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ANTIMICROBIAL POTENTIAL OF *HIPPOPHAE RHAMNOIDES* LINN (SEA BUCKTHORN) BERRIES, PULP AND TEA EXTRACTS AGAINST FOOD SPOILAGE AND FOODBORNE BACTERIAL STRAINS

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Abstract *The present study was carried out to evaluate the phytochemicals content and antibacterial activities of Hippophae rhamnoides Linn. (Sea buckthorn) pulp, dry berries, and tea (twigs, leaves, and dry berries). The content of phytochemicals (glycosides, tannins, saponins, flavonoids, phenols, and terpenoids) was measured using standard methods. The antibacterial activities were determined by applying well diffusion technique and Minimum Inhibitory Concentration (MIC) was determined using serial two-fold dilution against Gram-positive bacteria (Staphylococcus aureus, Listeria monocytogenes, and Bacillus cereus) and Gram-negative bacteria (Salmonella enterica and Escherichia coli). The results showed that tea methanol extracts had the highest extractive values (18±01), followed by whole berries extract (17± 0.1%) and pulp extracts (14±0.1%). The phytochemical screening showed that the glycosides found in average (++) quantity in methanol and ethanol 70% extracts of tea, pulp, and whole berries. Tannins were found in large quantities (+++) in methanol and ethanol 70% extracts. Saponins in large quantity (+++) were screened in ethanol 70% pulp and tea extracts and tea extract of methanol. The flavonoids and phenols in large quantities were detected in methanol extracts of whole berries and tea, with tea extract of ethanol 70% and whole berries extract of ethanol 70%. The average quantity (++) was detected in tea methanol and ethanol 70%. The antibacterial activities of Sea buckthorn tea extracts zone of inhibition (ZI) were in the range of 19±01mm - 09± 1mm and the MIC (mg/mL) range was 7.8125-62.5. The whole berries ZI (mm) range was 18±1 - 08±0 with MIC (mg/mL) range were 7.125-250 pulp ZI (mm) range was 16±1 - 07±0 and MIC range was 62.5 - 500. The methanol extracts were the most efficient extracts followed by ethanol 70%, ethanol, decoction, and infusion. While tea was the most active part followed by whole berries and pulp. The prominent antibacterial activities and phytochemical contents of Hippophae rhamnoides dry berries, pulp, and tea specify the significance of this plant utilization in food, health, agrochemicals, and agriculture practice.*

Keywords: *Sea buckthorn berries; phytochemicals; zone of inhibition; Gram-negative microbes; Gram-positive microbes; foodborne bacteria; food safety*

Introduction

Foodborne infections and diseases are sometimes life-threatening, expensive, and common illnesses, however mostly curable and are community health issues. Various ailments-causing agents could spoil food, creating food poisoning. Scientists have recognized more than 250 food-borne diseases. Several of them are infections, spread through parasites, viruses, and bacteria (Daniela, 2022). It has been calculated that 48 million citizens acquire a food-borne infection and food-borne diseases every year, 3000 die and 128,000 are admitted to hospitals. Every year it is estimated that 600 million persons catch diseases approximately one in ten citizens on the globe, from food spoilage with chemicals or microorganisms, and 420,000 pass away, consequential in the loss of thirty-three (33) million healthy lives per year (Daniela, 2022). In the early times human beings exploited remedial materials simply available to them and therefore herbs have been employed as medicines since the ancient era. Consequently, plants are the aged sources of valuable medication for human beings. The presence of phyto-compounds in the medicinal plants displayed various characteristics like wound healing, anti-ulcer, anti-diabetic, anti-atherosclerosis, anticancer, cell protectant, anti-hepatitis, anti-radicals, radio-protective, anti-inflammatory, immunostimulant, antibacterial and antifungal (Harshit *et al*., 2013). The *Hippophae rhamnoides* Linn a deciduous shrub, generally known as Sea buckthorn, produces yellow or orange fruits. This plant is native to Asia and Europe and is known for its notable therapeutic benefits. The fruits are utilized in the treatment of heart conditions, ulcers, and skin ailments. Additionally, sea buckthorn berries are highly nutritious and abundant in bioactive antioxidants (Javid and Bashir, 2015).

The *Hippophae rhamnoides* berries have reported significant medicinal properties. The fruits are used for the healing of heart, ulcer and skin ailments. The berries of Sea buckthorn are very nutritious and wealthy in vitamins having anti-radical compounds such as phenols, anthocyanins, carotenoids, ascorbic acid and vitamin E (Selvamuthukumaran and Farhath, 2014; Javid and Bashir, 2015). Numerous kinds of products like jams, jellies, juices, orange pigments and seed oils can be prepared from Sea buckthorn (Guo et al., 2017). Juice extraction processes have generated a press cake residue which might be used subsequent for oil extraction refinement (Eva et al., 2020). The Sea buckthorn antimicrobial properties of oil (Yue *et al*., 2017), stem and root (Jong et al., 2010), stem and bark (Javid *et al*., 2015), leaves (Bashir and Javid, 2013; Qadir et al., 2016), seeds (Chauhan and Varshneya, 2012; Negi *et al*., 2005) and berries (Qadir *et al*., 2016) have been reported. As an outcome, these *Hippophae rhamnoides* parts might be utilized to handle pathogens of food-borne and stored food stability (Netreba *et al*., 2024).

Foodborne pathogenic microbes and contamination of food are frequently handled through the utilization of commercial preservation. Still, despite their capacity to stop food epidemics and contamination, the repetitive application of synthetic preservatives has created the buildup of their precipitation remains in the food chain and the appearance of microbes confrontation to them as well as the negative effects of these substances on the customers' health (Nazir et al., 2017). According to these backgrounds, serious attempts to make available non-synthetic substitutes that are easy to prepare and use, efficient, powerful, and safe for customer health, and therefore was the importance of plant extracts as non-synthetic antimicrobial agents (Soheir et al., 2018). Nowadays, customers wish to prefer safer, healthy, and natural food due to the unsuitable application of anti-microbial compounds in the food production systems could affect unwanted remains in foodstuffs and the appearance of microorganisms that are resistant to antibiotics (Wu et al., 2022). Plenty utilization of synthetic medicines has become a new epidemic. These medicines have wiped out our normal immunity. They destroyed useful microbes in our intestines and caused the formation of super microbes that proved to be opposed to approximately any type of recommended medicines. Using non-natural food preservatives can create health problems, such as rising cancer risk and potential side effects. Thus, the application of herbal-based antibacterial and antifungal agents in food preservation has magnetized rising focus from consumers, food manufacturers, and scientists (Daniela, 2022).

With the growing interest in natural foods and their antibacterial properties, our research concentrated on Sea buckthorn berries and their capability to hold back the development of food-borne bacterial strains. Due to its therapeutic and nutritional benefits, sea buckthorn has gained global recognition. In light of the significance of this remarkable plant, the current study aimed to evaluate the phytochemicals screening, extractive values, and antibiotic potential of whole sea buckthorn berries, pulp, and tea extracts

Materials and Methods

Samples Collection and Preparation

Collection of Sea buckthorn berries, pulp, leaves, and twigs was carried out from Pakistan Council of Scientific and Industrial Research (PCSIR) Skardu-Pakistan at the stage of maturity, as judged by juiciness and appearance and stored at -20°C. Clean the berries manually to eliminate the damaged seeds and entire extra matter. Seeds were separated from frozen berries by pressing and the pulp portion dried at room temperature was further extracted.

