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Hematology

Evaluation of Antibacterial Activity of Sunflower Seed Extract on *Listeria monocytogenes and Shigella sonnei* Associated with Food-Borne- Infections

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Abstract **Original Research Article**

Introduction: The emerging upsurge in the rate of resistance of bacteria to conventional antibiotics and the high cost of orthodox medical treatment underpinned the need for medicinal plant as potential alternative therapy. This study aimed at evaluating the antibacterial activity of sunflower seed extract in the treatment of diarrhea. Ethanol and aqueous seed crude extract were screened for antibacterial activity in-vitro against *Listeria monocytogenes* and *Shigella sonnei.* Prior to the screening, plant phytochemical screening was conducted using standard methods. The antibacterial activity was carried out using agar well diffusion method and compared to the standard antibiotic ciprofloxacin. The minimum bactericidal concentration was determined by plating out from microtiter plates with no visible growth. The results of phytochemical screening reveal the presence of alkaloids, flavonoids, saponins, cardiac glycoside, anthraquinones and resins from prepared crude extract of sunflower. The ethanolic extract had antibacterial activity against the tests organisms with diameter zone of inhibition range from 31mm at 15.63mg/ml. This provides evidence for its usage as an alternative remedy for the treatment of diarrhea caused by *Listeria monocytogenes* and *Shigella sonnei*.

Keywords: Sunflower seeds, antibacterial, *Listeria monocytogenes* and *Shigella sonnei*.

BACKGROUND OF THE STUDY

Since the beginning of human civilization, foodborne infections have been a major cause of concern to public health. Foodborne pathogens are the leading cause of food poisoning and foodborne infections therefore constitute a major threat to food safety (WHO, 2015) [1]. In recent years, there has been an upsurge in the incidence of infections caused by foodborne bacteria, and this has become a key and significant worldwide health concern (Kaferstein, 2003) [2]. Food-contaminating pathogens have gained a great deal of interest, since they are now responsible for a staggering mortality rate of 420,000 per year (WHO, 2015) [3]. The Center for Disease Control stated that among the microorganisms responsible for food poisoning are; *Escherichia coli, Staphylococcus aureus, Shigella sonnei, Listeria spp., Clostridium perfringens, Campylobacter spp., and Salmonella spp.* (Scallan *et al.*, 2011) [4].

Listeria monocytogenes causes Listeria infection, which has negative consequences' especially among pregnant women as it increases the chances of miscarriage, among infant the Listeria infection has a high mortality rate. Although the incidence of this disease is generally low, it can have serious and even deadly repercussions for the health of newborns, children, and the elderly. *Listeria monocytogenes* is most especially found unpasteurized dairy products; it affects soft cheeses, prepackaged salad, and a variety of ready-to-eat meals; and it may flourish in refrigerated environments (WHO, 2015) [5].

Shigellosis is an acute diarrheal illness caused by four species of Shigella bacteria: *S. dysenteriae, S. flexneri, S. boydii*, and *S. sonnei* (Baker, 2018). Due to their distinctive characteristics, pathogenesis, and evolutionary history, *Shigella* species stand apart from other Enterobacteriaceae (Kotloff *et al.,* 2018) [6] Japan's Kiyoshi Shiga first isolated the highly virulent strain of *Shigella dysenteriae* that produces exotoxins in 1897 (Trofa *et al.,* 1999; Lampel *et al.,* 2018). *Shigella flexneri* was discovered in 1899, *Shigella sonnei* by Carl Olaf Sonne in 1906, and *Shigella boydii* was discovered in 1921 (Barceloux, 2008) [10]. *Shigella* species has additional serotypes and sub-serotypes due to its diverse antigenicity based on lipopolysaccharides (LPS) and cell wall O-antigen components. *Shigella dysenteries* (formerly *Shigella bacillus*) serotype 1 has 15 types, *Shigella flexneri* has 19, *Shigella boydii* has 20, but *Shigella sonnei* has only one serotype (Zaidi *et al*., 2014).

According to several studies, the geographic distribution of the *Shigella* spp. depends on how developed each country's economy is (Hale and Keusch 1996) [11]. A more recent genomic study revealed that *Shigella sonnei* originated from *Escherichia coli* in central Europe around the year 1500 AD and spread throughout the world via travelers (WHO., 2016). *Shigella sonnei* is now showing dominance over *Shigella flexneri* as a result of a number of mechanisms and is significantly dependent on the economic and industrial development of regions (WHO., 2016). *Shigella flexneri* infection rates are declining, but *Shigella sonnei* infections are on the rise and are now primarily found in low- and middle-income (LMIC) countries and Nigeria is not an exception (Lee *et al*., 2017).

Due to antibacterial resistance of *Shigella sonnei* and *Listeria monocytogenes* to antibiotics, research has shifted to plants for treatment. This project emphasizes on the use of sunflower seed extract as a medicinal plant.

Sunflower, *Helianthus annuus*, is a member of the Asteraceae family. There are 65 distinct species in the Helianthus genus (Andrew *et al.,* 2013). The name Helianthus, which derives from the Greek words helios (the sun) and anthos (a flower), means "sunflower" in English.

In all of North America, American Indian tribes frequently grew sunflower. According to available data, the plant was grown by locals in what are now Arizona and New Mexico around 3000 BC. Sunflower has been speculated to have been domesticated before corn by certain archaeologists (NSA, 2013) [15].

