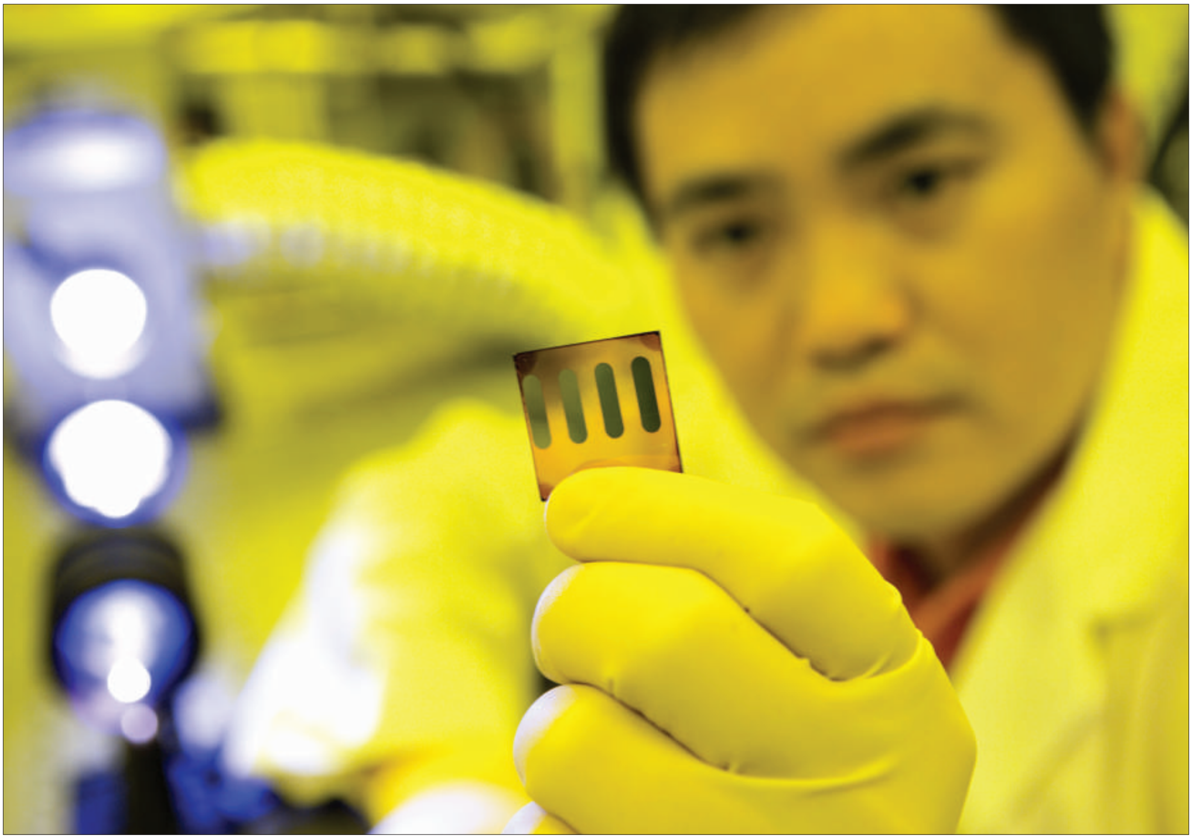

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Research Article
Screening the Antimicrobial Activity of Different *Sepia officinalis* (Cephalopoda: Sepioidea) Parts Collected from Alexandria Mediterranean Waters, Egypt Against Some Human Pathogens