Extraction

The drying of whole berries, pulp, and tea was conducted in a clean, dry, and aseptic room at the Food Pilot Plant of PCSIR Laboratories Complex in Peshawar, Pakistan. Once dried, the samples were ground into a fine, homogenous powder using a Commercial Laboratory Blender (Waring®, USA). Twenty grams (20g) of the powder were soaked in 250 mL of selected solvents (ethanol, methanol, and 70% ethanol) in a dark place for three (03) days at room temperature. The mixture was filtered using Whatman No. 1 filter paper (Whatman International, UK). The filtrate was then distilled using a Rotary Evaporator at a reduced 40°C to extract the solvents. The extracts were dried overnight at 40°C using a water bath (PCSIR-Pakistan). Extractive values were quantified by comparing the final dry weight of the extracts to the original weight of the ground-dried samples. The extracts were kept at 4°C in a cooled Incubator (Memmert-Germany) in airtight bottles for future experimental works.

Phytochemical screening

Stock solutions of Sea buckthorn berry extracts (1% w/v) were prepared and used to screen for phytochemical content following standard methods (Harborne, 1998; Bashir and Javid, 2013).

Tested microorganism

Bacterial cultures Gram-positive i.e. *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* (*L. monocytogenes*), and *Bacillus cereus* (*B. cereus*) and Gram-negative i.e. *Salmonella enterica* (*S. enterica*) and *Escherichia coli* (*E. coli*) were obtained from Microbiology Laboratory of PCSIR Laboratories Complex Peshawar, Khyber Pakhtunkhwa- Pakistan. These microbes were preserved in an incubator (Memmert-Germany) for 24 hours at 37°C culturing it in Nutrient Agar (NA) slants. The microbial suspension was prepared using Nutrient Broth (NB) and kept in an incubator (Memmert-Germany) for 24 hours at 37°C. These broth cultures were then stored in a cool incubator and refreshed weekly.

Determination of Antibacterial Assay

To activate the pathogenic bacteria, each microbe's pure cultures were transferred into NB and incubated at 37°C for 24 hours. The bacterial cell density in the suspension was in tune to approximately 7.5 x 10^7 Colony Forming unit (CFU/mL) using a standard of 0.5 McFarland (Georgescu *et al*., 2022). Each bacterial strains of one millimeter were shifted to uncontaminated Petri dishes. Melted NA at 45°C was added into every Petri dish, which was softly swirled in both counterclockwise and clockwise ways to consistently dispense the media and culture. A six millimeter sterilized cork borer was utilized to prepared wells in the inoculated and solidified NA plates. In every wells, 100 µL (125 mg/mL) Sea buckthorn extracts dissolved in dimethyl sulfoxide (DMSO) were added. The Petri plates were kept at low temperature (+4°C) for 02 hours to permit the extracts to absorb into the agar. Subsequently, all the Petri dishes were kept in an incubator (Memmert-Germany) for twenty four hours at 37°C. The inhibition zone was measured in millimeter (mm) after completion the incubation time to evaluate the antimicrobial activities, applying a Vernier caliper for accurate calculation (Bashir and Javid, 2013).

Minimum Inhibitory Concentration (MIC) Determination

A *Hippophae rhamnoides* extracts stock solution was prepared in DMSO in a concentration of 1000mg/mL and afterward applying a serial two-fold dilution method a dilutions were made. This created a range of experimental concentrations, including 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 mg/mL. The MIC was measured by incubation every Sea buckthorn concentration extracts with 100 µL of microbial density (1 x 10^8 CFU/mL) at 37°C for 24 hours in two milliliter of NB hold in test tubes. The lowest concentration that demonstrated no appearance microbial growth was measured the extract MIC (Bashir and Javid, 2013).

Statistical Analysis

Each test trial was conducted in triplicate, and the data were presented as average ± standard deviation (SD). A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

Extractive values

Extractive values of Sea buckthorn whole berries, pulp, and tea extracts are shown in Table 1. The results show that methanol extract is the most efficient solvent having an extractive value of 18±01% and 17±0.1% for tea and whole berries. Similarly, ethanol 70% is the second most efficient followed by ethanol. The decoction is more efficient as compared to the infusion extract. The tea extract is given maximum extractive values followed by whole berries and pulp. The extractive values gave the thought about extractability of substantive composition of the samples in various solvents. Extractive values showed that the methanol extractive value was greater as compared with other solvents. Herbs hold various behavior data regarding solubility of herbs molecular ingredients in various solvents therefore signifying the best solvents for obtaining bioactive compounds. Table 1 shows that methanol extracts produce extractive values followed by ethanol 70%, ethanol, decoction, and last on infusion. This could be attributed to the lofty polarity of the solvent methanol, which is capable of extracting a broader range of herbal ingredients compared to other solvents. The yield percentages for methanol, acetone, ethyl acetate, chloroform, and n-hexane were 13.27%, 6.20%, 8.65%, 3.59%, and 4.32% respectively. The extractive values demonstrate that the extractability of sea buckthorn pulp varies with different solvents. Methanol exhibited the highest extractability, followed by ethyl acetate and acetone (Nishat and Tripathy 2015). The highest yield (13.10%) was obtained from the polar components of Sea buckthorn berries when extracted using methanol, followed by 5.20% from non-polar components extracted with petroleum ether, and 3.7% from the intermediate polarity components extracted using chloroform (Saadia et al., 2011). These findings suggest that Sea buckthorn berries are rich in polar compounds that are soluble in methanol, which are likely to be polyphenolic (Egon, 1969).

Phytochemical Qualitative Screening

The mature and healthy Sea buckthorn berries (Fig.1) were collected from PCSIR Skardu, Gilgit Baltistan-Pakistan. Phytochemicals qualitative analysis results are illustrated in Table 2. The table showed the presence of glycosides, tannins, saponins, flavonoids, phenolics, and terpenoids in the berries, pulp, and tea extract of Sea buckthorn. Methanol is the most efficient extract followed by ethanol 70%, ethanol, decoction, and finally infusion extracts. The tannins are found in a large quantity (+++) in whole berries and tea extracts of methanol and ethanol 70%. The saponins large quantity (+++) is found in the tea extract. The flavonoids in large quantity (+++) are found in whole berries and tea extracts of methanol and tea extract of ethanol 70%. The phenolics' large quantity (+++) are reported in whole berries and tea extract of methanol and ethanol 70%. The terpenoids' average quantity (++) is screened in methanol and ethanol extracts of whole berries and tea sea buckthorn. The glycosides average quantity (++) are detected in the whole berries, pulp, and tea parts of Sea buckthorn methanol and ethanol 70%. Phytochemicals are organic compounds produced by plants, which can be either non-nutritive or nutritive. They are generally considered naturally occurring, non-nutritive substances derived from plants. The presence of these secondary metabolites can vary depending on the solvent used, likely due to the varying solubility of different solvents for specific phytoconstituents.

