Joint Technical Report, 2009). Therefore, the investigations on antimicrobial activity increased year after year and these studies were focused on discovering new antimicrobial agents, especially from plant sources.

The aim of this study was to determine the antimicrobial activities of the different aerial and root extracts of SC; to compare their activities and to answer whether, SC can be used in phytotherapy as antimicrobial agent.

## MATERIALS AND METHODS

### Plant material and extraction

The aerial parts and the roots of *S. cilicica* Boiss. and Kotschy (SC) were collected from Adana- Pozanti (Turkey), in September 2011 and identified by Assoc. Prof. Dr. Nur Tan (Istanbul). The voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 98085).

The dried and powdered aerial parts (SCA) and the roots (SCR) of SC (1 kg of each species) were extracted in a Soxhlet respectively with petroleum ether (PE), acetone (Ac), ethanol (EtOH). The extracts were concentrated *in vacuo*.

### Biological assays

#### *Microorganisms and media*

The antimicrobial activity of the extracts was evaluated against Gram positive and Gram negative reference standard microorganisms; *S. aureus* ATCC 25923*, S. epidermidis* ATCC 12228*, E. faecalis* ATCC 29212*, P. aeruginosa* ATCC 27853*, E. coli* ATCC 25922*, K. pneumoniae* ATCC 4352*, B. cereus* ATCC 11778*, B. subtilis* ATCC 6633*, P.mirabilis* ATCC 7002*, S. cholerasuis* ATCC 14028) and yeast *C. albicans* ATCC 10231 from the Institute of Microorganisms Culture Collection of Istanbul Medical Faculty/Istanbul/Turkey.

Ciprofloxacin (Bayer Türk Kimya San. Istanbul /Turkey), Amphotericin B (Gilead Sciences Ilac, Istanbul/Turkey), Dimethylsulfoxide (DMSO- Merck) Tryptic soy agar (TSA) from GBL, Istanbul/Turkey, Mueller Hinton broth (MHB), Mueller Hinton Agar (MHA) from TitanBiotek, Istanbul/Turkey and RPMI1640 medium from İnterlab /Sigma, İstanbul/Turkey were purchased.

#### *Antimicrobial assays*

The extracts were dissolved in DMSO and the volume was adjusted

**Table 1.** MIC and MBC (µg/ml) values of the root and aerial parts extracts parts in petroleum ether (PE), acetone (Ac) and ethanol (Et-OH) of *Salvia cilicica* Boiss.and Kotschy.

| *Salvia cilicica* (SC) | SC Root | | | SC Aerial | | | Ciprofloxacin | Amphotericin B |
|---|---|---|---|---|---|---|---|---|
| | Petroleum ether | Acetone | Ethanol | Petroleum ether | Acetone | Ethanol | | |
| | MIC*/MBC** µg/ml | | | | | | | |
| *S. aureus* | 78*/156** | 313 | 1250 | 625/2500 | 625/1250 | 1250/2500 | < 2 | ND*** |
| *S. epidermidis* | 78/156 | 78/156 | 2500 | 625 | 313/625 | 2500 | < 2 | ND |
| *E. faecalis* | 20 | 20/78 | 625/2500 | 78/156 | 156 | 2500 | < 2 | ND |
| *B. subtilis* | 20/40 | 156/313 | 313/1250 | 625/1250 | 313 | 1250/2500 | < 2 | ND |
| *B.cereus* | 40/78 | 78 | 313/625 | 78/156 | 78/156 | 625/1250 | < 2 | ND |
| *S. cholerasuis (typhi murium)* | 156 | 313 | 313 | 313 | 313 | 1250 | < 2 | ND |
| *P. aeruginosa* | 1250/5000 | 156/1250 | 1250 | 625/2500 | 625/2500 | 625/2500 | 9/20 | ND |
| *P. mirabilis* | 1250/5000 | 313/625 | 625/1250 | 1250/2500 | 1250 | 313/625 | 2/4 | ND |
| *K. pneumoniae* | 78 | 20 | 78 | 78/156 | 40 | 40 | < 2 | ND |
| *E. coli* | 313/625 | 625 | 625 | 625 | 625 | 625 | < 2 | ND |
| *C. albicans* | 2500 | 1250 | - | - | - | - | - | 2 |

*MIC = minimum inhibitory concentration; **MBC = minimal bactericidal concentration; ***ND= not done

to 10000 µg/ml. The antimicrobial activity of the extracts was carried out under laminar air flow (Nüve mn 120) by using micro dilution method as described in M7A7/2006 for bacteria and M27A3/2008 for yeast, and disc diffusion method as described in M02 A11/2012 for bacteria and M44A2/2009 for yeast by Clinical and Laboratory Standards Institute (CLSI,Wayne, Pennsylvania USA). Minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined for each extract. Ciprofloxacin (20 µg/ml) and Amphotericin B (20 µg/ml) were used as the positive control, respectively.

