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Editorial

Plagiarism vs Similarity Index: A Critical Insight

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The word Plagiarism is derived from the Latin term "*plagiarius*", meaning "kidnapper" [1]. Plagiarism severely violates publication ethics and professional conduct [2]. It may be defined as an unethical intentional or unintentional piracy of someone else idea/s or text without acknowledgement [1]. Intentional Plagiarism usually occurs when some educational credentials, professional promotions, or economic benefits might benefit the concerned author(s)—unintentional plagiarism results either from negligence or lack of awareness about plagiarism [3].

The first incidence of plagiarism was detected in the year 1979 in a scientific paper. A later number of papers were found to be plagiarised [4]. A report published in 2018 showed an increased number of retractions of scientific papers in the last two decades due to plagiarism [5]. The primary reason for the increase in plagiarism by the scientific community could be a mandatory requirement to publish for employment and promotions. In addition, lack of skill in scientific writing and stringent policies related to plagiarism [4]. The availability of advanced text formatting tools and free access to scientific information may also reason for increased cases of plagiarism [6].

In the early days, plagiarism detection was challenging for the publisher due to the unavailability of sophisticated screening technology for reviewing manuscripts against published hard copies of the articles. Advanced tools for detecting plagiarism, such as **iThenticate (Crossref), Turnitin, Grammarly,** and **Dupli Checker**, are available to compare manuscripts with published articles [3,4]. Recently, iParadigms has developed a plagiarism detection tool for individual authors to screen individual manuscripts against an extensive live database of scholarly literature [7].

Although instances of Plagiarism in scholarly article manuscript can easily be detected using such tools, they are limited to detecting text plagiarism, and thus Plagiarism of ideas remain undetected. In other words, these tools can detect similarities in the text and fail to detect actual plagiarism [4]. Accessibility to parts of published literature during its copyright period could be another limitation for tools to detection of Plagiarism [8]. In addition, certain commercial agencies unethically develop clones of published literature and indulge authors in manipulation [8]. Detection of Plagiarism may also encourage Smart Plagiarism [7]. Non-affordability of these high-cost subscription-based services could be another hurdle for researchers [5]. It is also challenging to detect similarity and Plagiarism in articles resubmitted for publication in other languages. Similarity in the articles with deliberate errors in text, and structure may also be difficult to detect [5]. Thus, the similarity screening tools need to be upgraded by providing more access to subscription-based publications [8].

The term 'Similarity Index' refers to the extent of overlap or matching between the submitted manuscript and already published scientific work [5]. The percentage of the similarity index is regarded as a standard measure against plagiarism [9]. Although many authors write their manuscripts for scientific publication honestly, however, depending on the number of words from the methodology section and quotations may contribute significantly to the similarity index. This may result in the rejection of a manuscript by the publisher even though the entire scientific content is novel and genuine [10].

Some research work may share the same research background, and thus text similarity between the separate papers may observe [7]. Notably, some well-known procedures or quotations cannot be changed but unintentionally end up with increased similarity [10]. The methodology adopted remains the same for specific studies and thus ends up with increased similarity [10]. One can avoid similarity

in scholarly articles by using quotation marks for verbatim copying and giving due credit to original contributor(s) at appropriate places [3].

In conclusion, Plagiarism detection tools aid in detecting textual similarity, which shall not be considered an alternative for the pear review process. Authors have put in sincere efforts to improve their ethical writing skills. The original contributor/author deserves due acknowledgement. Research organisations and professional institutes must provide the necessary facilities and encourage researchers to publish scholarly articles with original ideas and content. Similarly, a publisher should not judge the manuscripts solely based on the similarity index; due consideration shall also be given to the uniqueness and novelty of the work.

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Review Article

Nutraceuticals: An inspiring therapy for lifestyle diseases

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Abstract

Among natural sources, majorly we consider plant-based phytochemicals to offer safe and long-lasting effects. Phytochemicals or secondary metabolites are the extract obtained from herbal plants that serve as an excellent venture for their utilization as formulation. Different nutraceuticals (herbal formulations) have always been a part of the world. Everyone around the world could use these supplements for their benefit. Since the end of the eighth century and the beginning of the nineteenth century, the concept of using products has improved. Nutraceutical food supplement plays an essential role for humans to stay healthy and gets all the important nutrients used for the body's basic metabolism and further prevent all kinds of illnesses. The present review highlights the potential of nutraceuticals against various types of leading life-threatening disorders such as diabetes, obesity, asthma, cardiac conditions, arthritis, cancer, etc. This review focuses on the nutraceuticals that help manage certain many life-threatening disorders.

Keywords: Phytoconstituents; nutraceuticals; food supplements; life-threatening disorder

Introduction

'**Nutraceuticals**', a term given by Dr Stephen De Felice in 1989, became a newly accepted word in Oxford Dictionary. The term '**Nutraceutical**' is made by two words: "nutrire," i.e., "to nourish," and "pharmaceutical," which means "to cure." [1]. Nutraceuticals are biologically active substances that may be considered food or a part of food with nutritional benefits to human health in treating various diseases. Most diseases, such as obesity, diabetes, cardiac issues and so on, occur due to imbalanced diet and living style. Nutraceuticals are crucial in disease prevention as they promote good health [2]. India has been an abundant source of medicinal plant species, but traditional communities have used only a limited number of plant species for treating different diseases. Herbs and herbals are plant-derived products with no woody tissues and can be modified in many ways depending on individual purposes. For processing, the herbs are generally subjected to drying; however, excessive heating may reduce the activity potential of herbal products. Since ancient times, herbs have constituted a rich concentration of antioxidants and have been employed for flavouring and aroma. Most plant constituents, such as carotenoids, lycopene, catechins, polyphenols, etc., are well-known in nutraceuticals for disease management [3].

Many research studies have been done extensively on herbs and spices to observe their antioxidant and antimicrobial potential to establish their beneficial effects on humans. Herbs and spices contain many bio-actives, including flavonoids, alkaloids, tannins, phenolic diterpenes and related compounds, sulphur-containing compounds etc. Many plants/herbs have been used for medicinal purposes at home and are also available as marketed formulations. For instance, herbs such as *Allium sativum* (garlic) extracts, *Ocimum basilicum* (Tulsi*), Aloe barbadensis M.* (aloe vera*)* gel, *Zingiber officinale* (ginger*)* root, *Tinospora cordifolia (Giloy),* etc. have been known for excellent health benefits in reducing cholesterol, boosting immunity, anti-ulcer, wound healing, antimicrobial and antioxidant activities.

Despite the disparity in scientific evidence, the consumption of herbs has tremendously increased globally based on the health benefits reported by various studies [4].

Based on various reports, it is suggested that taking any food and nutritional supplements derived from herbs, roots, vegetables, fruits etc., improves health by reducing adverse results such as inflammation, radical scavenging features etc., during any infections in the body, thereby boosting the immune system. Eventually, using nutraceuticals can offer adjuvant therapeutic and prophylactic assistance to manage a wide variety of disorders. For example, Parsley contains flavonoids and shows diuretic and antipyretic action [5]. The reasons for the transition to nutraceuticals may be as follows [6,7].