Alkaloids

Alkaloids have been reported to exhibit analgesic, antimalarial, bactericidal, and antispasmodic activities (Zakia *et al*., 2015). Alkaloids are complex heterocyclic nitrogen-containing substances that are generally known for their antimicrobial activities. They are particularly effective against protozoan and viral infections. The mechanism of action for highly aromatic, planar quaternary alkaloids is often attributed to their ability to intercalate with DNA (Mahesha *et al*., 2015).

Glycosides

Glycosides belong to organic molecules, generally originating from herbs. A glycoside is composed of a sugar molecule bonded with a non-sugar molecule in a specific way. The non-sugar portion is referred to as the genin or aglycone. In most of the hundreds of herbs glycosides that have been discovered, the bond between the sugar and the aglycone is a hemiacetal bond, prepared by the reducing class generally ketone or aldehyde of the sugar and phenolic hydroxyl group or alcohol of the aglycone (Stephen, 1967).

Tannins

Tannins are recognized for their antimicrobial and antioxidant properties, as well as their cytotoxic and antineoplastic effects (Mahesha *et al*., 2015). According to the literature, tannins, particularly proanthocyanidins derived from berries, exhibit effective preventive action against urinary tract infections, especially those caused by *E. coli* (Suriyaprom et al., 2022; Hisano et al., 2010). This may provide a strong rationale for the effectiveness of various berry extracts against this microorganism. Among the tested strains, *S. typhi* was the most resistant (Georgescu et al., 2022).

Saponins

Saponins are known as varied class of glycosides make up of a hydrophilic molecule; compose of a lipophilic component known as genin and monosaccharides. They are described by their surfactant activities; create foaming solutions and allowing them to dissolve in H_2O. These are found in numerous herbs and are group into two classes: triterpenoid saponins and steroidal saponins. Various pharmacological of saponins have been reported such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-ulcer, hemolytic and hepatoprotective (Tagousop *et al*., 2018)**.**

Flavonoids

Flavonoids are a vital phytochemical compounds synthesized by *Hippophae rhamnoides.* They have produced by plants as result of reaction to pathogens infections and documented *in-vitro* efficiency as antibiotic substances against numerous germs (Gupta et al., 2011). Flavonoids are chief significant secondary metabolites occurred in herbs; they possess antioxidant activities (ROS) (Jong et al., 2010).

Phenolics

Phenolic molecules are extensively recognized as efficient anti-radical in nature because of its abilities to counteract reactive free oxygen and free radicals species like hydroxyl radicals, singlet oxygen and superoxide radicals (Anelise et al., 2014). Phenolic substance might be control microbial growth and metabolism, possessing either inhibition or stimulatory property influence on their dosage and the particular kind of phenolic compounds (Tamas-Krump et al., 2020). The polyphenols are a group of natural substances distinguished by their phenolic shape. This class comprise 04 basic subgroups: flavonoids, stilbenes, phenolic acids and lignans. The flavonoids could be further categorized into flavanones, flavones, flavonols and anthocyanidins (Kumar et al., 2023). Phenols are necessary phytcochemical substances that possess vital activities against bacteria and fungi (Mousmi and Handique, 2013).

Terpenoids

Terpenoids, comprising diterpenes, sesquiterpenes and triterpenes are documented in pharmaceutical industries for their antiseptic, anthelmintic, insecticidal and antibiotic properties (Zakia et al., 2015). The differences in findings between the current study and previous research (Bashir and Javid 2013; Javid et al., 2015) may be attributed to variations in geographical locations, where soil minerals and environmental factors significantly influence the phytochemical composition of the plant (Zakia et al., 2015). In light of this background, our research on Sea buckthorn is particularly intriguing due to the presence of several significant classes of bioactive phytochemicals in the plant. Additionally, it offers scientific validation for the use of these plant extracts in conventional health systems within our region. This preliminary phytochemical screening of Sea buckthorn berries, pulp, and tea strongly supports the need for further advanced research focused on the chromatographic isolation of these compounds in their pure form on an industrial scale.

Antimicrobial activities

Searching for natural products was a growing interest because it has safe, a source of novel medicines, and effective substitutes for synthetic drugs. In this study, we studied the antibacterial activities of Sea buckthorn tea extracts against microbes causing severe food poisoning or infections. Table 3 shows the antibacterial activity of *H. rhamnoides* tea. The results show that the maximum zone of inhibitions is recorded by methanol extract against *Bacillus cereus (*19±1mm), *Staphylococcus aureus (*18±0mm), and *Listeria monocytogenes (*17±0mm). The ethanol 70% extracts are the second most competent extracts as compared with ethanol, infusion, and decoction extracts. The Gram-negative microbes have reported a low zone of inhibition as compared with Gram-positive microbes. Infusion is the least active extract as compared with the other extracts.

Table 4 shows the antibacterial activity of *H. rhamnoides* whole berries extracts. The results showed that methanol extract was found more effective against *Bacillus cereus* with 18 ±1 mm *Listeria monocytogenes* 16±0 mm and Staphylococcus aureus 15±0 mm zone of inhibition (ZI). The methanol 70% extracts show a moderate zone of inhibition followed by ethanol, decoction, and infusion the least extracts to show the zone of inhibition. During the study, observed that methanol extract was found the best choice against Gram-positive microbes. While Gram-negative microbes were found to be resistant to the Sea buckthorn berries extracts. It was presumed that most antimicrobial bioactive compounds were found in methanol extract. For each microorganism, the Sea buckthorn berries extracts observe the variance in selectivity of antimicrobial activity. The antibacterial activity of Sea buckthorn pulp is shown in Table 5. The results show that pulp extracts are the least active as compared with the berries and tea extracts. In these results, it is clear that methanol shows the maximum zone of inhibition as compared with all the extracts.

Table 6 displays the MIC of the Sea buckthorn tea extracts against pathogenic bacteria. Lower MIC values were obtained for methanol extract against gram-positive bacteria. The infusion extract was counted maximum MIC (125 mg/mL) for *Escherichia coli*. Overall result showed that decoction and infusion extracts had high MIC values. The minimum Inhibitory Concentration (MIC) of Sea buckthorn Whole Berries is shown in table 7. The methanol extracts show a minimum MIC value of 7.8125 mg/mL against *Bacillus cereus* and the maximum MIC value of 250mg/mL is calculated against Listeria monocytogenes and *Escherichia coli* by infusion and decoction extracts. Minimum Inhibitory Concentration (MIC) of Sea buckthorn Pulp is shown in table 8. The results show that pulp has maximum MIC values as compared to the whole berries and tea extracts of Sea buckthorn. Most of the MIC values in these results show that they range from 62.5-500mg/mL. The Gram-negative microbes have shown maximum MIC values to inhibit these microbes as compared with Gram positive microbes. *Listeria monocytogenes* is a member of the genus *Listeria* and is a foodborne pathogen associated with illnesses that have a high mortality rate, particularly in susceptible populations. It is frequently implicated in outbreaks of human listeriosis. *L. monocytogenes* is a Gram-positive, non-spore-forming rod with an optimal temperature range of 30–37 °C. As one of the leading foodborne pathogens, it is the causative agent of listeriosis. This microorganism can withstand freezing temperatures but is inactivated by heating at 60 °C for 30 minutes (Daniela, 2022). The causative agent can grow at 4.4 °C or below temperatures, as opposed to a sanitizer, and remain on different surfaces. Once it enters processing plants, *Listeria monocytogenes* can stay alive and stay vigorous for extended age, even under adverse circumstances. Their aptitudes to prepare biofilms allow it to be a nonstop foundation of pollution, instead of a main risk to the foodstuff business (Daniela, 2022).