Helianthus annuus is primarily found in Nigeria today in the edges of farmlands, lawns, and the adjacent savanna and woodland zones. Its presence has been documented and confirmed in five states of south-western Nigeria: Lagos, Ogun, Oyo, Osun, and Ondo. However, it is noticeable around river courses and along roadside in the derived savannah of Guinea. States like Kaduna, Plateau, Akwa Ibom, Rivers, Cross River, and parts of Delta, Imo, and Anambra have reported seeing it (Perkins, 2002) [16].

STATEMENT OF PROBLEM

Due to rising standards of living, rising costs of health care to combat diseases and the bacteria causing these diseases, the complexity of available drugs, the resistances of microorganisms, and the finances to treat diseases in developing countries and locales (especially Nigeria) have been on the rise (Yousef *et al*., 2012) [17].

Listeria spp. isolated from soft cheese (wara) in Ekiti, South-West Nigeria, demonstrated 90 and 89 percent resistance to ampiclox (ampicillin/cloxacillin) and amoxicillin, respectively. (Oyinloye, 2016) [18].

In 2017, the World Health Organization (WHO) published its initial list of pathogens resistant to antibiotics (Tacconelli, 2017) [19]. Among the other 12 families of bacteria that pose a significant threat to the public's health, *Shigella* was a priority pathogen (Tillotson, 2018) [20].

The Research Hypothesis include:

H_o: Sunflower seed extract alongside the positive fluoroquinolone control have no significant levels of phytochemical constituents nor antibacterial activity on *Listeria monocytogenes* and *Shigella sonnei* as no correlation existed between the mean MIC tested for the two representative Gram positive and Gram negative organisms respectively

H_a: Sunflower seed extract alongside the positive fluoroquinolone control have significant levels of antibacterial activity on *Listeria monocytogenes* and *Shigella sonnei* as high correlation existed between the mean MIC tested for the two representative Gram positive and Gram negative organisms respectively.

LITERATURE REVIEW

SUNFLOWER *(Helianthus annuus* (L.))

One of the top seeds in the world for producing oil is the sunflower seed (*Helianthus annuus)*. The plants can withstand colder climates and are drought-resistant. Sunflower seeds are typically visible at the apical region after maturation. The outer layer of the seeds is referred to as epicarp, mesocarp the middle layer the endocarp, which is the innermost layer. The seeds are enclosed in an achene, which is made of a shell covering the kernel, made of lignin and cellulolytic components this makes about 80% of its overall weight. Uncooked sunflower seeds typically have approximately 25% oil, but thanks to plant breeding, it has 40% more added. Cold extraction can be used to obtain the seed oil as well as hot-pressing.

Sunflower seeds are high in vitamins E, B, folate, and niacin, as well as minerals calcium, copper, iron, magnesium, manganese, selenium, phosphorous, potassium, sodium, and zinc. Sunflower is known for its anti-inflammatory properties. Sunflower seeds are known to be high in vitamin E and magnesium, making them an excellent source of fat-soluble antioxidants for the body (Kumar *et al.,* 2017) [21]. Vitamin E's antioxidant qualities neutralize the free radicals that can harm fat-containing structures and molecules, including cholesterol, brain cells, and cell membranes (Nowicka & Kruk, 2017) [22].

Sunflower oil's main fatty acid components are oleic, stearic, linoleic, and palmitic acid. Carotenoids, waxes, lecithin, and tocopherols are also found in sunflower oil. (Kozłowska & Gruczyńska, 2018).23 Sunflower seeds have been shown to be medically beneficial for colds and coughs, as a substitute for quinine, as an anti-malaria agent, and as a diuretic and expectorant (Islam *et al.,* 2016) [24].

ACTIVE CONSTITUENTS OF SUNFLOWER AND ITS' HEALTH BENEFITS

Sunflower's health benefits extend beyond its antioxidant and anti-inflammatory characteristics, as well as its positive impact on cardiovascular health. It is also suspected to have anticancer capabilities. Selenium, a trace element, is abundant in sunflower seeds. This component is critical for boosting the body's defenses against malignant cells (Roy *et al.,* 2015) [25]. Numerous studies have revealed the intervention trials of the inverse connection between selenium intake and cancer growth in animal models (Cardoso *et al.,* 2017; Khan *et al.,* 2015) [26]. The presence of selenium in sunflower has caused DNA repair and generation in degraded cells, suppression of the growth of cancer cells, and stimulation of apoptosis, the self-destruction of chromosomes in the body in order to eliminate undesired or worn-out cells. In addition, the incorporation of selenium into the active areas of proteins such as glutathione peroxidase protects body cells from cancer (Pisoschi and Pop, 2015) [27].

Utilizing sunflower in the therapy or reduction of asthma and diabetes risk factors has proven their health benefits. Sunflower extracts have shown anti-asthmatic and anti-diabetic effects, according to reports (Gad and El-Ahmady, 2018) [28]. It has been observed that rats given oral dose of ethanolic extracts of sunflower seeds extract had an antihyperglycemic impact on their blood sugar levels (Saini & Sharma, 2013) [29]. In vivo antiasthmatic experiment of sunflower aqueous extract on ovalbumin-induced mice and hematoxylin and eosin staining of their lungs showed the extract's ability to reduce asthma in mice (Kim *et al.,* 2020) [30].

Sunflower seeds are considered to contain phytosterols with a chemical structure similar to that of cholesterol, and the presence of phytosterols in high quantities in the human diet tends to reduce blood levels of cholesterol, boost immunological response, and lower cancer risk factors (Farahmandfar *et al.,* 2018) [31].

Tocopherol and phytosterol, which are found in standard sunflower oil, are interesting because they have therapeutic effects on human health by lowering total plasma cholesterol and low-density lipoprotein (LDL) cholesterol levels (Rani *et al.,* 2017) [32].