- Consumers are becoming more worried about healthcare costs.
- Nutraceuticals are used by people disappointed with pharmaceutical agents to maintain well-being and avoid chronic disease.
- According to healthcare specialists, our excessively processed food supply, which originates from crops farmed with chemical fertilizers, pesticides, herbicides, and sometimes genetically engineered seeds, lacks the nutrients required for optimal health.
- People are more inclined to believe in preventing the disease.
- Individuals are suffering from long-time illnesses for which allopathic medicine has failed to provide a cure.
- Nutraceuticals are readily available and less expensive than pharmaceuticals, and they do not place an undue financial burden on their consumers because they are either food or parts." Instead of "Patients who are struggling financially.

Phytochemicals have a large apparent volume of dispersion and can cause organ accumulation. These herbals have recently been employed to make nutraceuticals that offer a supplementary regimen to the treatment therapy for better patient compliance and lesser side effects than synthetic preparations [8,9]. The importance of nutraceuticals and their application as cardioprotective, hepatoprotective, antiepileptic, anti-inflammatory and many more for managing life-threatening diseases have been reported. Since various kinds of literature are available on similar lines, this review mainly discussed the fundamentals of nutraceuticals. It highlighted some essential facts about the therapeutic use of nutraceuticals as complementary medicine to conventional therapies allowing dose reduction and minimizing adverse effects [10-15].

Mechanism of action of nutraceuticals

Herbal phytochemicals used in nutraceuticals show their therapeutic action by various mechanisms, including suppressing the over-expression of proteins, enzymes, amino acids, and hormones. These phytoconstituents starts the generation of protection enzyme. Also, these are quickly taken by normal tissue and taken after being of occurring naturally. Nutraceuticals are effective in disease management such as cancer, obesity, arthritis, etc. Moreover, it strengthens the body's defence mechanism and refines the immune response towards diseases [16,17]. Figure 1 represents several mechanisms of actions of nutraceuticals in disease management.

Classifications of nutraceuticals

The impact of nutraceuticals became more relevant as the current generation is more focused on approaches to preventive healthcare. There is much misunderstanding about words like "nutraceuticals," "functional foods," "dietary supplements," "designer foods," "medical foods," "pharmafoods," and "phytochemicals," among others. Their substitutable use by diverse entities on diverse events indicates that it tends to have a thin dividing line [18]. Nutraceuticals can be developed as solid dosage forms like capsules, tablets, and liquid formulations. It can be divided into a dietary fibre, probiotics, prebiotics, polyunsaturated fatty acids, antioxidants and other natural foods. Based on various characteristics, nutraceuticals can be categorized based on a natural food source, chemical

nature, mechanism of action and traditional and non-traditional nutraceuticals. Here, the categorization is done as traditional and non-traditional nutraceuticals [19].

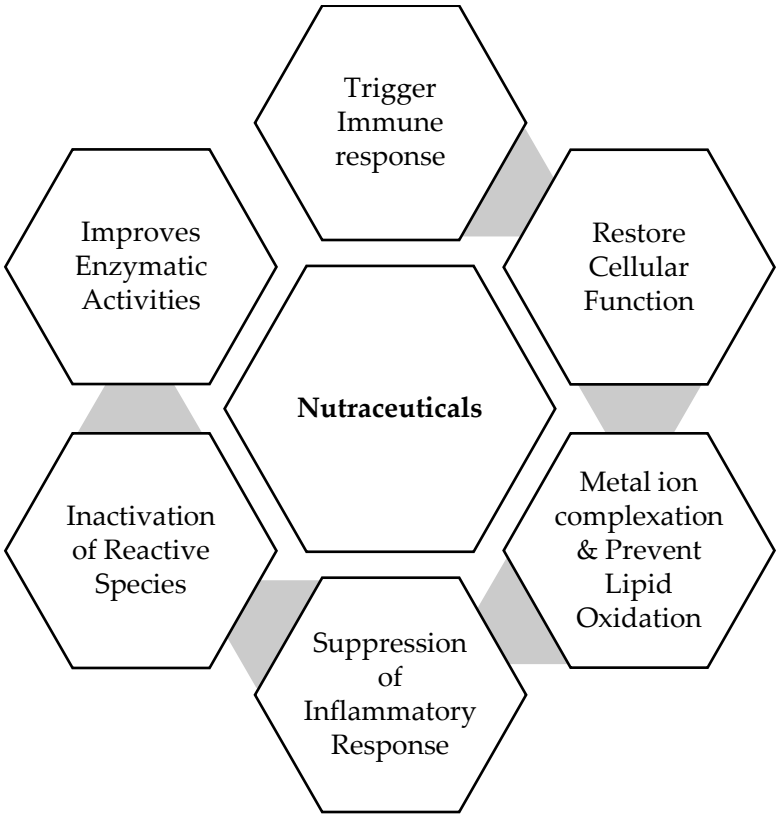

Figure 1. Several mechanisms of actions of nutraceuticals in disease management.

Traditional nutraceuticals

These include natural foods with potential health benefits. These are substances that have been derived directly from natural resources without modifying the food in any way. Vegetables, fruits, grains, dairy, meat, dairy products, and other foods may be included. For example, nutrient-dense foods like the lycopene found in tomatoes. Traditional nutraceuticals positively impact health by boosting immunity and lowering the risk of life-threatening disorders, including cancer and heart disease. For instance, the secondary metabolite flavonoid, which is rich in antioxidant characteristics and aids in suppressing tumours, lowers the risk of heart attacks and kidney-related problems. Chemical components (nutrients including vitamins, amino acids, minerals, etc.), probiotic bacteria, and enzymes are additional categories for both traditional food and nutraceuticals [20]. The following is a description of each functional category of nutraceuticals:

Dietary -fibre

Nondigestible carbohydrates and lignin, found naturally in plants, make up dietary fibre. Functional fibre is a type of nondigestible carbohydrate with physiological benefits for the body. Adult men should consume 38 gm of fibre per day, while adult women should consume 25 gm per day, according to the Dietary Reference Intake (DRI). There was insufficient information to identify an optimal upper consumption level for dietary or functional fibre [21].

Pro-biotics

Probiotics are live bacteria and yeasts that are good for your health, especially your digestive system. Bacteria are commonly associated with the transmission of diseases. On the other hand, your body is teeming with excellent and dangerous microorganisms. Probiotics are sometimes called "good" or

"useful" bacteria since they maintain your gut health. Probiotic Microorganism based preparations generally contain live microorganisms which improve an individual's health upon adequate consumption. Regularly consuming these products makes the intestine more friendly and promotes the body process like healthy digestion, absorption of specific nutrients and metabolism by removing toxic flora from the intestine—for example, appropriate consumption of *Bacillus bulgaris*.

Moreover, the antimicrobial property generally aids the altering impact on microflora resulting in better retention of supplements within the body tissues. Probiotics are beneficial in conditions like lactose intolerance as they initiate the formation of the related enzyme (ß-galactosidase), which helps in hydrolysing lactose into its sugar components. The few available probiotics are Milk (*Lactococcus lactis*), Yoghurt (*Streptococcus thermophilus*), and gastrointestinal tract (*Lactobacillus johnsonii*) [22].

Nutraceutical enzymes

Enzymes are proteins produced by cells inside a body that helps in regulating various metabolic pathways and act as a biocatalyst. Nowadays, enzyme supplements for treatment are much considered for managing several rare diseases like Hunter Syndrome, Gaucher disease, Pompe disease and Fabry disease. However, microbial sources are preferred over plant and animal sources for enzyme production due to the enzyme effect and cost effect. Nutraceutical microorganisms are food supplemented with bioengineered crops to augment the nutrients and their overall effect. For example, broccoli and rice are rich in vitamins and β-carotene. A few examples of enzymes obtained from microbial sources are Invertase, *Lactase,* and *Pectinase,* obtained from plant cell walls [23].