Today, there is an increasing interest in exploring natural products as safer and more effective alternatives to pesticides and antimicrobial agents, as well as a source of new compounds that can enhance human health and well-being. Among the various biological activities of *Hippophae rhamnoides* extracts, its antimicrobial properties significantly enhance the value of this plant as a medicinal resource (Eva et al., 2020). The increasing resistance of key food-borne microbes to conservative medications highlights the need for substitute antimicrobial agents. Herbs have long been used globally to care for a wide range of infections and ailments. Herbal extracts represent a valuable reservoir of potential antibacterial molecules that warrant additional searching. These plants possess various kinds of secondary metabolites or bioactive compounds, like phenolic compounds, terpenoids and alkaloids, which participate in a vital action in their antibiotic properties (Ashneel et al., 2023). The secondary metabolites like phenols act as protector device for plants against microorganisms, insects and herbivores (Cowan, 1999). The bioactive plants are an inborn source of anti-radicals and antimicrobial compounds, which build them feasible for using as food preservative and drugs (Ashneel et al., 2023). In the current study, all Sea buckthorn extracts hold microbial expansion inhibition against manifold food-borne germs, with changing levels of effectiveness. Varying degree of confrontation to the extracts were also scrutinized in the microbes.

The *Hippophae rhamnoides* fruits n-hexane, chloroform and aqueous extracts (06mg/mL) against S. aureus (Methicillin-resistance) observed zone of inhibition 22.93±1.27, 23.37±1.22 and 18.01±2.87 respectively (Qadir *et al*., 2016). The Sea buckthorn berries powder water extract (06mg/mL) showed a zone of inhibition (Disc Diffusion Test) against *S.*

aureus, B. cereus, E. coli, and *P. aeruginosa* were 18.31mm, 13.1mm, 16.0mm, and 10.01mm respectively (Sonu et al., 2022). Amongst all tested extracts, the extract of seed observed significant (11-14mm) and the extract of leaves calculated medium (07-10mm) activity against Gram-positive microbes. While, Gram-negative microbes (*E. coli* and *Pseudomonas fluorescens*) found no activity against all the extracts tested excluding *Agrobacterium tumefaciens* showed a few activities against *H. salicifolia* seed extract (Gupta *et al*., 2011). The inhibition zones for the microbes range from 2.33 ± 0.58 mm for *Hippophae rhamnoides* oil to 10.67 ± 4.93 mm for *Hippophae rhamnoides* tea. The antibacterial activities of *Hippophae rhamnoides* tea varied, with inhibition measuring 3.00 ± 1.00 mm against *S. aureus* and 10.67 ± 4.93 mm against *Y. enterocolitica*. For dry berries of *Hippophae rhamnoides*, the inhibition zones 4.00 ± 1.00 mm against *S. enterica* and *S. aureus* to 8.67 ± 1.53 mm against *L. monocytogenes*. The *Hippophae rhamnoides* oil displayed the maximum zone of inhibition against (8.00 ± 2.00 mm) and minimum zone of inhibition (2.33 ± 0.58 mm) was recorded against *S. enterica*. Further Sea buckthorn juice exhibited its minimum antimicrobial activities i.e. 3.33 ± 1.53 mm against *L. monocytogenes* and *B. thuringiensis,* whereas 6.00 ± 2.00 mm was observed maximum against *Y. enterocolitica*
(Eva et al., 2020). The significant antibacterial activity was observed by Sea buckthorn fruits. The extracts (concentrated) observed inhibition zone of C2=30 mm and C1=32 mm, while the hydroalcoholic extracts had H1 = 29 mm and H2 = 30 mm. In contrast, the powdered form exhibited inhibition zones of P1 = 27 mm and P2 = 27 mm (Daniela, 2022).

E. coli was the most sensitive microorganism to the berry extracts, particularly to the sour cherry extract (S2). Subsequent that, the S1 (blackcurrant) and S2 (Sea buckhorn) extracts documented medium to high antimicrobial activities against *E. coli* (Georgescu et al., 2022). The *Hippophae rhamnoides* fruits methanol extract (100mg/mL) reported a maximum antimicrobial activities, whereas extracts of water, chloroform and petroleum ether observed minimum zone of inhibition against all microbial strains. At minimum dosage (50 and 05 mg/mL), no one of these extracts possessed any inhibition zoneon the tested microorganisms (Saadia et al., 2011). Among the different Sea buckthorn components, the leaves had the most notable antibacterial activity, followed by moderate activity in the seeds, and the pomace showed the least antibacterial effect at a 50mg/mL concentration against spoilage and foodborne microbes (Richa et al., 2012). The methanol extracts from *Hippophae rhamnoides* leaves, seeds, and pomace demonstrated a wide range of antibacterial capabilities against the tested microbes, with an effectiveness of 64.71%. In dissimilarity, the water extracts observed a minimum antibiotic activities, with a weakness degree of 39.21% for tested microbes (Richa et al., 2012).

The *Hippophae rhamnoides* fruits powder showed a noteworthy antimicrobial potential against *Bacillus subtilis* and *S. aureus* with inhibition zones 19 to 29 mm and 22mm to 30mm respectively. The powder reported minimum antibiotic activities against *L. monocytogenes* (16 to 22 mm), *E. coli* (12 to 18 mm) and *Salmonella typhimurium* (13 to 19 mm) (Sandulachi et al., 2022). Similar results with the previous findings were showed only against *Bacillus subtilis*, wherever *Hippophae rhamnoides* extract (S1) showed medium activities 12–18 mm, and for *E. coli*, which observed medium activities with S2-*Hippophae rhamnoides* extract (Georgescu et al., 2022). The Sea buckthorn exhibited antimicrobial potential against microbe (Gram-negative), which bring into line with the research documented (Michel et al., 2012). The Sea buckthorn phenolic-rich fraction (PRF) and leaf extract (SLE) antimicrobial activities were evaluated against microbes (Gram-positive and Gram-negative). The SLE demonstrated the lowest (8.33 ± 1.12 mm) against *S. pneumoniae* and highest zone of inhibition zone of inhibition (15.23 ± 0.84 mm) against *S. dysenteriae*. In contrast, the PRF displayed the minimum zone zone (8.0 ± 0.47 mm) against *S. typhi* and the maximum zone (20.67 ± 1.54 mm) against *S. dysenteriae* (Yogendra et al., 2013). The findings of current study disagree from these research might be due to differences in climate, geography, plant parts used, microbial strains, extraction methods and plants species differences

The MIC was assessed through the microdilution technique. The findings showed that *Hippophae rhamnoides* oil (leaves, seed, and pulp) had an equal MIC value of 12.20 mg/mL against *S. aureus*. Amongst the oils, pulp oil demonstrated to be the main effectual against *B. subtilis* (0.19 mg/mL), *B. cereus* (3.05 mg/mL), and *B. coagulans* (0.10 mg/mL). In the case of *E. coli*, the oil of the seed exhibited a slow-down effect that was twice as strong as that of the pulp or leaves oil, with an MIC of 6.10 mg/mL (Yue *et al*., 2017). The *Hippophae rhamnoides* antimicrobial activities, assessed by measuring the inhibition zone diameter, ranged from 3.70 to 15.91 mm/g against *Bacillus pumilus* for whole *Hippophae rhamnoides* berries, and from 13.33 to 26.67 mm/g for *Hippophae rhamnoides* purees (Netreba *et al*., 2024). The diffusion tests discussed above are frequently used to evaluate bacterial susceptibility, but they are limited by providing only 'susceptible' or 'resistant' outcomes, making this method qualitative. For a more precise evaluation, the MIC of a natural product or antibiotic against the specific organism should be determined. Dilution methods are used to establish the low quantity of an antibiotic molecule that can inhibit or eliminate the microorganism. This is achieved by performing serial dilutions of the natural product or antimicrobial agent, typically in two-fold increments, in either agar or broth media (Daniela, 2022).