It has been claimed that sunflower seeds contain high levels of total phenolic compounds and tocopherols, active substances that have been used to stabilize foods containing fat and support critical biological processes related to oil bodies. Because they act as anti-inflammatory, anticancer, and antimicrobial agents, natural antioxidants from plant seeds have drawn a lot of attention as a source of bioactive substances with many health benefits (Menzel *et al.,* 2019). [33].

Methanolic sunflower seed extracts have been shown to have antimicrobial activity against some pathogenic Gram-positive and Gram-negative bacteria, including those that could cause food-borne illness, such as *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* (Menzel *et al.*, 2019) [34]. As a result, it is critical that sunflower extract be used as a natural food preservative agent (Thielmann, *et al.*, 2017) [35].

Its' being reported by Ahmad *et al.*, 2016 that *Helianthus annuus* methanol seed extract possess antimicrobial properties against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi,* and *Vibrio cholera. Helianthus annuus* seed extract had a high susceptibility/sensitivity effect on *Salmonella typhi,* a medium sensitivity effect on *Staphylococcus aureus* and *Vibrio cholera*, and a low sensitivity to *Bacillus subtilis*. (Ahmad *et al.,* 2016) [36]. According to the findings of Ahmad *et al.*, (2015) [37] and Bashir *et al*., (2015) [38], sunflower seeds have the ability to prevent bacteria from growing in water. As a result, it is believed that the use of sunflower plants (Helianthus annuus) for domestic water treatment is critical, particularly at the household level in underdeveloped rural communities.

SCIENTIFIC CLASSIFICATION OF *Helianthus annuus*

Kingdom: Plantae
Order: Asterales
Family: Asteracaea
Genus: *Helianthus*
Species: *annuus*

Figure 1: A picture of sunflower plant from
Source: www.healthline.com

Figure 2: A picture of sunflower seeds from www
Source: www.healthline.com

COMPLICATIONS OF *Listeria monocytogenes INFECTION*

Listeria monocytogenes is a foodborne pathogen that causes the illness listeriosis. Literature had it that in the United States, an estimated 1600 illnesses annually result due to this bacterium and about 2500 confirmed invasive human cases have been reported in countries of the European Union (EFSA and ECDC, 2019) [39] with an elevated mortality rate of about 20% in at-risk population.

Listeriosis outbreaks are linked to a wide range of foods, including meat, dairy products, and fresh produce. *Listeria monocytogenes* can flourish in unfavorable environmental conditions like low pH, low temperature, and high salt concentration. This trait makes it more likely to contaminate and grow on food products and presents some control challenges (Alan *et al.,* 2015; [40], Bahrami *et al.,* 2020 [41] and Rothrock *et al.,* 2019) [42]. As a result*, Listeria monocytogenes* in food is still regarded as a serious global food safety issue.

Most African countries, including Nigeria, have poor information on the epidemiology of listeriosis (Enurah *et al*., 2013) [43] compared to Europe and the United States, with few reports (Molla *et al*., 2004) [44].

PATHOGENESIS OF FOOD LISTERIOSIS

Any foodborne disease's pathogenesis depends on the pathogen's capacity to endure and spread from its animal or environmental source to humans via food. Food, and its inherent qualities like pH and water activity, as well as factors like how the food is processed, stored, and prepared, will affect a foodborne pathogen's ability to cause disease. As a result, food plays a central role in the pathogenesis of a foodborne disease like listeriosis. In order to survive and spread, a pathogen must also interact with its environment, whether it be the environment of a host, food, or the natural world. Each stage of the infectious process will be influenced by factors unique to the pathogen, host, and environment. However, in the end, it is their interactions that define the pathogenesis of a foodborne illness like listeriosis in the most comprehensive way [44].

SYMPTOMS

Fever, diarrhea, and vomiting are common gastroenteritis symptoms. The average incubation period before symptoms manifest is 18 to 20 hours. Because humans can be asymptomatic carriers, it is challenging to establish with certainty the frequency of noninvasive gastrointestinal listeriosis (Slutsker and Schuchat,1999) [45].

In humans, symptoms of neuropathic listeriosis can include fever, malaise, ataxia, seizures, altered mental status, meningitis, and encephalitis, while those of septicemic listeriosis typically include fever, malaise, fatigue, and abdominal pain. Adults with underlying immunosuppression are the ones who contract invasive listeriosis in humans most frequently. These people include the elderly, women who are expecting, people who have cancer, people who have had organ transplants, people who have AIDS, and anyone who is undergoing immunosuppressive therapy. Additionally, newborns are particularly vulnerable to contracting invasive listeriosis from an infected mother. The onset of listeriosis in newborns can be either early or late (Slutsker and Schuchat, 1999) [46].

TREATMENT

Ampicillin, Amoxicillin, and Penicillin G were once the recommended treatments, but resistance has been noted; this is why the study was conducted to determine whether the extract has antibacterial activity [46].

Shigella sonnei

Shigella sonnei, a Gram-negative pathogenic bacteria with a facultative anaerobic state, is the main culprit behind the endemic bacillary dysentery condition known as shigellosis (Niyogi 2005; CDC 2013) *Shigella sonnei* infection of the gastrointestinal tract may result in severe fluid loss, fever, nausea, vomiting, diarrhea, and abdominal cramps (Niyogi, 2005) [47].