Pre-biotics

Prebiotics are chemicals that promote the growth or activity of beneficial microorganisms (such as bacteria and fungi). In concept, however, it is a broader phrase that can refer to various body parts. Prebiotics, like probiotics, are conceptually transitional between foods and medications as a functional dietary component. They usually face an intermediate level of regulatory scrutiny, depending on the country, especially regarding health claims. Prebiotics are all fibre, but not all fibre is prebiotic [24,25].

Poly-unsaturated fatty acids

The first double C-bound position of polyunsaturated fatty acids (PUFAs) differs between omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids. Needed fatty acids are two PUFAs that the human body cannot generate yet are required for physiological health. As a result, they have to be obtained through dietary means. One is linoleic acid, which belongs to the n-6 family [25].

Anti-oxidants

Free radical damage to cells is thought to have a significant part in the ageing process and disease progression. Antioxidants are the body's first line of defence against free radical damage and are crucial for overall health and happiness. Free radicals can be stabilised or deactivated by antioxidants before they harm cells. Antioxidants are essential for cellular and systemic health and well-being [25,26].

Poly-phenols

They are phytochemical compounds found naturally in plant-based food like fruits, vegetables, whole grains, cereals, tea, and coffee; more than 8000 polyphenolic compounds have been identified in whole plant foods, including phenolic acids, flavonoids, stilbenes. These chemicals are secondary metabolites produced by plants to protect them from UV radiation, oxidants, and infections [26].

Spices

Since the dawn of time, spices have played a crucial role in the culinary art of flavouring food. They are aromatic vegetable parts more for flavouring food than for supplying nutrition. They may be left whole, shattered, or ground up. Spices play a significant role in the economy of the producing nation because they are used extensively as pesticides in native medicines, pharmaceuticals, Nutraceuticals,

aroma therapy, beverages, perfumes, dental preparations, cosmetics, and other products in addition to flavouring and seasoning [26,27].

Dietary-supplements

It is a liquid or capsule-based item which have nutrients extracted from foods which have been concentrated. Vitamins, herbs or other botanicals, enzymes, glandular, and metabolites are all "dietary ingredients" in these products. Dietary products can only be used to help the body's primary function but not to treat any disorder or illness, except for some of the well-defined exceptions that achieve this goal by utilising nutraceuticals' ability to detoxify our body and restore proper digestion and eating habits [28].

Functional-food

They are "food or any food product that, in addition to the usual nutrients it provides, can give a benefit to health." They are designed to make it possible for consumers to eat nutrient-dense foods in their natural state as opposed to consuming liquid or capsule-based nutritional supplements. Nutrification is the process of adding nutrients to functional foods [28,29].

Medicinal food

Under the supervision of a health practitioner, this food should be consumed or administered internally for the precise dietary treatment of a condition with a specific nutrient requirement. Food that does not include any ingredients that promote disease or contain a nutrient the body cannot naturally produce due to a specific disease state is defined by medical evaluation based on accepted scientific principles [29].

Non-traditional nutraceuticals

Nutraceuticals or food products belonging to this category are synthesized artificially with the help of biotechnology to add bioactive to the products for enhancing food properties and human health. For example, cereals infused with minerals and vitamins and rice enriched with β-carotene contain a provitamin A bioactive, which boosts antioxidant activity. Based on the processing method, non-traditional nutraceuticals may be arranged into fortified nutraceuticals and recombinant nutraceuticals.

Fortified nutraceuticals

These constitute food or nutraceutical products prepared from agricultural breeding or adding compatible nutrients to main components to improve their overall health value. For instance, fortified orange juice can improve glycaemic control if calcium is added to food products like orange juice. A few fortified examples are minerals added to cereals, flour fortified with iron, calcium and folic acid, and orange juice fortified with calcium cholecalciferol fortified milk are generally used for managing vitamin D deficiency.

Recombinant nutraceuticals

These foods and nutraceuticals are produced by using biotechnology and genetic recombination. The crops are genetically modified to produce products containing recombinant compounds and proteins, making them more beneficial to health, for example, golden rice, golden mustard, iron rice, multivitamin corn and gold kiwi fruit. Gold kiwi fruit consists of a recombinant gene that helps increase ascorbic acid levels and carotenoid and lutein to improve immunity. Also, it is considered a rich source of potassium, vitamin and fibre. In addition, food products such as cheese, bread, yoghurt and many other energy-providing foods are produced with the help of biotechnology [30,31].

Nutraceuticals over pharmaceuticals in disease management

The term ‘Nutraceuticals’ is well understood & widely accepted; however, it does not have any standard definition. The ideal definition is ‘food or a component of food (including beverages) that has

medical or health benefits, such as disease prevention or therapy. Many natural nutraceuticals offer preventive benefits for various conditions in rising markets, including cardiovascular disorders, diabetes, obesity, allergies, and cancer [32]. Ayurveda, since ever, relies on strategies to inculcate the use of phytochemical extractives, alone or in combinations, in many immunostimulants and other disease ailments. Nutraceuticals containing poly herbals constituents are beneficial to the body in regulating internal processes. Phytochemicals have been proven to be effective in accelerating the production of protective enzymes as well as inhibiting the over-expressed proteins, enzymes, hormones etc., and, in this way, boosting immunity instead of affecting healthy cells (to certain concentrations) [33]. Nutraceuticals used for the treatment or prevention of different diseases are mentioned below.

Cardio-vascular disorders

Supplements like dietary fibres, Anti-oxidants, Omega-3, vitamins, and minerals are used to prevent and treatment of Cardiovascular disorders. Polyphenol found in grapes protects from arterial disorders. Onion, grapes, apples, and cherries have flavonoids that can inhibit the ACE and give strength to the capillary, which are used as an oxygen and another nutrient carrier for every cell of the body. Rice bran improves cardiovascular health by lowering serum cholesterol levels, lowering the level of LDL and increasing the level of HDL [32].

Diet-related disorders

Diet-related diseases are on the rise in Western countries, owing to the increased availability of high-calorie foods. Obesity, diabetes, and neurodegeneration are all important diet-related diseases, with low-grade inflammation as a shared pathogenic denominator [34].

Heart-attack and lung-cancer

In addition to its fiber, corn also contains a lot of folates, which benefits heart health. It is predicted that consuming the recommended daily amount of folate will reduce the occurrence of heart attacks by 10%. Corn also contains cryptoxanthin, a naturally occurring carotenoid pigment. Regular use of cryptoxanthin has been proven to reduce the incidence of lung cancer by 27% [35].

Diabetes

People with diabetes may benefit from the ethyl esters found in N-3 fatty acids. Docosahexaenoic acid plays a role in neuro visual development and insulin resistance. Diabetic neuropathy can be treated with lipoic acid, an antioxidant. The dietary fiber psyllium has been used to treat diabetes and reduce lipid levels in hyperlipidemia [36].

Obesity

As a worldwide epidemic, obesity can be defined as excessive body fat. Heart disease, coronary artery disease, high blood pressure, high cholesterol, respiratory illness, osteoarthritis, cancer, and infertility are all well-known risk factors [35,36].