The MIC (µg/mL) of the aqueous extract of Sea buckthorn berries powder against *Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa* 250, 125, 4, and 300 respectively (Sonu et al., 2022). The MIC values of Sea buckthorn seed extracts in methanol for *B. cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *L. monocytogenes*, and *Y. enterocolitica* were 200mg/Kg, 300mg/Kg, 300mg/Kg, 300 mg/Kg, and 350mg/Kg respectively. These findings suggest that sea buckthorn seeds could be a potential resource for medicinal applications and food preservation (Negi *et al*., 2005). Methanol extracts MIC values of (seed, leaves, and pomace) of Sea buckthorns against the tested microbes were inferior (125 to 4000 µg/mL) than that of extracts water which was 2000 > 4000 µg/mL (Richa et al., 2012). The MIC of Sea buckthorn required slowing down the expansion and reproduction of Listeria was determined to be 2.6 mg/mL. Based on these findings, it was concluded that the most active compounds are present in the *H. rhamnoides* species (Daniela, 2022).

E. coli was determined to be the mainly vulnerable to *H. rhamnoides* tea, exhibiting a MIC 50 1086.37 µg/mL and a MIC 90 value of 1687.93 µg/mL for the Sea buckthorn berry extract. *Y. enterocolitica* and *Salmonella enteritidis* documented the minimum susceptibility to *H. rhamnoides* tea, having MIC 90 and MIC50 values of 2860.52 µg/mL and 1583.90 µg/mL respectively, for *H. rhamnoides* fruits extracts. *Bacillus thuringiensis*, *S. aureus* and *L. monocytogenes* showed the minimum susceptibility to *H. rhamnoides* berries and tea extract, with MIC 90 value of 8142.60 µg/mL and with MIC 50 values of 7660.07 µg/mL. The Sea buckthorn juice and oil displayed antimicrobial activities at a dosage not more than 10.240 µg/mL against Gram-negative and Gram-positive bacteria. Amongst the bacteria tested, *Y. enterocolitica*, *S. enteritidis* and *E. coli* observed minimum vulnerability to *H. rhamnoides* (Eva et al., 2020). The MIC findings variation depending on the extracts applied in the study, with *Staphylococcus aureus* and *Bacillus subtilis* demonstrated the maximum confrontation to all extracts (Saikia and Handique, 2013). The results showed that *S. aureus* and *Bacillus thuringiensis* were among the maximum resisted to the effect of Sea buckthorn and all tested extracts showed powerful antibacterial potential against microbes (Eva et al., 2020). This observable fact can be clarified by the shape dissimilarities between the outer membranes of Gram-positive and Gram-negative. The microbes (Gram-negative) exhibited an outer container that is build up of lipoproteins and lipopolysaccharides, which is selectively porous and manage admittance to the main arrangement. As an outcome, microbes (Gram-negative) are generally little susceptible to plants extracts as contrast to Gram-positive microbes (Koohsari et al., 2015).

The antibacterial activities are liable an outcome of the ability of flavonoids to synthesize composites with extracellular and soluble proteins in bacterial cell walls through distracted communications, like as hydrogen bonding, hydrophobic effects and covalent bonding. Though, their antimicrobial activities might be accounted to its capabilities to restrain microbial adhesion, enzyme activity and protein transport. Moreover, these substances could also straight destroy microorganisms in the course of their actions (Gill *et al*., 2012). As a result, the outcome of the antibacterial potential showed in these finding of *H. rhamnoides* fruits extracts are connected to its polyphenolic substances. The antimicrobial activities might be accounted to flavonoids, which are recognized for their antibacterial and antifungal activities (Bashir and Javid, 2013). Sure methanol extracts from herbs reported high efficiencies against most of the tested microbes compared to other kind of extracts (Ahmet et al., 2011, Lin et al., 1999, Mousmi and Handique, 2013). Generally, bacteria (Gram-negative), possessed a minimum vulnerability to plants extracts contracts to Gram positive bacteria (Chan *et al*., 2007). This effect is supported by evidence that Gram–Ve microbes possess an external layer composed of lipopolysaccharides and lipoproteins, which creates a choosey porous barrier that controls the right of entry to the core configurations (Mousmi and Handique, 2013). The antimicrobial activities of herbal extracts are often accredited to the occurrence of various phytochemical substances, such as tannins, polyphenols, and flavonoids (Bashir and Javid, 2013). This finding aligns with earlier studies indicating that extracts from different divisions of the Sea buckthorn (stem, leaves, roots, and seeds), possess antioxidant and antimicrobial properties (Harshit et al., 2013; Selvamuthukumaran and Farhath, 2014; Camelia et al., 2008; Jong et al., 2010; Mousmi and Handique, 2013; Gill et al., 2012; Bashir and Javid, 2013).

The antibiotic potential of *H. rhamnoides* extracts is likely attributed to their capacity to interact with soluble and extracellular proteins, as well as cell walls of bacteria, through distracted interactions like hydrophobic effects and hydrogen bonding, along with the formation of covalent bonds. Consequently, their antimicrobial mechanism may involve the inactivation of enzymes, microbial adhesins, and convey proteins in the cell envelope. Consequently, the it was showed that *Hippophae salicifolia* might act as valuable resources of bioactive antimicrobial substances for applied as natural nutraceutical products and natural preservatives (Gupta *et al*., 2011). The research on antimicrobial activities of a lone plants species might be yield various results based on numerous aspects. The herbs bioactivity might be affected by precise components of the plants utilized for extraction, as various parts might possess distinctive substances. Moreover, geological places act as noteworthy actions, as various climates conditions, stress factors, climate outlines and soil kinds can influence the ingredients and efficiencies of these substances (Ashneel *et al*., 2023).

The system by which the fruits extracts exercise its antibacterial activities are diverse; they might prevent protein synthesis, DNA synthesis inhibition, cell membrane disruption and among other actions (Netreba *et al*., 2024). The antimicrobial activities of Sea buckthorn extracts are probable accredited to their capabilities to synthesize complexes with extracellular and soluble proteins, as well as microbes cell walls, during nonspecific connections such as hydrophobic and hydrogen bonding, in adding formation of covalent bond. Therefore, its antibacterial mechanisms might be engage the inactivation of enzymes, microbial adhesions and convey proteins within the cell membrane, among other targets. There is also evidence suggesting that these extracts can directly inactivate microorganisms (Gupta *et al*., 2011). Sea buckthorn has the potential to serve as a natural alternative to artificial additives in foodstuff products, offering purposeful benefits (Netreba *et al*., 2024).