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Abstract
Objective: This study was aimed to evaluate the antimicrobial activity of different parts of *Sepia officinalis* (*S. officinalis*) which was collected from Alexandria Mediterranean waters, Egypt against the most common human pathogens and detection of the most active part and extract. **Materials and Methods:** Different parts from *Sepia officinalis* were screened for antimicrobial activity *in vitro*. Acetone, chloroform, ethanol, methanol and aqueous extracts were tested against some pathogens, four Gram-negative bacteria: *Escherichia coli* (*E. coli), Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*), *Vibrio cholera* (*V. cholera*) and two species of clinically human pathogenic Gram positive: *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) and two fungal strains such as *Aspergillus fumigatus* (*A. fumigatus*) and *Candida albicans* (*C. albicans*). **Results:** Maximum antibacterial activity was noted in methanol extract of ink against *P. aeruginosa* and *S. typhi* (18 and 15 mm, respectively), followed by methanol and chloroform extracts of nidamental gland and ink against *P. aeruginosa* (14 and 13.2 mm, respectively). On the other hand, the minimum antifungal activity was observed against the tested fungi but chloroform and methanol extracts were also the best solvents. No antimicrobial activity was detected in aqueous extracts of different parts. **Conclusion:** The screening result confirms that ink and nidamental gland extracts had a good antimicrobial activity to play a vital role in future for medical applications.

Key words: *Sepia officinalis*, antimicrobial activity, ink, nidamental gland, human pathogens

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sea food is considered a good source of animal protein since it had a high content of polyunsaturated fatty acids. Cephalopods are one of the most important protein food resources[1]. They are classified under phylum Mollusca[2]. Cuttlefishes, Squids and Nautili are the most important representatives of the Cephalopods. This class includes about 1000 known species, which represent about 2.07% from phylum Mollusca[3].

The accessory nidamental glands (ANG) are organs in the reproductive system of females cephalopod taxa (loliginidae, sepiidae and sepiolidae) associated with egg laying and located at the anterior end of the nidamental gland. They are also closely associated with the ventral surface of ink sac[4]. These glands play an important role in protecting the eggs from pathogens or predators by coating them with symbiotic bacteria and also act as indicator for maturity stages[5], whereas its colour changes from white in immature females and to yellow, orange and finally from orange to red in case of mature stage and then to pink after spawning[1]. Ink is a bioactive secondary metabolite secreted by cephalopods as a self-defense mechanism to escape from their enemies and avoid dangers and risks[6].

Marine organisms are the next generation of medicines[7] since they have a vast array of new pharmaceutical compound with novel activities that will provide a new source for drug against microbial pathogens which has resistance to conventional antibiotic therapies. Marine invertebrates, including molluscs have wide spectrum of antimicrobial activity due to their bioactive compound[8,9]. The ink and mantle tissue extracts of cuttlefish and squid posed antibacterial effect[10-12].

In recent year, Roper *et al.*[5] stated that cephalopod ink is good source for biomedical and industrial applications.

Sepia officinalis Linnaeus, 1758 is one of the nine Cephalopoda species and best known cuttlefish species which were recorded in Alexandria Mediterranean waters, Egypt[1]. The specie was cosmopolitan recorded in Mediterranean sea, Tunisian waters, Adriatic sea, Spanish Catalonian sea, Turkish waters and Eastern Atlantic from Baltic and North seas to South Africa[5]. In Egypt, it was previously recorded in Abu Qir Bay by Steuer[13] and in the Eastern Harbor, Abu Qir Bay (7-36 m depth) to Rosetta East and to Sidi Krir West (10- 45 m depth) by Riad[1] (Fig. 1).

The present study aimed to screen the antimicrobial activity of many extracts of different parts of mature females *S. officinalis* (mantle tissue and waste materials (e.g. accessory nidamental, nidamental gland and ink) against some human pathogenic bacteria and fungi *in vitro*.

Fig. 1: Dorsal view of *S. officinalis*, Class: Cephalopoda cuvier, 1798; Subclass: Coleoidea bather, 1888; Order: Sepioidea naef, 1916; Family: Sepiidae kersstein, 1866 and Genus: *Sepia officinalis*

MATERIALS AND METHODS

Sepia officinalis were collected at the end of March (spring season), the antimicrobial testes take from 7-10 days *in vitro*.