Cancer

Flavonoids that inhibit estrogen-producing enzymes prevent estrogen-related malignancies. “Phytoestrogens” (hormonal activity) can be used to prevent a wide range of breast or prostate cancer. Curcumin from curry and soya flavones in soy foods have cancer-fighting qualities. Lycopene is found in high concentrations in the skin, testes, adrenals, and prostate, where it fights cancer [37,38].

Anti-inflammatory activity

Curcumin (diferuloylmethane) is an anti-carcinogenic, anti-oxidative, and anti-inflammatory polyphenol found in turmeric. Spinach, cucumber, turmeric rhizomes, and beet-root have an antitumor effect. Gamma linolenic acid is a fatty acid found in green leafy vegetables, almonds, and vegetable oils

such as evening primrose oil and blackcurrant seed oil that is used to alleviate inflammation and auto-immune diseases [38].

Alzheimer's disease

β - carotene, lycopene, and turmerin may protect against some diseases by counteracting the harmful effects of oxidative stress, mitochondrial malfunction, and different types of brain degeneration [38].

Parkinson's disease

The disease can be prevented by adding Vitamin E to the diet. Although exploratory studies have shown some promising benefits with nutritional supplements, it is essential to note that there is not enough scientific data to recommend them for Parkinson's disease right now. Patients should be aware that over-the-counter medications have side effects, can combine with different medications, and are expensive [39].

Osteo-arthritis

Osteoarthritis is the most frequent type of arthritis in the U.S., a crippling joint illness affecting approximately twenty-one million people. Joint discomfort caused by osteoarthritis and other joint problems may cause people to become less active, resulting in an imbalance in energy and gaining weight. Weight gain can aggravate existing problems by putting additional strain on joints. Nutraceuticals have both nutritional & pharmacological qualities, & they tend to modulate gene expression and Nitrous oxide and Prostaglandin E2 generation, which could explain their anti-inflammatory effects [39,40].

Adrenal-dysfunction

Adaptogenic products are non-synthetic plants which can normalize physiology; they can affect the basic functionality of the human body; they merely have a minor effect on regular bodily functioning, encouraging non-specific stress resistance. Examples are *Ocimum Eleutherococcus senticosus*, Ginkgo biloba etc. [41] Table 1 represents the nutraceutical used for different medical conditions and their natural origin.

Table 1. Various nutraceuticals in different disorders with natural origin.

Disease	Nutraceuticals	Source	Reference
Cardiovascular health	Co-q-10	Soyabeans, olive oils	[42]
	Melatonin	Bone marrow, Pineal, Glands	
	Dha	Fish oil	
	Reservatrol	Grapes, Red wine	
	Caretonoids	Carrot, Sweet Potato	
	Catechin	Tea extracts.	
Joint health	Glucosamine	Found in Ligaments, tendons , cartilages,	[43]
	Chondroitin	Proteoglycans of articular cartilage	
Eye health	Dha	Linseed (flax oil), Fish oil.	[43]
	Pycnogeal	Barley	
	Loteins	Spinach	
Diabetes	Green tea	Plant Camellia Sinensis	[43]
	Resveratrol	Red Wine, Grapes	
	Cinnamon supplements	Cinnamon extracts	
Obesity	Bottle guard	Plants	[43]
	Black gram	Plants	
	Green tea	Plants	
	Vitamin c	Supplements, Citric fruits	
	Fenugreek	Spices	
Cancer prevention	Dha	Flax seeds, Fish oil	[44]
	Resveratrol	Red Wine, Grapes	
	Lycopene	Tomato	

In addition, many plant-origin phytochemicals show proven activity in different disease conditions such as Allergy, inflammation, cardiovascular disease, obesity, skin care and cancer prevention etc. Many phytochemicals have been investigated as treatment options which show potential effects in desired dose [45]. Tables 2 [46-48] and Table 3 [23,49] represent a few examples of reported phytochemicals, their actions in various disorders, and a few examples of commercially available nutraceutical products, respectively.

Table 2. Some herbs & plants are listed with their therapeutic effects and doses.

Plants	Therapeutic properties	Dosage aprrox.
Allium sativum. L (Garlic)	Antimicrobial, hypolipidemic, antioxidant Antineoplastic, antithrombotic, antiatherogenic effects.	Cloves: 2-5 gm fresh; 0.4-1.2 gm dried powder
Citrus aurantium. L (Bitter Orange)	Stimulates the of CNS, assistance in weight loss	Fruit: 60-120 mg
Cissus quadrangularis Linn (Hadjora)	Anti-inflammatory, anti-obesity, analgesic, antibiotic, antimicrobial, hypoglycemic.	Stem: 100-500 mg
Coleus forskohlii	Treating disorders such as glaucoma, heart failure, bronchial asthma.	Root: 50-300 mg
Commiphora muklul Engl. (Indian Belellium tree)	Hypolipidemic, anti-inflammatory, antitumor.	Resin: 50-100 mg
Coriandrum sativum L. (Coriander)	Hypogylcemic, hypolipidemic, hypotenstive.	Leaf: 5 mg
Costus igneus. Nak (Fiery spiral ginger)	Diurectic , hypotensive, hypoglycemic.	Leaf: 5000 mg
Cyperus Rotundus L. (Nutgrass)	Anti-inflammatory, antidiabetic, hypocholesterolemic.	Leaf: 1-3 gm
Foeniculum vulgare. Mill (Fennel seeds)	Antispasmodic, secretolytic, secretomor, antibacterial.	Seeds: 1-7 gm
Garcinia Cambogia (Malabar Tamarind)	Anti-obesity, Anti-inflammatory, antiulcer.	Fruits: 200-500 mg
Glycyrrhiza glabra Linne (Licrorice)	Anti-inflammatory, antiulcer, anti-depressive.,	Root: 2-4 gm
Piper nigrum. L (black pepper)	Anti-inflammatory, antioxidant, analgesic.	Seeds: 2-5 gm
Phyllanthus embilica. L (Amla)	Anabolic, antibacterial, antipyretic, antiviral, antioxidant.	Seeds: 3-6 gm
Vitis vinifers. L (Grape)	Anti-inflammatory, antithrombotic, cardioprotective effects.	Seed extract 100-500 mg

Table 3. Examples of few commercially available nutraceutical products.

Product	Category	Manufacturer
Proteinex®	Protein supplement	Pfizer Ltd., Mumbai, India
Omega woman	Immunity booster	Wassen, Surrey, U.K.
Calcirol D-3	Calcium supplement	Cadilla Healthcare Ltd. Ahemdabad, India
Chyawanprash	Immunity booster	Dabur India Ltd.
GRD	Nutritional supplement	Zydus Cadila Ltd., Ahemdabad, India

Future prospects of nutraceuticals

Finding a new molecule is more complex, costly and riskier than already available drug molecules. Also, synthetic drugs' side effects and ineffectiveness have emerged in need to establish nutraceutical products as a supplementary alternative therapy. In the last two decades, there has been a rapid increase in nutraceutical use due to increased public awareness of health-related issues. Nutraceuticals help manage severe health conditions by providing sufficient nutrients to the body upon consumption, improving overall health and preventing diseases. Numerous pharmaceutical firms are endeavouring to produce nutraceuticals due to the immense and rapidly rising demand. Anti-arthritic, cold and cough, sleeping conditions, digestion, avoidance of some diseases, osteoporosis, blood pressure, cholesterol management, pain relievers, depression, and diabetes are medical conditions managed by nutraceuticals. Nutraceutical consumers believe that nutraceuticals or dietary supplements are much safer than those synthetic compounds. However, it is equally important to understand that their presumption could be wrong, and medical diagnosis is required to prescribe conventional medicines

for critical conditions. Recently, treatment with nutraceuticals for managing health has been considered prophylactic in many diseases. For instance, carnitine and flaxseed oil are prescribed for managing cardiac conditions and antioxidants such as green tea and soy products are for cancer prevention. Several manufacturing units are launching new nutraceutical products into the market for its expansion point. Many companies have introduced nutraceuticals to the market such as Novartis launched "functional food", Dean farms launched "Columbus healthier eggs" (rich in fatty acids) in 1988, and Allied Bakeries launched burgeon bread in 1997 which contains soy and flaxseed and also enriched with natural plant estrogen to treat menopausal symptoms [19]. Recently, government financial support for clinical investigations of herbal and nutraceuticals has been increased to strengthen the research to establish a vital piece of evidence towards the use, effectivity, quality and safety of nutraceuticals against certain life-threatening disorders.