The findings could enhance the acceptance of *Hippophae rhamnoides* as a valuable source for the pharmaceutical, foodstuff, agriculture, and cosmetics industries. Its antibacterial properties position Sea buckthorn as a cost-effective raw material for natural antibacterial agents in the food, agricultural, pharmaceutical, and medical sectors, particularly in developing countries like Pakistan

Conclusion

With the rapid pace of modern life and profit-driven industries that reduce production and distribution costs through the use of preservatives, additives, and antibiotics, many people are consuming more fast food and convenience meals. This trend often leads to significant health issues and a weakened immune system among the general population. Over the past few decades, there has been a growing interest in organic food. In this context, it is crucial to develop reliable natural alternatives to synthetic compounds, to reduce the reliance on synthetic additives and antibiotics as preservatives. The outcomes revealed that extracts of *Hippophae rhamnoides* berries have effective antibacterial behavior that would have useful possessions on human being fitness, and the extract of methanol is better with better antimicrobial activities. The pronounced antibacterial properties of *Hippophae rhamnoides* parts designate the significance of this plant appliance in agricultural practice and health promotion.

Fig. 1. Sea buckthorn Berries

Table 1. Extractive values of Sea buckthorn with different solvents

	Extract Yield (% w/w)		
	Whole berries	**Pulp**	**Tea**
Methanol	17±0.1	14±01	18±01
Ethanol	13±0.2	10±0.5	15±0.3
Ethanol 70%	12±0.5	11±0.2	16±0.4
Infusion	06±0.3	05±0.11	08±0.5
Decoction	07±01	05±0.1	09±0.2

Each value is a mean of three replicates ±standard deviation.

Table 2. Phytochemicals Screening of Sea buckthorn.

Phytochemicals	**Plant Parts**	**Ethanol**	**Methanol**	**Ethanol 70%**	**Infusion**	**Decoction**
Glycosides	Whole berries	+	++	++	+	+
	Pulp	+	++	++	+	+
	Tea	++	++	++	+	+
Tannins	Whole berries	++	+++	+++	+	++
	Pulp	+	++	++	+	+
	Tea	++	+++	+++	+	++
Saponins	Whole berries	+	++	+	ND	ND
	Pulp	+	++	++	+	+
	Tea	++	+++	+++	+	+
Flavonoids	Whole berries	++	+++	++	+	++
	Pulp	+	++	+	+	+

	Tea	++	+++	+++	+	+
Phenolics	Whole berries	++	+++	+++	+	++
	Pulp	+	++	+	+	+
	Tea	++	+++	+++	+	+
Terpenoids	Whole berries	ND	++	++	+	+
	Pulp	+	+	+	**ND**	**ND**
	Tea	++	++	++	+	+

ND = not detected; + symbol illustrate phytochemicals finding level in extracts; + + + = large quantity; + + = average quantity; + = small quantity.

Table 3. Antibacterial Activity of Sea buckthorn Tea

Bacteria	**Extracts/Zone of Inhibition in millimeter (mm)**				
	Ethanol	**Methanol**	**Ethanol 70%**	**Infusion**	**Decoction**
Gram-positive					
Bacillus cereus	12±1	19±1	16±0	12±1	14±0
Listeria monocytogenes	13±1	17±0	15±0	10±0	11±0
Staphylococcus aureus	14±1	18±0	13±1	11±0	13±1
Gram-negative					
Escherichia coli	10±1	15±0	14±1	10±0	11±0
Salmonella enterica	09±01	13±1	10±1	--	--

Data are mean (n=3) ± SD, -- = No zone of inhibition. Different superscripts in each column indicate significant differences in the mean at P < 0.05.

Table 4. Antibacterial Activity of Sea buckthorn Whole Berries

Bacteria	**Extracts/Zone of Inhibition in millimeter (mm)**				
	Ethanol	**Methanol**	**Ethanol 70%**	**Infusion**	**Decoction**
Gram-positive					
Bacillus cereus	11±1	18±1	15±0	11±1	13±0
Listeria monocytogenes	12±1	16±0	14±0	09±0	10±0
Staphylococcus aureus	13±1	15±0	12±1	10±0	12±1
Gram-negative					
Escherichia coli	09±1	14±0	13±1	09±0	10±0
Salmonella enterica	08	12±1	09±1	--	--

Data are mean (n=3) ± SD, -- = No zone of inhibition. Different superscripts in each column indicate significant differences in the mean at P < 0.05.

Table 5. Antibacterial Activity of Sea buckthorn Pulp

Bacteria	**Extracts/Zone of Inhibition in millimeter (mm)**				
	Ethanol	**Methanol**	**Ethanol 70%**	**Infusion**	**Decoction**
Gram-positive					
Bacillus cereus	09±1	16±1	13±0	09±1	10±0
Listeria monocytogenes	10±1	14±0	12±0	08±0	09±0
Staphylococcus aureus	12±1	15±0	11±1	07±0	10±1
Gram-negative					
Escherichia coli	08±1	12±0	11±1	07±0	08±0
Salmonella enterica	07±	11±1	08±1	--	--

Data are mean (n=3) ± SD, -- = No zone of inhibition. Different superscripts in each column indicate significant differences in the mean at P < 0.05.

Table 6. Minimum Inhibitory Concentration (MIC) of Sea buckthorn Tea.

Bacteria	**Extracts/Zone of Inhibition in millimeter (mm)**				
	Ethanol	**Methanol**	**Ethanol 70%**	**Infusion**	**Decoction**
Gram-positive					
Bacillus cereus	31.25	7.8125	15.625	31.25	15.625
Listeria monocytogenes	31.25	7.8125	15.625	62.5	62.5
Staphylococcus aureus	15.625	7.8125	31.25	62.5	62.5
Gram-negative					
Escherichia coli	62.5	15.625	15.625	125	62.5

Salmonella enterica	125	62.5	125	NA	NA

NA=Not applied.

Table 7. Minimum Inhibitory Concentration (MIC) of Sea buckthorn Whole Berries.

Bacteria	**Extracts/Zone of Inhibition in millimeter (mm)**				
	Ethanol	**Methanol**	**Ethanol 70%**	**Infusion**	**Decoction**
Gram-positive					
Bacillus cereus	62.5	7.8125	15.625	125	62.5
Listeria monocytogenes	31.25	15.625	15.625	250	250
Staphylococcus aureus	62.5	62.5	62.5	125	62.5
Gram-negative					
Escherichia coli	125	31.25	31.25	250	250
Salmonella enterica	125	31.25	125	NA	NA

NA=Not applied.

Table 8. Minimum Inhibitory Concentration (MIC) of Sea buckthorn Pulp.

Bacteria	**Extracts/Zone of Inhibition in millimeter (mm)**				
	Ethanol	**Methanol**	**Ethanol 70%**	**Infusion**	**Decoction**
Gram-positive					
Bacillus cereus	125	62.5	62.5	125	125
Listeria monocytogenes	62.5	62.5	125	250	125
Staphylococcus aureus	62.5	62.5	125	250	125
Gram-negative					
Escherichia coli	250	250	250	500	500
Salmonella enterica	500	250	500	NA	NA

NA=Not applied.