### *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

The inoculums were prepared by diluting overnight cultures in saline (approx.$10^8$ CFU/ml for bacteria and $10^7$ CFU/ ml for *C. albicans*). Bacteria and yeast were grown overnight on nutrient agar and on sabouraud dextrose agar (SDA) plates, respectively. The inocula were prepared from overnight grown cultures (bacteria for 24 h 37°C and yeast for 24-48 h 35°C) and the formed turbidity was adjusted to 0.5 McFarland units approximately $10^8$ CFU/ml for bacteria and $10^7$ for yeast.

The MIC and MBC values of extracts were determined on the basis of microbroth dilution method in 96 multi-well microtitre plates. The 50 µl Müller Hinton Broth (MHB) for bacteria and 50 µl RPMI1640 for yeast were added to the wells starting from the second well and continuing up to the twelfth. The crude plant extracts of 10000 µg/ml were added to the first and second wells. Two-fold serial dilutions were made achieving a final concentration ranging from 5000-9.76 µg/ml. One row of positive controls for Ciprofloxacin (final concentrations from 512 to 1 µg/ml) and for Amphotericin B (final concentrations from 250 to 0.5 µg/ml) were added to each plate. In addition, an extra row of DMSO was used as a vehicle control to determine it's possible inhibitory activity.

After incubation (bacteria at 37°C for 24 h and yeast at 35°C for 24-48 h) the microtitre plates were examined visually for microbial growth which appeared as visible turbidity. In each row, the well containing the least concentration and showed no visible growth was considered the MIC. MBC and MFC were determined by taking samples from the wells which showed no visible growth. The bacterial samples were inoculated on TSA plates and incubated at 37°C for 24 h while the yeast sample was inoculated on Sabouraud plates and incubated at 35°C for 24-48 h.

### *Disc diffusion technique*

Antimicrobial activity was determined using bacterial cultures adjusted to 0.5 McFarland turbidity standard and inoculated onto TSA plates (diameter: 15cm). *C. albicans* was adjusted to the concentration of $10^6$ CFU/ml. Cultures of *C. albicans* were suspended in sterile solution of 0.9% normal saline and the cultures were inoculated onto sabouroud dextrose agar plates.

Sterile filter paper discs (5 mm diameter for both bacteria and yeast) were impregnated with either 20 µl of extract dilutions, 10 µl ciprofloxacin (each disc containing 5 µg) or 10 µl amphotericin B (each disc containing 10 µg).

Bacterial cultures were then incubated at 37°C for 24 h and yeast at 35°C for 24-42 h. The antimicrobial activity was determined by measuring the inhibition zone on each paper disc.

## RESULTS AND DISCUSSION

The extracts of SCA and SCR showed varying antimicrobial activities against the reference standard bacteria and yeast. The antimicrobial activity was summarized in Table 1 for micro dilution method and in Table 2 for disc diffusion method. The experiments were performed in duplicate and the results were expressed as average values.

In general, all the extracts (except EtOH extracts) showed a significant antimicrobial activity via MIC and MBC tests, but especially SCR-PE and -Ac extracts showed the highest antimicrobial activities against the tested Gram positive bacteria, and SCR-Ac extract had

**Table 2**. Antimicrobial activity (zone of inhibition, mm) of various extracts *Salvia cilicica* Boiss. and Kotschy against reference strains.

| *Salvia cilicica* (SC) | SC Root | | | SC Aerial | | | Ciprofloxacin | Amphotericin B |
|---|---|---|---|---|---|---|---|---|
| | Petroleum ether | Acetone | Ethanol | Petroleum ether | Acetone | Ethanol | | |
| *S. aureus* | 12 ± 0.28 | 9 ± 0.71 | - | - | 7 ± 0.63 | - | 33 ± 0.91 | ND** |
| *S. epidermidis* | 13 ± 0.14 | 11 ± 0.21 | - | - | 8 ± 0.63 | - | 30 ± 0.77 | ND |
| *E. faecalis* | 14 ± 0.71 | 12 ± 0.35 | - | 7 ± 0.49 | 9 ± 0.42 | - | 23 ± 0.98 | ND |
| *B. subtilis* | 15 ± 0.21 | 15 ± 0.28 | - | - | 11± 0.35 | - | 35 ± 0.21 | ND |
| *B. cereus* | 12 ± 0.57 | 11 ± 0.35 | 7 ± 0.56 | 8 ± 0.21 | 8 ± 0.49 | - | 32 ± 0.21 | ND |
| *S. cholerasuis* | 12 ± 0.28 | 10 ± 0.49 | - | 8 ± 0.49 | 9 ± 0.35 | - | 40 ± 0.42 | ND |
| *P. aeruginosa* | - | - | - | - | - | - | 30 ± 0.21 | ND |
| *P. mirabilis* | - | 11 ± 0.56 | - | - | - | - | 34 ± 0.91 | ND |
| *K. pneumoniae* | 10 ± 0.84 | 10 ± 0.63 | - | 7 ± 0.71 | 7 ± 0.49 | 6 ± 0.56 | 32 ± 0.42 | ND |
| *E. coli* | - | 8 ± 0.49 | - | 9 ± 0.98 | 9 ± 0.56 | 6 ± 0.35 | 40 ± 0.28 | ND |
| *C. albicans* | 10 ± 0.21 | 9 ± 0.35 | - | - | - | - | - | 26 ± 0.63 |