Conclusion

This review paper provides information on nutraceuticals that can potentially cure different diseases. Nutraceuticals have demonstrated their ability to provide health advantages and disease prevention and should be consumed following their acceptable recommended consumption. In today's environment, where everyone is taking medicines independently, they play a critical part in therapeutic growth. However, the growth is contingent on maintaining quality, purity, safety, and efficacy. Not only formulation but the constituents play tremendous roles in treating the ailments alone or in combination with other natural constituents and chemotherapeutic agents. All this proves to be a significant milestone in treating severe conditions in unique ways.

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Declaration of interest

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Research Article

Role of Fluvastatin sodium loaded polymeric nanoparticles in the treatment of Hyperlipidemia: Fabrication and Characterization

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Abstract

The present work is focused on the formulation and evaluation of polymeric nanoparticles (NPs) loaded by Fluvastatin sodium (FLS). This drug has many disadvantages, including bioavailability of up to 24 to 44% and a half-life of approximately 30 min to 60 min. Also, it comes under Biopharmaceutical classification system (BCS) class III, but it is sparingly soluble in water. Polymeric NPs were prepared using gum rosin and chitosan as polymers which Solvent Evaporation and Inotropic Gelation method prepared. Prepared polymeric NPs were characterized for respective parameters like drug entrapment efficiency, particle size, zeta potential, Differential Scanning Calorimetry, X-Ray Diffraction, Scanning Electron Microscopy, Transmission Electron Microscopy, In vitro and In vivo drug release study. The average Particle size was lay within the range of 271 nm to 313.3 nm for the Solvent Evaporation method (SE) and 123.3 nm to 382 nm for the Inotropic gelation method (IG). Drug entrapment efficiency (DEE) of SE3 was found to be 86.1%, and IG3 had 70.2%. In vitro release study showed sustained action for both methods at the end of 48 h; an increase in the polymer resulted in decreased drug release. From the study, it can be concluded that prepared polymeric NPs from both methods show significant hypolipidemic activity. Stability studies showed that formulations are stable at the end of 3 months.

Keywords: Fluvastatin sodium; solvent evaporation method; inotropic gelation method; drug entrapment efficiency; polymeric nanoparticles

Introduction

Nowadays, the most vitality-possessed branch is nanotechnology: which provides an opportunistic pathway for various fields like biomedicine, medicine, pharmacy, biotechnology, engineering, chemical engineering, and polymer science. It plays a significant role in pharmaceutical sciences as a tool for diagnosis and treating aid [1]. Nano is a Latin word whose meaning is 'dwarf'. Nanotechnology involves a complete size range, one thousand millionths of a meter, i.e., 1 nm = 10^{-9} meters. Nanotechnology is a branch of science that studies processes occurring at the molecular level and non-lengthy scales. According to various literature, the term NPs refer to particulate dispersions, solid dispersions, or solid particles with a size from 10 nm to 1000 nm in which entrapped/encapsulation or attachment of drug can be performed [2].

Recent studies reveal that polymeric NPs have manifested several new properties and activities due to their nanoscale size and stability. Polymeric NPs ranging from 1 to 1000 nm can carry active compounds entrapped inside or surface adsorbed onto their core [3]. The size of polymeric NPs lies between 20 and 250 nm. They can be classified into three types based on how they have been encapsulated: Linear polymeric NPs – covalent for drug conjugation, micellar polymeric NPs –

amphiphilic block copolymers involved in the formulation, Hydrogels – encapsulation of hydrophilic drug [4].

Pharmaceutical formulations that contain NPs formulated with biopolymers are gaining importance. A particular interest is paid to polysaccharide NPs due to their multifunctional, nontoxic, and biocompatible properties. Since they usually have multiple functions, they improve the permeability and solubility of many powerful drugs. The penetration strength of polysaccharide–loaded drugs inside tissues and their biocompatibility make them more effective [5]. Chitosan is the most abundant natural polysaccharide. This is the most important naturally occurring cationic polymer approved for tissue engineering, drug delivery, and gene transfer by the FDA and EMA [4,6]. Gum rosin is a thick resin produced by pine trees and coniferous plants. Acid and modified acid forms of resins, like dimers and decarboxylated acids, are present. They provide better biocompatibility and biodegradability [5].

Since the beginning of time, humans have been affected by hyperlipidemia. A systemic correlation between blood lipids, hyperlipidemic conditions, and their complications was established in 2002 by coronary heart epidemiology studies [7]. Various inherited and acquired disorders result in hyperlipidemia, indicated by raised levels of lipids in the body. Across the globe, it is a common disorder, which is especially prevalent in the western hemisphere. Because this disorder usually does not have symptoms, it is likely to be diagnosed early and prevent the development of the disease [8].

As the first synthetic statin, FLS is used in treating hyperlipidemia as the first-line therapy. Bioavailability is up to 40 % for this amorphous powder. FLS has a relatively short half-life of 3 h and belongs to BCS Class III. The substance is wholly absorbed even when food is present. A single drug dose is excreted nearly 95% via the biliary route and less than 2% as the parent compound. The standard dose of FLS is 8-10 mg per day and the log P value is 3.86. FLS is therefore selected as the best candidate for fabricating polymeric NPs using solvent evaporation and inotropic gelation using biodegradable polymers like gum rosin and chitosan to approach sustained release action [9,10].

Material and methods

Pure FLS drug was gifted as a sample from Biocon ltd. Bangalore. Gum rosin (**CAS NO** – 8050097, 98% pure, Sigma Aldrich, Bangalore Karnataka), polyvinyl alcohol (35 – 50 cps, **CAS NO** – 9002895, 98% pure, Fischer scientific, Mumbai, Maharashtra), Ethanol 95% (**CAS NO** – 64175, 99 % pure, CSS chemical co. Ltd, Mumbai, Maharashtra), Chitosan from Shrimp shells (**CAS NO –** 9012764, 99% pure, Loba Chemie, Mumbai, Maharashtra) and Sodium tripolyphosphate (STPP) (**CAS NO** – 7758294, 99% pure, Fischer scientific, Mumbai, Maharashtra) were used in the preparation of NPs. All the ingredients used in the preparation of polymeric NPs possess analytical grades.

Table 1. Formula for the preparation of polymeric NPs by the solvent evaporation method.