Cephalopod sample collection and identification: Cuttlefish (*S. officinalis*) samples were collected by hand-picking from Abo Qir Bay, Alexandria Mediterranean waters, Egypt by the help of fisherman. Samples were brought to the laboratory and were identified using the taxonomical technique used by Riad[1].

Extracts preparation: *Sepia officinalis* samples were washed with sterile water. The abdomen of cephalopods was open and the mantle tissue was removed and cut into small pieces. The other parts (e.g. accessory nidamental gland, nidamental gland and ink) were separated aseptically and carefully removed the preserved and kept in refrigerator at 0-2°C. Some of the tested parts were air dried. The fresh and dry samples were kept at room temperature in a glass bottle with acetone, ethanol, chloroform, methanol and water for 7 days then homogenized with previous tested solvents. The tested extracts were centrifuged at 10,000 rpm for 20 min at 4°C to collect the supernatant and concentrated under vacuum in a rotary evaporator at low temperature according to Nithya *et al.*[14] with modification.

Microbial cultures: Two species of Gram positive pathogenic bacteria viz., *Bacillus subtilis* and *Staphylococcus aureus*; four species of Gram negative bacteria viz., *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*, *Vibrio cholera* and two fungal pathogenic strains *Aspergillus fumigatus* and *Candida albicans* were inoculated and maintained on sterile nutrient and Czapek-Dox medium, respectively then incubated and maintained at 37°C.

Antimicrobial activity assay: The antimicrobial activities of the tested extracts of cuttlefish were determined by disc diffusion method[15]. Autoclaved MH-agar and Czapex-Dox agar plates were inoculated with 150 CFU mL^{-1} of bacterial inoculums and 0.75×10^{6} fungal spores mL^{-1}, respectively then spreaded with sterile swab and sterile filter paper discs (Whatman No. 1) of 6 mm impregnated with 250 µL of different tested extracts were placed on the surface. The petri plates were incubated at 37°C for 24 h for bacterial and at 28°C for 48-72 h for fungal species and the diameter of the inhibitory zone around the disc was measured and expressed in mm[16]. The standard antibiotic ampicillin (10 mg mL^{-1}) and the tested solvents were used as a positive and negative control.

Statistical analysis: Results were expressed as Mean±SD (n-5). In general, data were analyzed by one way ANOVA test using SPSS windows version 11.5 with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Screening of the antimicrobial activity of mantle tissue and wastes materials (ink, accessory nidamental gland and nidamental gland) extracts from the cuttlefish *Sepia officinalis* was carried out against six bacterial and two fungal human pathogenic species *in vitro* (Fig. 2 and 3). The present data showed that there was significant difference in antimicrobial activity of all extracts against all bacteria and fungi species. The variation in antimicrobial activity depended on *Sepia* parts, solvent efficiency used and the tested microbe and this was in accordance with Cannel[17] stated that the differences in mollusca antibacterial activity may be related to solvents used and the compounds extracted.

The results showed that most extracts a higher antibacterial activities than recorded by ampicillin (positive control), but few extracts had antifungal inhibition better than control. Generally, the aqueous extract of all *S. officinalis* parts did not have antimicrobial activity against all tested microbial species. The methanol extracts of different *S. offinalis* parts showed the highest antimicrobial activity against all the tested pathogenic, followed by chloroform extracts. In this regard, the highest antibacterial activity was detected in ink methanol extract against *P. aeruginosa* (18±1.3 mm) and *S. typhi* (15±1.2 mm, respectively), followed by nidamental gland methanol (14±0.8 mm) and ink chloroform extracts against *P. aeruginosa* (13.2+0.8 mm, respectively). As shown in Fig. 3, the methanol extract of *S. officinalis* ink and nidamental gland showed the maximum antifungal activity against *C. albicans* (3.5±0.4 and 3.2±0.5 mm, respectively) followed by chloroform extracts of both parts (2.9±0.4 and 2.5±0.3 mm) against *C. albicans*. On the other hand, other extracts had lower antifungal activity than ampicillin against the both pathogenic fungi.