*(-) No measurable zone; **ND= Not done.

higher antimicrobial activity against the Gram negative bacteria in comparison to other extracts (Table 1). The SCR-Ac and SCA-Ac extracts showed MIC's ranges of 20-625 µg/ml and MBCs ranges of 20-1250 µg/ml against *S. aureus, S. epidermidis, E. faecalis, B. subtilis, B. cereus, S. cholerasuis* and *K. pneumoniae*. The SCR-PE extract resulted in MIC's ranges of 20-313 µg/ml and MBC's ranges of 20-625 µg/ml against *S. aureus, S. epidermidis, E. faecalis, B. subtilis, B. cereus, S. cholerasuis* and *E. coli*. However, these activities were weak in comparison to the positive control Ciprofloxacin. Furthermore, the SCA-EtOH and SCR-EtOH extracts showed MIC' and MBC's ranges 40-1250 µg/ml against *B. cereus, S. cholerasuis, K. pneumoniae* and *P. mirabilis*.

No antimicrobial activity of all extracts except SCR-Ac was observed against *P. aeruginosa.* The most remarkable result of the antimicrobial activities is that, all extracts of SC (except EtOH extracts) have a good inhibitory effect against *S. cholerasuis* and *B. cereus*. Hence no antifungal activity was observed against *C. albicans.*

The results of disc diffusion method are summarized in Table 2. The SCR-PE and SCR-Ac indicated significant antimicrobial activity against *E. faecalis, B. subtilis, S. aureus, S. epidermidis, S. cholerasuis, K. pneumoniae*, and yeast *C.albicans.* No antimicrobial activity of all extracts was observed against *P. aeruginosa* with the disc diffusion. However, all of the above mentioned activities were weak in comparison to the positive control Ciprofloxacin (23-40 mm zone) and Amphotericin B (26 mm zone). Overall, all extracts are more active in paper disc diffusion test against Gram positive bacteria than Gram negative bacteria. The PE and Ac extracts of SC-R showed the highest antimicrobial activity, followed by SCA-Ac and SCA-PE.

It has been estimated, that plants provide over 100,000 secondary metabolites, small-molecule compounds (Dixon, 2001) and for thousands of years medicinal plants have played a significant role in the treatment of a wide range of medical conditions, including infectious diseases. Some naturally occurring chemical compounds serve as models for a large percentage clinically proven drugs, and many are now being re-assessed as antimicrobial agents.

Haznedaroglu et al. (2001) have tested the antimicrobial activity of the essential oil of *Salvia tomentosa* and showed it remarkably inhibited the growth of tested Gram-positive (*S. aureus, S. epidermidis* and *E. faecalis*) and Gram negative (*E. Coli and E. cloacea*) bacteria except *P. aeruginosa.*

The antibacterial effect of essential oil of *Salvia heldreichiana* was shown against *E. coli, S. lutea* and *S. typhimurium*. In the same study, the oil of *S. cryptantha* inhibited the growth of *S. lutea* (Akin et al., 2010).

Karatas and Ertekin (2010) showed that all essential oils of *Salvia palaestina, Salvia multicaulis, Salvia syriaca* and *Salvia ceratophylla* possessed a good antibacterial activity against *B. subtilis, E. coli* and *S. aureus*, additionally essential oils of *Salvia multicaulis* possessed antibacterial activity against *P. aeruginosa* and essential oils of *Salvia syriaca* against possessed antibacterial activity *P.aeruginosa* and *S. pyogenes*.

Ibrahim et al. (2013) have tested methanol extracts of *Salvia libanotica* against *S.aureus* ATCC25923 standard strain and a clinical isolate of methicillin-resistant *S.aureus* (MRSA) and *S.libanotica* was at 4 mg/ml affective against MRSA.