		Formulation code		
Sl. no.	**Ingredients**	**SE1**	**SE2**	**SE3**
1	FLS (mg)	100	200	100
2	Gum Rosin (mg)	300	400	400
3	Ethanol (ml)	10	10	10
4	Polyvinyl alcohol (mg)	500	500	1000
5	Distilled water (ml)	100	100	100

Fabrication of polymeric NPs by the solvent evaporation method

Formulation contents are mentioned in Table 1. Accurately weighed FLS and gum rosin were dissolved in ethanol to obtain solution I. Simultaneously polyvinyl alcohol was added to distilled water to obtain solution II. This surfactant solution was homogenized for 5 min at 25000 rpm to dissolve Polyvinyl alcohol. Solution I was slowly added to solution II. Then it was subjected to homogenization for 10 min at 15000 rpm. The dispersion was sonicated for 5 min (15 cycles with 30 sec of halt). Each formulation was stirred at a magnetic stirred at 100 rpm for 6 h to evaporate ethanol, centrifuged at -20°C at 15000 rpm for 20-30 min, and the supernatant liquid was discarded to obtain pellets. The pellet was redispersed in cryoprotectant (0.5% mannitol) solution and pre-frozen at pre glycolic path at -40°C.

It was lyophilized at -80°C for 4 h (primary drying) and -40°C for 24 h (secondary drying). The obtained spongy NPs were stored in an air-tight container to avoid moisture [11].

Fabrication of polymeric NPs by inotropic gelation method

Ingredients required for the formulation are mentioned in Table 2. 0.1%, 0.2%, and 0.3% of chitosan solution (chitosan of shrimp shells) were prepared by using 1% v/v acetic acid solution and subjected to sonication for 20-30 min to obtain a clear solution (if not filter with muslin cloth). Then the pH of the chitosan solution (pH 3.4) was altered to 5 to 6 by adding 0.1N NaOH solution. STPP solution was added in deionized water/ distilled water at various concentrations of 0.1%, 0.15% and 0.20% while stirring at 750 rpm for 30 min to get respective STPP solutions. FLS was dissolved in a 1:1 ratio of Ethanol: Water to obtain a clear solution. This solution was added dropwise with a #26 syringe (0.45 mm) to 40 ml of chitosan solution and sonicated for 1 h cycles. 20 ml of STPP was added 0.75ml/min under stirring at 1000 rpm at 25°C and stirred for 30 min. Further, it was bath sonicated for 30 min at 50 HZ and centrifuged at 9000 rpm for 2 h. Pellets were dispersed in 2% mannitol, pre-frozen at -40°C for 15-30 min, then lyophilized at -40°C for 24 to 48 h [12].

Table 2. Formula for the preparation of polymeric NPs by inotropic gelation method.

Sl. no.	Ingredients	Formulation code		
		IG1	IG2	IG3
1	FLS (mg)	100	100	100
2	Ethanol: Water (ml)	1:1	1:1	1:1
3	Chitosan (mg)	100	200	300
4	Acetic acid 1% v/v	1%	1%	1%
5	Sodium tripoly phosphate (STPP) (mg)	100	150	200
6	Distilled water (ml)	100	100	100

Characterization

Solubility study of polymeric NPs

According to the drug solubility profile of FLS, the polymeric NPs prepared from both methods was subjected to a solubility test by using solvents in which FLS drug is soluble, like distilled water, methanol, ethanol, and DMF. The sample equivalent to 1 mg was dissolved in 10 ml of solvent and sonicated for 2 min [13].

Drug entrapment efficiency

Solvent evaporation method (SE)

NPs equivalent to 5 mg of FLS were taken, and 80 ml of 7.4 pH buffer was added. It was kept aside overnight by sealing it with silver foil. It was stirred and heated gently at 40°C for up to 15 min. The rest of the 20 ml buffer was added. Further, aliquots were prepared according to Beer's range, and absorbance was measured at 305 nm spectrophotometrically [11,12].

Inotropic gelation method (IG)

NPs equivalent to 5 mg of FLS was dissolved in a 7.4 pH buffer with continuous stirring. The solution was filtered through 0.45 micron-sized filter paper. It was diluted according to Beer's range, and measurement was recorded spectrophotometrically at 305 nm [10]. The calculated according to the formula:

$$\% \text{ DEE} = \frac{\text{content of drug practiclly obtained}}{\text{Theoratical drug calcuated}} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR)

After a baseline correction with dried potassium bromide to confirm compatibility, FTIR analysis was carried out on purified drug and NPs obtained by mixing with Potassium Bromide (KBr). In an evacuated die, pellets were pressed to 5 x 106 Pa to produce clear transparent pellets with an approximate diameter of 1.5 to 2 cm and thickness of 0.2 cm. An integrated Fourier transform

spectrometer (Bruker, USA) was used to record spectra at room temperature, scanning between 4000 cm^{-1} to 400 cm^{-1} [14].

Characterization by Particle size, Poly dispersity index, and Zeta potential

The average particle size, poly dispersity index, and zeta potential of the fabricated NPs from both methods were measured by a particle size analytical instrument (Nano Zeta Sizer, Malvern Instruments, UK) using techniques of Dynamic Light Scattering for size analysis and combination of Laser Doppler Velocimetry and Phase Analysis Light Scattering for zeta potential. Values of these both give Polydispersity index (PDI) [15].

Transmission electron microscopic studies (TEM)

Using a JEOL JEM 2010F UHR operating at 200 kV, HR-TEM photographs were obtained for polymeric NPs from SE2. The technique involves preparing the suspension of NPs in polyol obtained from the SE method, then placed on the amorphous carbon membrane of the transmission electron microscope grid and allowing it to evaporate solvent at room temperature. In order to eliminate most of the organic molecules, the grid was again thermally treated under an ultrahigh vacuum at 150 °C. With this treatment, high-resolution images were greatly improved without modifying particle crystallinity. We analysed the TEM images with a digital camera and the SAISAM and TAMI's software (Micro vision Instruments) to determine the particle size distribution [16].

Scanning electron microscopic studies (SEM)

NPs of IG3 were coated with a thin gold layer by sputtering. The microstructure was observed in a scanning microscope attached to an energy dispersive analysis of X-ray (EDAX) instrument (Joel 6390LA/ OXFORD XMX N) that operated at an acceleration voltage of 20 kV [17].

Differential scanning calorimetry (DSC)

DSC thermograms of pure medicines and NPs were taken using heating rates of 10 K/min from 20°C to 300°C in 25 ml/min under nitrogen flow. The aluminium crucibles were covered with a perforated lid before the samples were sealed. An empty aluminium crucible was used as the reference [18].

X-ray diffraction (XRD)

At room temperature, XRD patterns were collected using a Cu-Kα radiation diffraction system (Bruker D8 Advance diffractometer). Using a LYNXEYE XE-T detector and Kβ radiation which has monochromaticity, the instruments were utilized to scan samples of pure drug and NPs over 2-degree ranges of 3-50°C [19].

In vitro drug release study

In vitro drug release study of polymeric NPs was performed by diffusion study, i.e., equilibrium dialysis membrane method. The experiment was performed in a 1.2 pH buffer for 2 h and then using a buffer solution of pH 7.4 for the rest of the hours (till the end). 10 mg FLS equivalent to polymeric NPs (Equivalent to the dose of the drug) was taken and dissolved in 10 ml of methanol for the IG method and 10 ml of distilled water for the SE method. This suspension/solution was placed in the diffusion setup where gelatin paper was used as an equilibrium membrane and dialyzed against a 1.2 pH buffer for 2 h. At fixed time intervals, a 2 ml sample was withdrawn, and absorbance was measured at 305 nm. After 2 h, dialyzed against 7.4 pH buffer and 2 ml sample was withdrawn, and absorbance was read at 305 nm UV spectrophotometrically [19].