References

Daniela Cojocari. 2022. In vitro antibacterial effect of various berries on Listeria monocytogenes as food borne pathogen. Agrobiodivers Improv Nutr Health Life Qual, 6, 2022(1): 67–74. https://doi.org/10.15414/ainhlq.2022.0008.

Ahmet M, Nimet Y, Demet Y, Ali K. 2011. Antioxidant and antimicrobial activity of Turkish endemic *Sonchus erzincanicus* extracts. Turk J Biol., 35: 243-250.

Anelise SNF, Carla RFV, Matheus S, Claudia ALC, Maria CV, Zefa VP. 2014. Evaluation of Antioxidant Activity, Total Flavonoids, Tannins and Phenolic Compounds in *Psychotria L*eaf Extracts. Antioxidants., 3: 745-757.

Ashneel Ajay Singha, Zafiar Tasmeen Naaz, Edward Rakaseta, Marcha Perera, Vrinda Singh, Wilson Cheung, Francis Mania, Swastika Nath. 2023. Antimicrobial activity of selected plant extracts against common food borne pathogenic bacteria. Food and Humanity. Vol 1, pp. 64–70.

Bashir A, Javid A. 2013. Physiochemical, minerals, phytochemical contents, antimicrobial activities evaluation and fourier transform infrared (FTIR) analysis of *Hippophae rhamnoides L.* leaves extracts. Afr J Pharm Pharacol., 7(7): 375-388.

Camelia P, Cristiana D, Nicorescu V. 2008. Antioxidant activity of sea buckthorn (*Hippophae Rhamnoides*) extracts compared with common food additives. Rom Biotech Lett., 13(6): 4049-4053.

Chan EWC, Lim Y, Omar M. 2007. Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. Food Chem., 104(4):1586–1593.

Chauhan, S., Varshneya, C. 2012. The profile of bioactive compounds in sea buckthorn berries and oil. Int. J. Theor. Appl. Sci., 4: 216–220.

Cowan MM., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. Vol 12,pp. https://doi.org/10.1128/cmr.12.4.564.

Egon S (1969). Thin Layer Chromatography: A Laboratory Hand-book. Toppan Company, Ltd., Tokyo, Japan, pp. 873-904.

Eva Ivanisova, Martina Blaskova, Margarita Terentjeva, Olga Grygorieva, Olena Vergun, Jan Brindza, Miroslava Kacaniova. 2020. Biological Properties of Sea Buckthorn (*Hippophae Rhamnoides* L.) Derived Products. Acta Sci. Pol. Technol. Aliment. 19(2):195–205. Doi.org/10.17306/J.AFS.2020.0809.

Georgescu, C.; Frum, A.; Virchea, L.-I.; Sumacheva, A.; Shamtsyan, M.; Gligor, F.-G.; Olah, N.K.; Mathe, E.; Mironescu, M. 2022. Geographic Variability of Berry Phytochemicals with Antioxidant and Antimicrobial Properties. Molecules. 27: 4986. https:// doi.org/10.3390/molecules27154986.

Gill NS, Sharma R, Arora R, Bali M. 2012. Antioxidant and Antibacterial Activity of *Hippophae rhamnoides* Leaf Extracts from dry Temperate Agro-climatic Region of Himachal Pradesh. J Plant Sci. DOI: 10.3923/jps.2012.

Guo, R., Chang, X., Guo, X., Brennan, Ch. S., Li, T., Fu, X., Liu, R H. 2017. Phenolic compounds, antioxidant activity, antiproliferative activity and bioaccessibility of sea buckthorn (*Hippophae rhamnoides* L.) berries as affected by in vitro digestion. Food Funct., 8, 4229. Doi.org/10.1039/C7FO00917H.

Gupta, S. M., Gupta, A. K., Ahmed, Z., Kumar, A. 2011. Antibacterial and antifungal activity in leaf, seed extract and seed oil of sea buckthorn (*Hippophae salicifolia* D. Don) plant. J. Plant Pathol. Microbiol., 2, 105. https:// doi.org/10.4172/2157-7471.1000105.

Harshit V, Mandeep S, Rajesh C, Akanksha P. 2013. Assessment of antimycotic activity of seabuckthorn (*Hippophae rhamnoides*) leaf exacts against common fungi associated with skin dermatitis. Vet World., 6(4): 205-208.

Harshit V, Mandeep S, Rajesh C, Akanksha P. 2013. Assessment of antimycotic activity of sea buckthorn (*Hippophae rhamnoides*) leaf exacts against common fungi associated with skin dermatitis. Vet World. 6(4): 205-208. doi:10.5455/vetworld.2013.205-208.

Hisano, M.; Bruschini, H.; Nicodemo, A.C.; Srougi, M. 2012. Cranberries and lower urinary tract infection prevention. Clinics. 67: 661–667.

Horborne JB. 1998. Phytochemical Methods- A guide to modern techniques of plant analysis. 3rd Edition, Chapman and Hall Int. Ed., New York.

Javid A, Bashir A. 2015. Comparative antitumor and anti-proliferative activities of *Hippophae rhamnoides* L. leaves extracts. J Coast Life Med., 3(3): 228-232.

Javid Ali, Bashir Ahmad, Said Hassan, Muhammad Siddique, Farrah Gul, Shafaat Ullah. 2015. Proximate composition, mineral contents, phytochemical constituents, antimicrobial activities and Fourier transforms infrared spectroscopy analysis of bark, stem and seed of *Hippophae rhamnoides* Linn. Journal of Coastal Life Medicine 2015; 3(6): 486-490. Doi: 10.12980/JCLM.3.2015J5-2.

Jong HJ, Ji WL, Kyoung SK, Ju-Sung K, Sang NH, Chang YY, Ju KL, Yong SK, Myong JK. 2010. Antioxidant and antimicrobial activities of extracts from a medicinal plant, Sea Buckthorn. J Korean Soc Appl Biol Chem., 53(1): 33-38.

Koohsari, H., Chaemi, E. A., Sadesh Sheshpoli, M., Jahedi, M., Zahiri, M. 2015. The investigation of antibacterial activity of selected native plants from North of Iran. J. Med. Life, 8, 38–42.

Kumar, A.; P, N.; Kumar, M.; Jose, A.; Tomer, V.; Oz, E.; Proestos, C.; Zeng, M.; Elobeid, T.; K, S.; et al. 2023. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. 28, 887. https://doi.org/ 10.3390/molecules28020887.

Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK, Staden JV. 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol., 68: 267-274.

Mahesha MP., Kanivebagilu AV., Airody VA. 2015. Extraction, characterization and biological studies of phytochemicals from *Mammea suriga*. Journal of Pharmaceutical Analysis. 5(3):182–189. Doi.org/10.1016/j.jpha.2015.01.002.

Michel, T., Destandau, E., Le Floch, G., Lucchesi, M. E., Elfakir, C. 2012. Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (*Hippophae rhamnoides* L.) leaf, stem, root and seed. Food Chem., 131, 754–760. Doi.org/10.1016/j.food chem.2011.09.029.

Mousmi S, Handique PJ. 2013. Antioxidant and antibacterial activity of leaf, bark, pulp and seed extracts of sea buckthorn (*Hippophae salicifolia* D. Don) of Sikkim Himalayas. J Med Plants Res., 7(19):1330-1338.