To the best of our knowledge, the antimicrobial activity of *S. cilicica* has not been previously reported. In our study, generally all of the extracts, except EtOH, showed a significant antimicrobial activity via MIC and MBC tests. SCR-PE and SCR-Ac showed the highest activity against Gram positive bacteria, while the latter SCR-Ac extract

has proven to possess higher antimicrobial activity against Gram negative bacteria. Our results suggest that *S. cilicica* may be useful in the treatment of infectious diseases caused by *S. aureus, S. epidermidis, B. subtilis, B. cereus K. pneumoniae* and *S. cholerasuis*.

## Conclusions

This is the first study to show the antimicrobial activity of SC and has opened up the possibility of the use of this plant in drug development for alternate therapy for the treatment of infections. However, further large-scale trials regarding on more pathogenic organisms, animal tests and toxicological investigations are required to provide more conclusive proof of their antimicrobial activity.

## Conflict of interests

The authors did not declare any conflict of interest.

## ACKNOWLEDGEMENTS

The authors would like to thank Özden Özceviz from Adana for the collection of the plant material. This work was supported by the Research Fund of Istanbul University. Project number: 22239.

### REFERENCES

Akin M, Demirci B, Bagci Y, Baser KHC (2010). Antibacterial activity and composition of the essential oils of two endemic *Salvia* sp. from Turkey. Afr. J. Biotechnol. 9:2322-2327.

Askun T, Tumen G, Satil F, Ates M (2009). Characterization of the phenolic composition and antimicrobial activities of Turkish medicinal plants. Pharm. Biol. 47:563-571.

Baytop T (1999). Turkiye'de Bitkiler Ile Tedavi. *(*2nd ed.). Nobel Tip Kitapevleri Ltd Sti., Istanbul.

Davis PH (ed.) (1988a). Flora of Turkey and the East Agean Islands. Vol VII. Edinburgh University Press, Edinburgh.

Davis PH (ed.) (1988b). Flora of Turkey and the East Agean Islands. Vol X. Edinburgh University Press, Edinburgh.

Deans SG, Simpson EJM (2000). Antioxidants from *Salvia officinalis*. In: Kintzios SE Sage. Harwood Academic Publishers, pp. 185-192.

Demirci B, Baser KHC, Yildiz B, Bahcecioglu Z (2003). Composition of the essential oils of six endemic *Salvia* spp. from Turkey. Flavour Fragr. J. 18:116-121.

Dixon RA (2001). Natural products and plant disease resistance. Nature 411:843-847

ECDC/EMEA Joint Technical Report (2009). The bacterial challenge: Time to react a call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new antibacterial agents. ISBN: 978-92-9193-193-4 doi: 10.2900/2518. http://www.who.int/mediacentre/factsheets/fs194/en/index.html

Farjam MH (2012). Comparative study of the antimicrobial activity of essential oil and two different extract from *Salvia urmiensis* Bunge. Asian Pac. J. Trop. Biomed. 2:1680-1682.

Haznedaroglu MZ, Karabay NU, Zeybek U (2001). Antibacterial activity of *Salvia tomentosa* essential oil. Fitoterapia 72:829-831

Ibrahim A, Aqel AA, Aljamal A (2013). Effect of the methanol extracts of *Salvia libanotica, Rosmarinus officinalis, Capparis spinosa* and *Achillea fragrantissima* against two strains of *Staphylococcus aureus*. Afr. J. Microbiol. Res. 7(29):3750-3753.

Kamatou GPP, van Vuuren SF, van Heerden FR, Seaman T, Viljoen AM (2007). Antibacterial and antimycobacterial activities of South African *Salvia* species and isolated compounds from *S. chamelaeagnea.* S. Afr. J. Bot. 552-557.

Karataş H, Ertekin S (2010). Antimicrobial activities of the essential oils of four *Salvia* species from Turkey. J. Med. Plants Res. 4:1238-1240.

Tan N, Kaloga M, Radtke OA, Kiderlen AF, Oksuz S, Ulubelen A, Kolodziej H (2002). Abietane diterpenoids and triterpenoic acids from *Salvia cilicica* and their antileishmanial activities. Phytochemistry 61: 881-884.

Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M (2005). Antimicrobial and antioxidant activities of the essential oils and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem. 90:333-340.

Walker JB, Sytsma KJ (2007). Staminal Evolution in the Genus *Salvia* (Lamiaceae): Molecular Phylogenetic Evidence for Multiple Origins of the Staminal Lever. Ann. Bot. 100:375-391.