The present data indicated the methanol and chloroform were the best solvents for antimicrobial extraction as compare with other used solvents. Similar observations were described by Mohanraju *et al.*[18], who detected the antibacterial activity of the methanolic extract of cephalopods tissues against some human pathogens. Ramasamy *et al.*[19] reported that the methanolic extract of *Sepia prashadi* body tissue exhibited antimicrobial activity against many pathogenic strains. In the same years, Nithya *et al.*[14] recorded that the chloroform ink extract of *Sepia pharaonis* had highest antibacterial activity against *P. aeruginosa* (10 mm). Rajaganapathy *et al.*[20] demonstrated the activity of ink methanol extract of *S. pharaonis* and *S. inermis* against *C. albicans*. On the other hand, the acetone extract of *S. officinalis* ink had the minimum antibacterial activity against all the tested bacteria. Nithya *et al.*[14] detected the acetone extracts showed lowest activity against *K. pneumoniae* (2.5 mm).

The most extracts of all extracts demonstrated higher activity against Gram negative bacteria than Gram positive bacteria. In these connection, Nair *et al.*[21] detected that the inhibition activity of ANG being more against Gram -ve bacteria than Gram +ve strains. Nirmale *et al.*[22] stated the precipitated and freeze-dried ink showed more pronounced antibacterial activity against Gram -ve bacteria (e.g. *Salmonella,* spp. *E. coli, V. cholera*, *V. parahaemolyticus* and *Pseudomonas* spp.) and a less pronounced activity against Gram +ve bacteria (e.g. *Staphylococcus* spp. and *Micrococcus* spp.).

In the present study, the comparison of *S. officinalis* parts showed that the antimicrobial activity was higher in ink and nidamental gland than accessory nidamental gland and