In vivo antihyperlipidemic drug action in albino rats

The polymeric NPs SE2 and IG2, which provided maximum *in vitro* drug release, were selected for the in vivo activity. The plasma lipid profiles were determined in the healthy albino rats weighing between 200-300 gm of either sex. For the study, animals were provided with regular pelletized food and water. The rats were divided into the following five groups: SE2, IG2, normal, control, and standard.

The course of treatment lasted 14 days. The normal group was fed with standard food pellets, the control group was fed with pure 2 ml coconut oil daily, and the standard group was fed pure FLS (2 mg/ kg body weight) daily. The test groups SE2 and IG2 were given the polymeric NP solution (2 mg/kg body weight) prepared in distilled water and distilled water with tween 80, respectively. Dosing was done daily for up to 14 days. The blood sample was collected on the 0, 3, 8 and 14 days by retro-orbital injection and centrifuged at 3000 rpm for 20 min at -20°C. The plasma was estimated for cholesterol and triglycerides using diagnostic kits (Erba Ltd. India). Fixed volumes mentioned in the leaflets of kits of the standard were prepared for reference, and a working reagent was added to the plasma of each group and kept aside for 10 min and simultaneously blank was prepared by omitting the sample and absorbed spectrophotometrically at 505 nm and 546 nm for cholesterol and triglycerides respectively [20].

Comparative pharmacokinetic drug release study

A comparative drug release kinetic study is carried out using Higuchi's and Kores Meyer and Poppa's models.

Higuchi Model-drug release from polymer matrix can be predicted

$$Q = K\sqrt{t}$$

Q = concentration of drug release;
K = slope;
√t = Square root of time

Graph plotted for Q v/s √t gives a straight line with slope K [18]. The data of in vitro release in an excel sheet model is checked using the concept mentioned above, or KinetDS3 software was utilized.

Kores Meyer Equation or Peppa's Model

$$\mathbf{C_t / C_\alpha = Kt^n}$$

Where,

Ct / Cα = The fraction of drug released at time 't'.
K = Constant indicating the properties of the drug/polymer system.
n = Diffusion exponent related to the mechanism of the release.

Above equation can be easily simplified,

$$\mathbf{\log C_t / C_\alpha = \log K + n \log t}$$

Using above equation and data of in vitro release in excel sheet model is verified Or KinetDS3 software was used [21].

Stability studies

Accelerated stability studies were performed to estimate the drug loss from the polymeric NPs. The study was carried out for the formulations of both methods, characterized by in vitro drug release. Formulations were stored at -2°C in the refrigerator and room temperature (29°C). They were then characterized for entrapment efficiency and *in vitro* drug release study after storing at room temperature and -2°C. They were estimated for one-month intervals for 3 months to verify both parameters [20].

Results

Solubility study of NPs

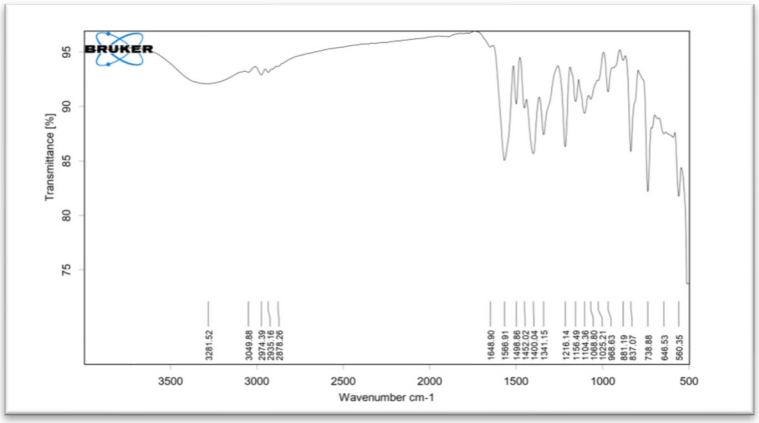
Table 3. Solubility studies of polymeric NPs.

Solvent name	NPs from SE method	NPs from IG method
Distilled water	Soluble	Insoluble
Methanol	Soluble	Soluble
Ethanol	Insoluble	Insoluble
Dimethyl formamide	Sparingly soluble	Sparing soluble

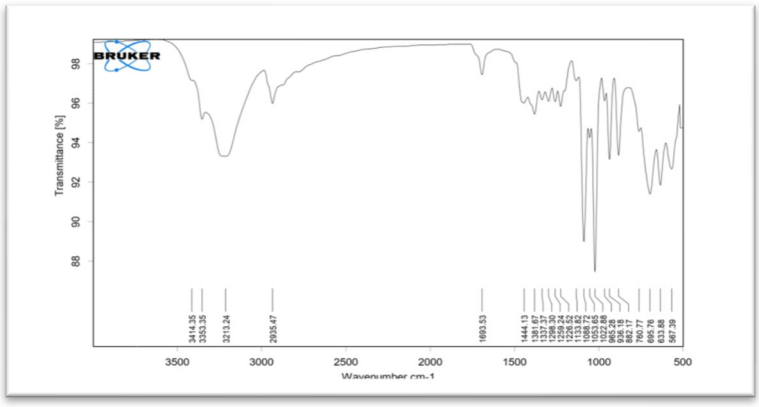
The solubility study for the polymeric NPs was based on the solubility profile of the Fluvastatin sodium drug. According to various literature, FLS is soluble in ethanol, methanol, and sparingly soluble in dimethyl formamide (Table 3).

FTIR studies

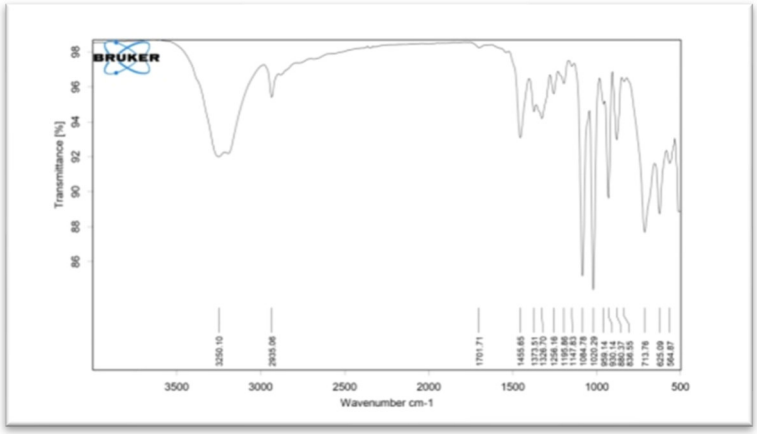
The FTIR estimation was performed to verify the compatibility between FLS and polymers used in the preparation. The spectrum obtained for a pure sample of FLS, SE3 and IG2 NPs, respectively, is shown in Figure 1.



FTIR Spectrum of Fluvastatin Sodium.



FTIR Spectrum of SE3 polymeric NPs



FTIR Spectrum of IG2 polymeric NPs

Figure 1. FT – IR spectrums of Pure drug, polymeric NPs from Solvent evaporation and Inotropic gelation method.