TRANSMISSION

Serious outbreaks and transmission are caused by the extremely low infectious dose rate of 1–100 cells. Water, food, wild animals, birds and insects are among the reservoirs and modes of transmission is via contaminated food and water (Bridle, 2013) [48]. The main host is humans, and fecal-oral route of transmission is the main method of transmission. When compared to other bacterial entero-pathogens, *Shigella* spp. infections are the most contagious and are thought to be the most infectious. (DuPont, 2014) [49] however, the bacterium cannot continue to spread without a host for very long (Niyogi, 2005) [50].

TREATMENT

WHO had recommended fluoroquinolone for the purpose of effective treatment, But fluoroquinolone resistance has also emerged as a result of their extensive use as antibiotics. Fluoroquinolone-resistant *Shigella sonnei* first appeared in the late 1990s (The and Baker, 2018) [51].

BRIEF HISTORY OF MEDICINAL PLANTS

Most of our ancestors came from animistic cultures, which believed that all things- including plants- hold a spirit. And this is also true today: indigenous people worldwide still revere much of the natural world as sacred, and safeguard the plant spirits within- as is still done today in the sacred groves of Africa (Musa, 2019) [52]. The earliest records of herbs are found from Sumerian civilisation, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from Ancient Egypt, c. 1550 BC, describes over 850 plant medicines. The Greek physician Dioscorides, who worked in the roman army, documented over 1000 recipes for medicines using over 600 medicinal plants in De material medica, c. 60 AD; this formed the basis of pharmacopoeias for some 1500 years. A large number of diverse types of plants grow in different parts of the country. India is rich in all three levels of biodiversity namely, species diversity, genetic diversity and habitat diversity. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cause specific ailments has been in vogue since ancient times. Ayurveda, Unani, Siddha and folk (also called tribal) medicines are major systems of Indian medicines [52].

PHYTOCHEMICAL COMPONENTS WITH ANTIMICROBIAL ACTIVITY

ALKALOIDS

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms cause alkalinity of these compounds. The nitrogen atoms are usually situated in some ring or cyclic system. For example, indole alkaloids are those that contain nitrogen atom in indole ring system. Generally based on structures, alkaloids can be divided into classes like indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, and terpenoids and steroids. Alkaloids are an interesting group of compounds with a wide variety of activities, undesirable and desirable, on animal and human organisms. Alakaloids have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anaesthetic, hypnotic, psychrotropic, and antitumor activity and many others. Nowadays alkaloids from plants rather than from animals are still of great interest to organic chemists, biologists, biochemists, pharmacologists, and pharmacists. Well-known alkaloids include morphine, strychnine, quinine, atropine, caffeine, ephedrine, and nicotine (Kurek, 2019) [47].

TANNINS

Tannins are complex chemical substances derived from phenolic acids (sometimes called tannic acid). They are classified as phenolic compounds, which are found in many species of plants, from all climates and all parts of the globe. They are large molecules that bind readily with proteins, cellulose, starches and minerals. Tannins are found commonly in the bark of trees, wood, leaves, buds, stem, fruits, seeds, roots, and plant galls. Unripened fruits are high in tannin content, as fruit ripens, tannin content reduces. Tannins also play a major role in medicine and human health. Witch hazel (*Hamamelis virginia)* is a source of tannin used in a number of skin care products. Medical research has shown that tannin found in cranberries is highly effective against preventing urinary tract infections by preventing *Escherichia coli* from adhering to walls of the urinary tract. Similarly, this anti-adhesive property may reduce the ability of *Helicobacter pylori* to cause stomach ulcers. Recent research has also shown that polyphenolic compounds can also reduce LDL (Low Density Lipoprotein) cholesterol and improve cardiac health (U.S Forest Services, 2021).

SAPONINS

Saponins are naturally occurring compounds that are widely distributed in all cells of legume plants. Saponins, which derive its name from their ability to form stable, soap-like foams in aqueous solutions, constitute a complex and chemically diverse group of compounds. In chemical terms, saponins contain a carbohydrate moiety attached to a triterpenoid or steroids. Clinical studies have suggested that these health-promoting components, saponins, affect the immune system in ways that help to protect the human body against cancers, also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning (Shi, 2004).

TERPENES

Terpenes are aromatic compounds found in many plants, though many people commonly associate them with cannabis use because cannabis plants contain high concentrations of them. Terpenes also offer some health benefits to the human body. Terpenes play a protective role in the plant, helping the plant to recover from damage; others act as a part of the plant’s immune system to keep away infectious germs. Examples of terpens are limonene which contains therapeutic properties such as anti-inflammatory, antioxidant, antiviral, antidiabetic and anticancer properties. Limonene appears to modulate the way certain immune cells behave, which may protect the body from a range of disorders. Limonene is also safe for people to take as a supplement. Pinene acts as a bronchodilator, allowing more air into the lungs. It also has anti-inflammatory effect and may fight against infectious germs when inhaled. Linalool, myrcene, humulene, beta-caryophyllene, terpinoline etc are just a few of the types of terpenes that have medical importance as therapeutic agents (Johnson, 2020).