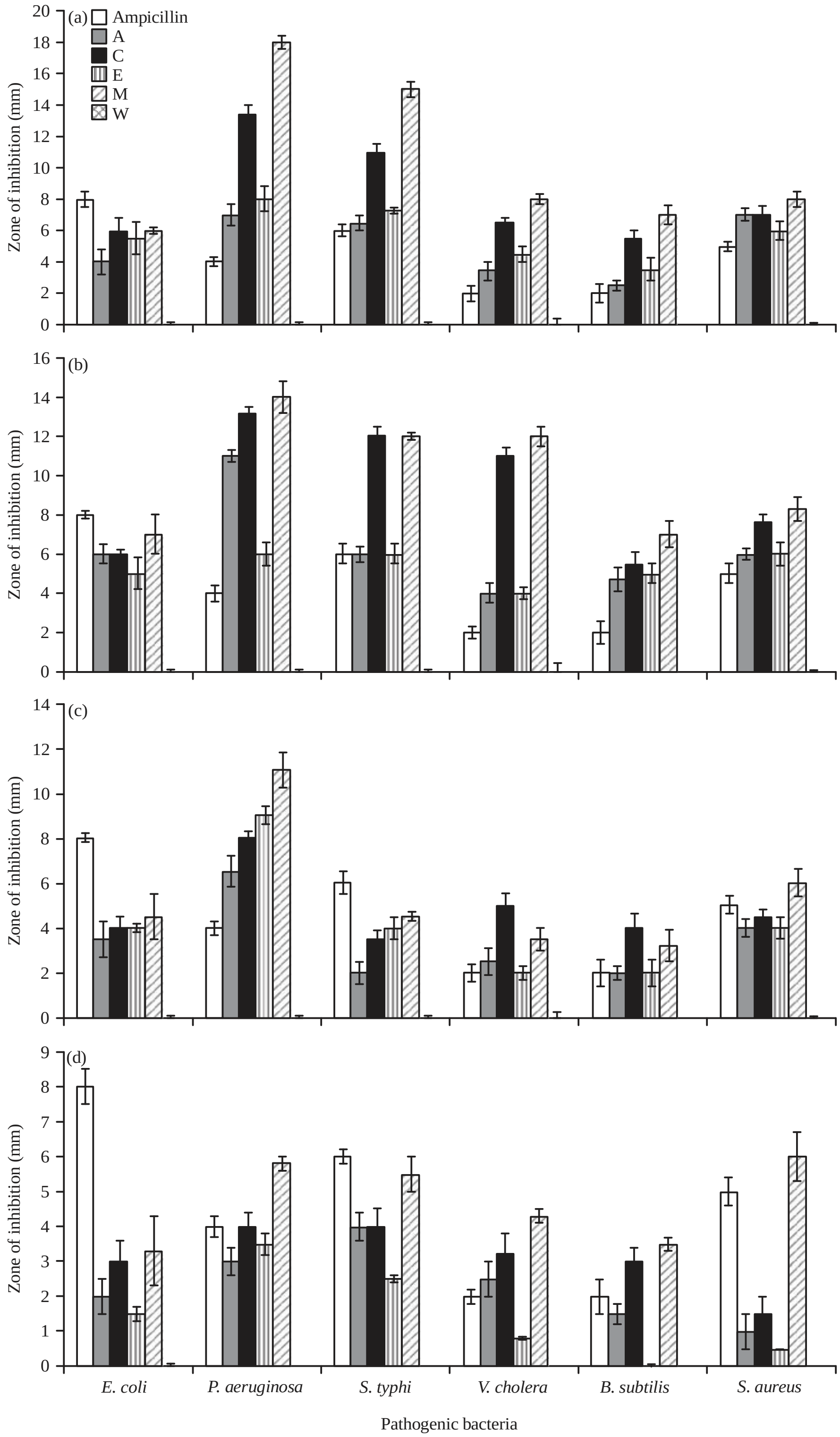

Fig. 2(a-d): Antibacterial activity of the (a) Crude ink, (b) Nidamental gland, (c) ANG and (d) Mantle tissue extracts of *Sepia officinalis* against human pathogenic bacteria spp. A: Acetone, C: Chloroform, E: Ethanol, M: Methanol, W: Water