Nazir, F., Salim, R., Yousf, N., Bashir, M., Naik, H. R. and Hussain, SZ. 2017. Natural antimicrobials for food preservation. J. Pharma. Phytoch. 6(6): 2078–2082.

Negi, P.S., Chauhan, A.S., Sadia, G.A., Rihinishree, Y.S., Ramteke, RS. 2005. Antioxidant and antibacterial activities of various sea buckthorn (*Hippophae rhamnoides* L.) seed extracts. In Food Chemistry. 92: 119 124. Doi.org/10.1016/j.foodchem.2004.07.009.

Netreba, N.; Sandulachi, E.; Macari, A.; Popa, S.; Ribintev, I.; Sandu, I.; Boestean, O.; Dianu, I. 2024. A Study on the Fruiting and Correlation between the Chemical Indicators and Antimicrobial Properties of *Hippophae rhamnoides* L. Horticulturae. 10: 137. https://doi.org/10.3390/ horticulturae10020137.

Nishat Anjum and Y.C. Tripathi. 2015. Flavonoid Constituents, Total Polyphenol and Antioxidant Efficacy of *Hippophae rhamnoides* L. Berries. Proceedings of 7th Conference of the International Seabuckthorn Association on "Seabuckthorn: Emerging Technologies for Health Protection and Environmental Conservation" V. Singh, Ed. In-Chief-2015: pp. 383-393. New Delhi-India.

Qadir, M. I., Abbas, K., Younus, A., Shaikh, RS. 2016. Report – Antibacterial activity of sea buckthorn (*Hippophae rhamnoides* L.) against methicillin resistant *Staphylococcus aureus* (MRSA). Pakistan J. Pharm. Sci., 29(5): 1711–1713.

Richa Arora, Sunil Mundra, Ashish Yadav, Ravi B. Srivastava and Tsering Stobdan. 2012. Antimicrobial activity of seed, pomace and leaf extracts of sea buckthorn (Hippophae rhamnoides L.) against foodborne and food spoilage pathogens. African Journal of Biotechnology Vol. 11(45), pp. 10424-10430, 5 June, 2012. DOI: 10.5897/AJB11.4150.

Saadia Chaman, Nawazish-I-Hussain Syed, Zeeshan Danish and Farrakh Zia Khan. Phytochemical Analysis, Antioxidant and Antibacterial Effects of Sea Buckthorn Berries. Pak. J. Pharm. Sci., Vol.24, No.3, July 2011, pp.345-351.

Saikia, M., Handique, PJ. 2013. Antioxidant and antibacterial activity of leaf and bark extracts of Seabuckthorn (*Hippophae salicifolia* D Don) of North East India. Int. J. Life Sci. Biotechnol. Pharma Res., 2, 81–91.

Sandulachi, E.; Macari, A.; Cojocari, D.; Balan, G.; Popa, S.; Turculet, N.; Ghendov-Mo¸ sanu, A.; Sturza, R. 2022. Antimicrobial properties of sea buckthorn grown in the Republic of Moldova. J. Eng. Sci. 29: 164–175.

Selvamuthukumaran M, Farhath K. 2014. Evaluation of shelf stability of antioxidant rich seabuckthorn fruit yoghurt. Int Food Res J., 21(2): 759-765.

Soheir S. Abd-El Salam, Mohamed F. Ghaly, Mohamed H. Yassin, Attia A.A. and Sally E. Sallam. 2018. Plant Extracts as Inhibitors of Foodborne Pathogenic Bacteria. Egypt. J. Microbiol. Vol. 53, pp. 127 – 139. DOI: 10.21608/ejm.2018.3902.1058.

Sonu R Tanwar, Pratiksha R Phadke, Hrutuja M Maydeo, Kirti J Mhatre. 2022. Antibacterial Activity of Sea Buckthorn (*Hippophae rhamnoides* L.) against Pathogenic Microbes. International Journal of Advanced Research in Science, Communication and Technology. Volume 2, Issue 3, pp. 211-215. DOI: 10.48175/IJARSCT-3089.

Sonu R Tanwar, Pratiksha R Phadke, Hrutuja M Maydeo, Kirti J Mhatre. 2022. Antibacterial Activity of Sea Buckthorn (*Hippophae rhamnoides* L.) against Pathogenic Microbes. Volume 2, Issue 3:pp. 211-215. DOI: 10.48175/IJARSCT-3089.

Stephen KSIM. 1967. *Medicinal Plant Glycosides* (pp. 1–9). University of Toronto Press. http://www.jstor.org/stable/10.3138/j.ctvfrxh8t.4

Suriyaprom, S.; Mosoni, P.; Leroy, S.; Kaewkod, T.; Desvaux, M.; Tragoolpua, Y. 2022. Antioxidants of Fruit Extracts as Antimicrobial Agents against Pathogenic Bacteria. Antioxidants. 11: 602.

Tagousop, C.N., Tamokou, JdD., Kengne, I.C. *et al.* 2018. Antimicrobial activities of saponins from *Melanthera elliptica* and their synergistic effects with antibiotics against pathogenic phenotypes. *Chemistry Central Journal*. **12**, 97. https://doi.org/10.1186/s13065-018-0466-6.

Tamas-Krump OM., Adriana U., Rodica M., Cornelia DA., Otilia B., Latiu C., Luca VE., Ognean L.2020. Antibacterial Potential of Honeydew Honey in Combination with Natural Oils. Agricultura.1-2:113-114. DOI: 10.15835/agrisp.v113i1-2.13692.

Wu, B., Liu, X., Nakamoto, S.T., Wall, M. 2022. Antimicrobial activity of ohelo berry (*Vaccinium calycinum*) juice against Listeria monocytogenes and its potential for milk preservation. In Microorganisms. 10, 548. https://doi.org/0.3390 microorganisms10030548.

Yogendra Kumar, M. S., Tirpude, R. J., Maheshwari, D. T., Bansal, A., Misra, K. 2013. Antioxidant and antimicrobial properties of phenolic rich fraction of sea buckthorn (*Hippophae rhamnoides* L.) leaves in vitro. Food Chem., 141, 3443–3450. Doi.org/10.1016/j.foodchem.2013.06.057.

Yue, X.-F., Shang, X., Zhang, Zh.-J., Zhang, YN. 2017. Phytochemical composition and antibacterial activity of the essential oils from different parts of sea buckthorn (*Hippophae rhamnoides* L.). J. Food Drug Anal. 25(2):327–332. Doi.org/10.1016/j.jfda.2016.10.010.

Zakia Khanam, Chew Shwu Wena, Irshad Ul Haq Bhat. 2015. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). Journal of King Saud University–Science. 27:23–30. http://dx.doi.org/10.1016/j.jksus.2014.04.006.

Declaration

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Authors contribution

JA**:** Conceptualization of the study, experimental design, and manuscript writing. AH**:** Data acquisition, statistical analysis, and result interpretation. HS**:** Supervision of laboratory procedures and validation of experimental protocols. MS; BJA; MY; RZ; and AM**:** Literature may be fictitious or non-fictitious, a key revision of the manuscript, and a final blessing of the satisfied. IU**:** Technical support, resource management, and administrative oversight. All authors have read and approved the final manuscript.

Conflict of Interest

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Data Availability statement

All authenticated data have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this paper.

Consent for publication

Not applicable