FLAVONOIDS

Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects. These molecules are found in a variety of fruits and vegetables. Flavonoids are polyphenolic molecules containing fifteen carbon atoms and are soluble in water. They consist of two benzene rings, either through an oxygen bridge or directly, which gives a third middle ring. Flavonoids are abundant in plants, in which they perform several functions. The abundance of flavonoids coupled with their low toxicity relative to other plant compounds means they can be ingested in large quantities by animals, including humans. Examples of foods that are rich in flavonoids include onions, parsley, blueberries, bananas, dark chocolate and red wine. Flavonoids are important antioxidants and promote several health benefits. They provide anti-viral, anti-cancer, anti-inflammatory and anti-allergic properties. One flavonoid called quercetin can help to alleviate eczema, sinusitis, asthma and hay fever. Studies have shown that flavonoid intake is inversely related to heart disease with these molecules inhibiting the oxidation of low-density lipoproteins and therefore reducing the risk atherosclerosis developing. Flavonoids are also abundant in red wine, which some have theorized is the reason why the incidence of heart disease may be lower among the French (who have a relatively high red wine intake) compared to other Europeans, despite a higher consumption of foods rich in cholesterol (French paradox) (Robertson, 2021).

CAROTENOIDS

Carotenoids are pigments in plants, algae and photosynthetic bacteria. These pigments produce the bright yellow, red, and orange colours in plants, vegetables, and fruits. Carotenoids act as a type of antioxidant for humans. They protect the body from disease and enhance the immune system. Provitamin, a carotenoid can be converted into vitamin A, which is essential for growth, immune system function, and eye health. Eating carotenoid-rich foods can protect the healthy cells in the eye and prevent the growth of cancerous cells. Antioxidants protect cells from free free-radicals, or substances that destroy or damage cell membranes. Increasing carotenoids via your diet can increase the amount of antioxidants and protective cells in your body. Similarly, carotenoids have been associated with reducing the risk of skin cancer. Some carotenoids can break down into vitamin A, a nutrient that protects against premature skin damage from sun exposure. Both are risks for melanoma, premature wrinkles and unhealthy skin (Anthony, 2018).

CHARACTERISTICS OF PHYTOMEDICINE

Phytomedicine has some characteristics that make them unique and different from synthetic drugs;

- The active principle is frequently unknown
- The availability and quality control are frequently problematic
- Standardization stability and quality control are feasible but not easy
- Have a wide range of therapeutic use and are suitable for chronic treatments.
- Well-controlled double blind clinical and toxicological studies to prove their efficacy are rare when compared with synthetic drugs but well-controlled randomized clinical trials revealed they do exist
- Cheaper than synthetic drugs

WHY THE DEMAND FOR PHYTOMEDICINE?

Phytomedicine obtained from herbal sources are in great demand in the developed world as they are able to cure many infectious diseases. These plant-based drugs provide outstanding contribution to modern therapeutics. They have proved their efficacy for primary health care because of their safety and lesser side effects. They also offer therapeutics for age-related disorders like memory loss, osteoporosis, immune disorders etc. The new found success with cases which were given up as hopeless by the allopathic doctors as their side effects free treatment (Mukeshwar, 2011) [48].

CHALLENGES IN THE DEVELOPMENT AND USE OF PHYTOMEDICINE

The lack of quality control, identification and standardization of chemical compounds and drug formulations, as well as the challenges of applying the same methodologies across dissimilar medicinal products presents a major challenge in the use and production of herbal drugs. The research design and conduct are not scientifically and ethically sound, without even taking into consideration the medical philosophies and practices that accompany the use of traditional herbal medicine (Juntra, 2019). Other challenges include the risk of side effects due to overdosage and interaction with conventional drugs, the risk of contamination is also there in that the work places used to prepare these herbal drugs are not sterilized and might lead to more problems for people who take them for ailments, there is also a lack of corroborative research between traditional medicine practitioners. There is absence and inadequate records of what is available and many plant species are actually going into extinction due to overharvesting of the plants to prepare this herbal drugs.

POSSIBLE SOLUTIONS

The quality and stability of phytomedicine is achieved by the use of fresh plants, regulated physical factors like temperature, light, water availability, cultivation of plants in place of wild-harvested plants, because they show smaller variation in their constituents. The standardization of phytomedicine can also be achieved by the use of chromatography, infra-red and ultraviolet (UV) spectrometry (Calixto, 2000) [49].

The African pharmacognosists, pharmacologists, pharmacists, physicians have to learn, acquire, document and use traditional medicine to help curtail the extinction of plants and human resources. Workshops and seminars should be conducted to bring these traditional herbal medicine practitioners together so they can exchange useful knowledge. Restrictive laws such as the Witchcraft Act of 1901 should be redacted and new legislation should be passed to promote herbal remedy uses. Standardised methods should be introduced so as to make appropriate concentrations of herbal drugs for the use of treatment for ailments.

METHODS

STUDY DESIGN

The study is a laboratory experimental study to determine the antibacterial activity of the crude extract of sunflower seed to *Listeria monocytogenes* and *Shigella sonnei*. Sampling method used was random sampling. The fresh seeds of sunflower (*Helianthus annuus)* were harvested from identified sunflower plants within Bingham University Community, dried for 48 hours in the Microbiology laboratory. The dried seeds were then blended using the USHA Mixer Grinder, labelled and stored in polythene bags for analysis. The extraction was carried out using hot water, cold water and ethanol as solvent., Phytochemicals screening were also carefully done using standardized methods alongside McFarland turbidity standards (Vinakaya *et al.*, 2009). The fluoroquinolone (Ciprofloxacin) was used as the positive control while water was the negative control.

RESULTS

1.0 Qualitative phytochemicals present in Ethanol and Hot water extract of sunflower seed extract is as presented in the Table 1 below.