Values represent mean values standard deviation

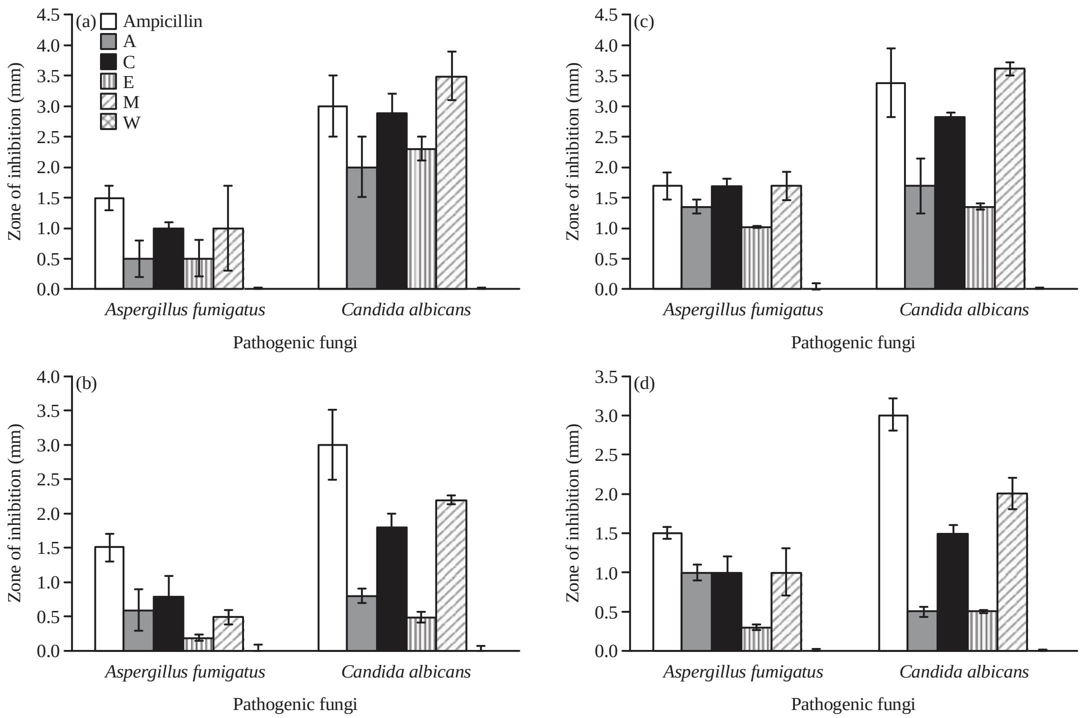

Fig. 3(a-d): Antifungal activity of the (a) Crude ink, (b) Nidamental gland, (c) ANG and (d) Mantle tissue extracts of *Sepia officinalis* against human pathogenic fungi. A: Acetone, C: Chloroform, E: Ethanol, M: Methanol, W: Water
Values represent mean values standard deviation

mantle tissue (Fig. 2 and 3). The crude ink of *S. pharaonis* exhibited antibacterial activity with the inhibition zone ranging from 6-20 mm[23]. Peruru *et al.*[24] detected the antimicrobial activity of melanin isolated from *S. officinalis* ink. Edward and Annappan[25] and Patil *et al.*[26] demonstrated that antibacterial activity of *Sepia* ink. Early, Lane[27] stated that the fluid from the ink sac of cephalopods had the antibiotic activity.

Mantle tissue of *S. officinalis* exhibited the lowest antimicrobial activity against all the tested bacteria as compared with other parts and ampicillin (positive control) (Fig. 2 and 3d). A very few studies were carried out on the antibacterial activity of cephalopod tissues and ANG. The antimicrobial activity of mantle tissue and ANG may be related to their fatty acid contents. Present study results were in agreement with Maktoob and Ronald[28] and Blunt *et al.*[29], who identified the bioactive compounds in molluscs as peptide, sterols, terpenes, polypropionates, nitrogenous compounds, macrolides, fatty acid derivatives and alkaloids which had specific activities e.g. antimicrobial activity. Ehrenberg[30] stated that ANG of the ripe female *S. pharaonis* had antibacterial activities. The antimicrobial activity of ANG may be related to the orange red xanthophyll "sepiaxanthin" or due to the higher levels of unsaturated fatty acids[31,32]. Sherief *et al.*[33] detected the antimicrobial activity of *S. aculeata* and *S. pharaonis* ANG extracts. Ozogul[34] reported that the major fatty acids found in *S. officinalis* were palmitic acid, stearic acid, eicosapentaenoic acid and docosahexaenoic acid. Unfortunately, there is no available data about antimicrobial activity of nidamental gland, so it must further studies to detect the bioactive compounds with antimicrobial activities in it.

CONCLUSION

The present research indicated the possible use of waste material like ink and nidamental gland of *Sepia officinalis* as a valuable natural antimicrobial agent that may instead of commercial antibiotics. This study acts as the baseline information in pharmaceutical and medical fields. Further investigation on the bioactive compounds and their cytotoxicity is still needed.

SIGNIFICANT STATEMENT

This study discovers that the medical importance of *Sepia officinalis* parts will help the researchers to discover a cheap and a new sources of antimicrobial substance against human pathogenic, whereas the extracts of ink and nidamental glands from *S. officinalis* explained a good antimicrobial activity which confirmed their vital role in future for therapeutic fields.

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