Table 1: Qualitative phytochemicals present in Ethanol and Hot water extract of sunflower seed extract

Parameters	Hot Aqueous Extract of Sunflower Seed	Ethanolic extract of Sunflower Seed
Alkaloids	+	+
Flavonoids	+	+
Tannins	-	+

Parameters	Hot Aqueous Extract of Sunflower Seed	Ethanolic extract of Sunflower Seed
Saponins	+	+
Cardiac glycoside	+	+
Phenol	-	+
Phlobatanins	-	-
Antraquinones	+	-
Cardenolides	-	-
Resins	+	+

Keys: + = Present
▪ = Negative

Table 2: Showing the microorganisms and their inhibition zone diameters for different concentrations of the Sunflower seed extract in agar well

Extract	Concentrations of the Extracts (mg/mL)	Inhibition zone diameters (IZD) in mm	
		L. monocytogenes	*Shigella sonnei*
Hot Water Cold water Ethanolic	250mg/ml	42 mm 37 mm 36 mm	40 mm 39 mm 35 mm
Hot Water Cold water Ethanolic	125mg/ml	40 mm 41 mm 39 mm	38 mm 36 mm 34 mm
Hot Water Cold water Ethanolic	62.5mg/ml	38 mm 37 mm 38 mm	32 mm 33 mm 33 mm
Hot Water Cold water Ethanolic	31.25mg/ml	35 mm 35 mm 35 mm	27 mm 28 mm 28 mm
Hot Water Cold water Ethanolic	15.63mg/ml	31 mm 33 mm 32 mm	19 mm 18 mm 17 mm
Positive control (Cipro)		34.5mm	37.5mm

ANALYSIS

Conc Combined Extracts .(Mg/ml)	Mean Zone of Inhibition for *Listeria monocytogenes* (mm)	Rank	Mean Zone of Inhibition for *Shigella sonnei* (mm)	Rank	Difference (D)	D^2
250.0	38.3	6	38	7	-1	1
125.0	40.0	7	36.0	5	2	4
62.5	37.7	5	32.7	4	1	1
31.25	35.0	4	27.7	3	1	1
15.63	32.0	2	18.0	2	0	0
Ciprofloxacin Positive Cottrol (15.63)	34.5	3	37.5	6	-3	9
Water Negative Control (0)	0	1	0	1	0	0 $\Sigma D^2 = 16$

r = 1 - 6 Σ D 2 // N(N2 -1) = 1 -0.3 = 0.7 High correlation

We have no sufficient evidence to reject the researchers/ alternative hypothesis which states;

“ H_a: Sunflower seed extract alongside the positive fluoroquinolone control have significant levels of antibacterial activity on *Listeria monocytogenes* and *Shigella sonnei* as high correlation existed between the mean MIC tested for the two representative Gram positive and Gram negative organisms respectively” Hence it is retained.

Table 3: Presents results of minimum Inhibitory Concentration of sunflower seed extract

Extract (sun flower seed)	*Listeria monocytogenes (mm)*	*Shigella sonnei (mm)*
Hot Aqueous extract	31.25	62.5
Cold Aqueous extract	125	125
Ethanol	15.65	12.5
Control (Cipro)	0.12	0.12

Table 4: Minimum Bactericidal Concentration of sunflower seed extract on the test organisms

Extract (sun flower seed)	*Listeria monocytogenes (mm)*	*Shigella sonnei (mm)*
Hot Aqueous extract	250	250
Cold Aqueous extract	-	-
Ethanol	250	-
Control (Cipro)	125	62.5

Discussion, Conclusion and Recommendation

DISCUSSION

This study results shows the presence of significant number of phytochemicals among which are alkaloid, flavonoids, phenols, saponins, cardiac glycoside and resins. Ethanol extract of sunflower seed presence more phytochemicals compared to hot water extract, this is probably due to the relative difference in polarity of these two different solvents. This finding reports of the presence of active phytochemicals agrees with the findings reported by Ahmad *et al.*, 2018 in whose report stated the presence of active phytochemicals in sunflower seed extract, it however differs from Srinivasan *et al.*, 2001, who opined that these bioactive components are found more in aqueous rather than ethanolic extract as shown in this study. Other school of thought are of the opinion that irrespective of the solvent, the bioactive compounds are present either way. The presence of these bioactive compound is responsible for the observe use of this plant seed in traditional medicine.

Based on Clinical and laboratory standards institute (CLSI) standards for zones of inhibition ≥20 mm means the organism is susceptible to the extract while between 15-19mm means the organism susceptibility or sensitivity is intermediate while ≤14mm means the organism is resistant to the extract. From the work done for zones of inhibition, both *Listeria monocytopenia* and *Shigella sonnei* both are inhibit by the extract at minimal concentrations as seen in tables two and three with aqueous extract expressing much of the sensitivity with diameter zone of inhibitions of 31 mm and above at minimal concentration. This finding thereby affirms to the null hypothesis that sunflower seed extract contains bioactive constituents with potential to inhibit and or kill bacteria as seen in this study reports. These experimental findings could be the basis upon which the observe medicinal use of this plant seed in traditional medical practice.

CONCLUSION

I hereby conclude by accepting the alternate (H_a) hypothesis which state ‘Sunflower seed extract has significant levels of phytochemical constituents and antibacterial activity on *Listeria monocytogenes* and *Shigella sonnei’* and rejects the null hypothesis

RECOMMENDATION

Following the remarkable presence of bioactive compounds in sunflower seed extract and the sensitivity/susceptibility expressed by the test isolate to the extract, I will recommend further studies on this plant seed especially

- Toxicity study to ascertain its toxic level in a living cell
- In-vitro studies to enable possible assessment of likely side effects in experimental animals.
- Quantitative analysis of the bioactive compounds

References

1. Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodbourne Pathogens & Disease*, *2*(2), 115-129.
2. Zhao, X., Zhao, F., Wang, J., & Zhong, N. (2017). Biofilm formation and control strategies of foodborne pathogens: food safety perspectives. *RSC advances*, *7*(58), 36670-36683.
3. World Health Organization (WHO). (2018). World Health Organization Fact Sheets. Available online at: https://www.who.int/news-room/fact-sheets/detail/ food-safety.
4. Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*, *17*(1), 7-15. Available at: https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html.

5. World Health Organisation. (2020). FOOD SAFETY. Retrieved from https:// www.who.int/news- room /facts-sheets/detail/food-safety. Date: 03/09/2022.
6. The, H. C., Thanh, D. P., Holt, K. E., Thomson, N. R., & Baker, S. (2016). The genomic signatures of Shigella evolution, adaptation and geographical spread. *Nature Reviews Microbiology*, *14*(4), 235-250.
7. Trofa AF, Ueno-Olsen H, Oiwa R, Yoshikawa M (1999) Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. Clin Infect Dis 29(5):1303–1306. https://doi.org/10.1086/313437..
8. Lampel, K. A., Formal, S. B., & Maurelli, A. T. (2018). A brief history of Shigella. *EcoSal Plus*, *8*(1). https://doi.org/10.1128/ecosalplus .esp-0006-2017
9. Barceloux, D. G. (2008). Shigella species (Shiga Enterotoxins). *Medical Toxicology of Natural Substances. Hoboken, New Jersey, USA: John Wiley & Sons, Inc*, 150-155. https://doi.org/10.1002/9780470330319.ch20.
10. Wu, Y., Lau, H. K., Lee, T., Lau, D. K., & Payne, J. (2019). In silico serotyping based on whole-genome sequencing improves the accuracy of Shigella identification. *Applied and environmental microbiology*, *85*(7), e00165-19. https://doi.org/10.1128/AEM.00165-19.
11. Anderson, M., Sansonetti, P. J., & Marteyn, B. S. (2016). Shigella diversity and changing landscape: insights for the twenty-first century. *Frontiers in cellular and infection microbiology*, *6*, 45.
12. Holt, K. E., Baker, S., Weill, F. X., Holmes, E. C., Kitchen, A., Yu, J., ... & Thomson, N. R. (2012). Shigella sonnei genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. *Nature genetics*, *44*(9), 1056-1059.
13. Qiu, S., Xu, X., Yang, C., Wang, J., Liang, B., Li, P., ... & Song, H. (2015). Shift in serotype distribution of Shigella species in China, 2003–2013. *Clinical Microbiology and Infection*, *21*(3), 252-e5.
14. Thompson, C. N., Duy, P. T., & Baker, S. (2015). The rising dominance of Shigella sonnei: an intercontinental shift in the etiology of bacillary dysentery. *PLoS Negl Trop Dis*, 9(6), e0003708.
15. Andrew, R. L., Kane, N. C., Baute, G. J., Grassa, C. J., & Rieseberg, L. H. (2013). Recent nonhybrid origin of sunflower ecotypes in a novel habitat. *Journal of Molecular Ecology*, 22(3), 799-813.
16. NSA. (2013). All about sunflower. National Sunflower Association. Retrieved from http://www. Sunflower nsa.com/all-about/history/.
17. Perkins, E. G. (2002). Effect of lipid oxidation on oil and food quality in deep frying: Angelo AJS(ed) lipid oxidation in food, 18, 310-321. *Acs symposium series no 500 ACS Publications American Chemical society Washington DC.*
18. Oyinloye, J. M. A. (2016). Detection and molecular characterization of Listeria species in ‘Wara’, a west African local cheese sold in Ekiti state. *Int J Curr Microbiol App Sci*, *5*(6), 941-948.
19. Tacconelli, E., Magrini, N., Kahlmeter, G., & Singh, N. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organ*, 27, 318–327.
20. Tillotson, G. (2018). A crucial list of pathogens. *Lancet Infect Dis*, 18(3), 234-236.
21. Nowicka, B., & Kruk, J. (2017). Vitamin E-occurrence, biosynthesis by plants and functions in human nutrition. *Mini Reviews in Medicinal Chemistry*, 17(12), 1039–1052.
22. Kozłowska, M., & Gruczyńska, E. (2018). Comparison of the oxidative stability of soybean and sunflower oils enriched with herbal plant extracts. *Chemical Papers*, 72(10), 2607–2615.
23. Islam, R. T., Hossain, M. M., Majumder, K., & Tipu, A. H. (2016). In vitro phytochemical investigation of Helianthus annuus seeds. *Bangladesh Pharmaceutical Journal*, 19(1), 100–105.
24. Roy, R. D., Hossan, M. S., & Rahmatullah, M. (2015). A review of anticancer potential of elephantopus scaber and its phytoconstituents. *World Journal of Pharmacology and Pharmaceutical L Science*, 4(10), 86–94.
25. Khan, S., Choudhary, S., Pandey, A., Khan, M. K., & Thomas, G. (2015). Sunflower oil: Efficient oil source for human consumption. *Emergent Life Sciences Research*, 1, 1-3.
26. Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, 97, 55–74.
27. Gad, H. A., & El-Ahmady, S. H. (2018). Prediction of thymoquinone content in black seed oil using multivariate analysis: An efficient model for its quality assessment. *Industrial Crops and Products*, 124, 626– 632.
28. Saini, S., & Sharma, S. (2013). Antidiabetic effect of Helianthus annuus L., seeds ethanolic extract in streptozotocin nicotinamide induced type 2 diabetes mellitus. *International Journal of Pharmacology and Pharmaceutical Science*, 5(2), 382–387.
29. Farahmandfar, R., Asnaashari, M., Pourshayegan, M., Maghsoudi, S., & Moniri, H. (2018). Evaluation of antioxidant properties of lemon verbena (Lippia citriodora) essential oil and its capacity in sunflower oil stabilization during storage time. *Food Science and Nutrition*, 6(4), 983–990.
30. Rani, R., Sheoran, R., & Sharma, B. (2017). Perspectives of breeding for altering sunflower oil quality to obtain novel oils. *International Journal* of *Current Microbiology* and *Applied Sciences*, 6(8), 949–962.

31. Menzel, C., González-Martínez, C., Chiralt, A., & Vilaplana, F. (2019). Antioxidant starch films containing sunflower hull extracts. *Carbohydrate Polymers*, 214, 142–151. https://doi.org/10.1016/j.carbpol.2019.03.022.
32. Thielmann, J., Kohnen, S., & Hauser, C. (2017). Antimicrobial activity of Olea europaea Linné extracts and their applicability as natural food preservative agents. *International Journal of Food Microbiology,* 251, 48–66.
33. Ahmad, S., AbdEl-Salam, N. M., & Ullah, R. (2016). In vitro antimicrobial bioassays, DPPH radical scavenging activity, and FTIR spectroscopy analysis of Heliotropium bacciferum. *BioMed research international*, *2016*.
34. Bashir, T., Zia-Ur-Rehman Mashwani, K. Z., Haider, S., & Shaista Tabassum, M. (2021). 02. Chemistry, pharmacology and ethnomedicinal uses of Helianthus annuus (Sunflower): A Review. *Pure and Applied Biology (PAB)*, *4*(2), 226-235.
35. EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control). The European Union One Health 2018 Zoonoses Report. EFSA J. 2019, 17, 5926.
36. Allen, K. J., Wałecka-Zacharska, E., Chen, J. C., Katarzyna, K. P., Devlieghere, F., Van Meervenne, E., ... & Bania, J. (2016). Listeria monocytogenes–An examination of food chain factors potentially contributing to antimicrobial resistance. *Food Microbiology*, *54,* 178-189.
37. Bahrami, A., Baboli, Z. M., Schimmel, K., Jafari, S. M., & Williams, L. (2020). Efficiency of novel processing technologies for the control of Listeria monocytogenes in food products. *Trends in Food Science & Technology*, *96*, 61-78.
38. Rothrock Jr, M. J., Micciche, A. C., Bodie, A. R., & Ricke, S. C. (2019). Listeria occurrence and potential control strategies in alternative and conventional poultry processing and retail. *Frontiers in Sustainable Food Systems*, *3*, 33.
39. Enurah, L. U., Aboaba, O. O., Nwachukwu, S. C. U., & Nwosuh, C. I. (2013). Antibiotic resistant profiles of food (fresh raw milk) and environmental (abattoir effluents) isolates of Listeria monocytogenes from the six zones of Nigeria. *African Journal of Microbiology*, 7, 4373-4378.
40. Molla, B., Yilma, R., & Alemayehu, D. (2004). Listeria monocytogenes and other Listeria species in retail meat and milk products in Addis Ababa, Ethiopia. *Ethiopian Journal of Health Development*, *18*(3), 208-212.
41. Slutsker, L., & Schuchat, A. (1999). Listeriosis in Humans. In: Listeria, Listeriosis and Food Safety, pp. 75–95.
42. Niyogi, S. K. (2005). Shigellosis. *J Microbiol*, 43, 133-143.
43. CDC. (2013). National enteric disease surveillance: Shigella annual report, 2011. Centers for Disease control and Prevention (CDC) Agency of the U. S. Department of Health and Human Services, CDC, Atlanta, GA.
44. Bridle, H. (2013). Waterborne pathogens. Detection methods and applications book. Academic Press. Elsevier ISBN:978–0- 444-59543-0. https://www.elsevier.com/books/waterborne -pathogens/bridle/978-0-444-59543-0
45. DuPont, H. L. (2014). Acute infectious diarrhea in immunocompetent adults. *N Engl J Med*, 370(16), 1532–1540.
46. The, H. C., & Baker, S. (2018) Out of Asia: the independent rise and global spread of fuoroquinolone-resistant Shigella. *Microb Genom*, 4(4). https://doi.org/10.1099/mgen.0.000171.
47. Kurek, J. (Ed.). (2019). Alkaloids: Their importance in Nature and Human life. Books on Demand.
48. Mukeshwar, P., Mousumi, D., Shobit, G., & Surender, K. C. (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. *Journal of Pharmacognosy and phytotherapy*, *3*(2), 27-37.
49. Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of medical and Biological research*, *33,* 179-189.
50. Adegoke, A. A., Iberi, P. A, Akinpelu, D. A., Aiyegoro, O. A., & Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllantus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*, 3(3), 6-12.
51. Cappuccino J. G., & Sherman, N. (2014). A laboratory manual 10th edition, Sunny rockland person, 12-17.
52. Sofowora, A. (1993). Recent trends in research into African medicinal plants. *Journal of ethnopharmacology*, *38*(2-3), 197-208.
53. Sofowora, A. (1993). Screening plants for bioactive agents. In: Medicinal plants and traditional medicinal in Africa.2nd Edition. Spectrum books Ltd. pp 134-156.
54. Srinivasan, D., Perumalsamy, L., Nathan, P., & Sures, T. (2001). Antimicrobial activity of certain indian plants used in folklore medicine. *Journal of Ethno-pharmacy*. 94: pp 217-222.