% Drug entrapment efficiency, Particle size, Zeta potential, and PDI

% Drug entrapment efficiency of SE polymeric NPs was obtained between the range of 46.8% to 86.1 %. In comparison, the DEE of IG NPs was found to be in the range of 44.6% to 70.2%, Graphically shown in Figure 2.

Particle size and PDI of the SE technique were found to be in the range of 271 to 313.3 nm and 0.453 to 0.781, respectively, whereas IG techniques fetched particles size and PDI in the range of 123.3 to 382 nm and 0.322 to 0.63 respectively.

Table 4. Measurement values of average particle size, PDI, Zeta potential, and %DEE.

Formulation Code		Particle size in nm	PDI	Zeta potential in mV	%DEE
SE	SE1	297.7	0.453	0.104	46.8
	SE2	271	0.562	26.1	82.3
	SE3	313.3	0.781	3.21	86.1
IG	IG1	123.3	0.422	11.1	44.6
	IG2	168.6	0.326	0.764	58.2
	IG3	382	0.630	9.09	70.2

Zeta potential can be used to represent a particle's surface charge. The results of the SE method were found to be in the range of +0.140 to + 26.1 mV, whereas the IG method results fall within the range of 0.764 to 11.1mV. The value for DEE, particle size, PDI, and Zeta potential is shown in Table 4.

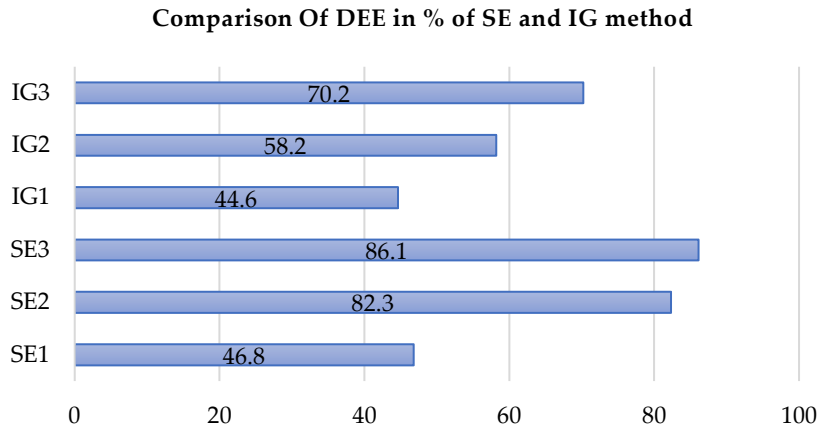
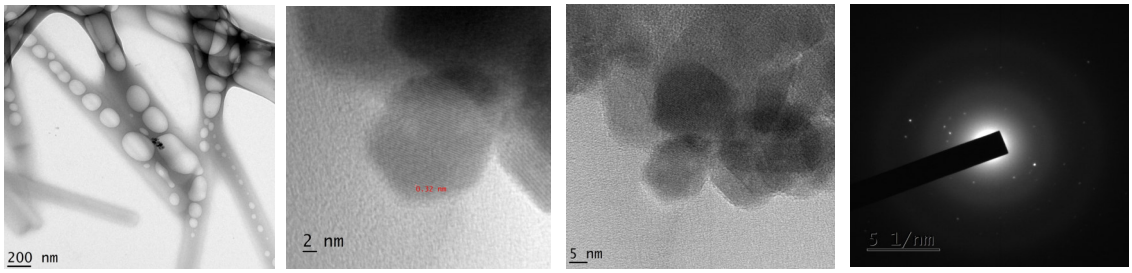


Figure 2. Comparison of % DEE of SE and IG methods.

Transmission electron microscopy (TEM)

High resolution transmission electron microscopy (HR-TEM) with the selected area electron diffraction (SAED) technique was used to characterize the SE3 polymeric NPs. The polymeric NPs had an elliptical shape with a smooth surface.

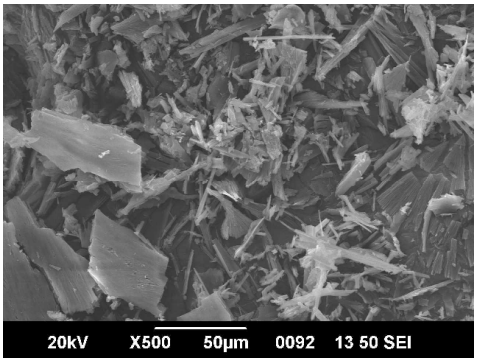


Elliptical shape Unidirectional planes Meso porosity SAED image

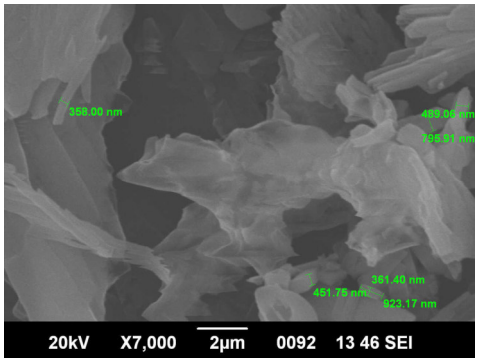
Figure 3. TEM with SEAD images of SE3 polymeric NPs.

Scanning electronic microscopy (SEM)

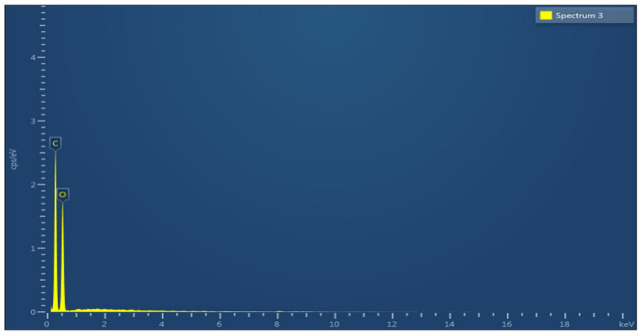
IG3 NPs were characterized by Field emission scanning electron microscopy (FE-SEM) with energy dispersive x ray analysis. The polymeric NPs from the IG method were found to be irregular in shape. The texture was found to be smooth.



Irregular shape



Smooth Texture



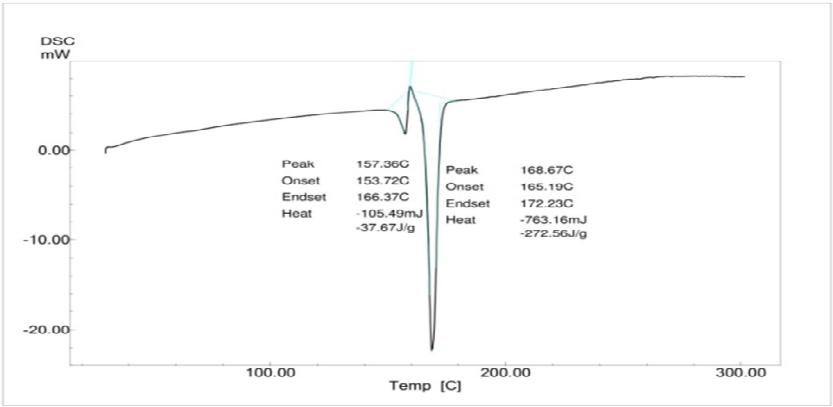
EDX image

Element	Line Type	Atomic %	Wt%
C	K series	57.02	49.9
O	K series	42.98	50.1
Total:		100	100

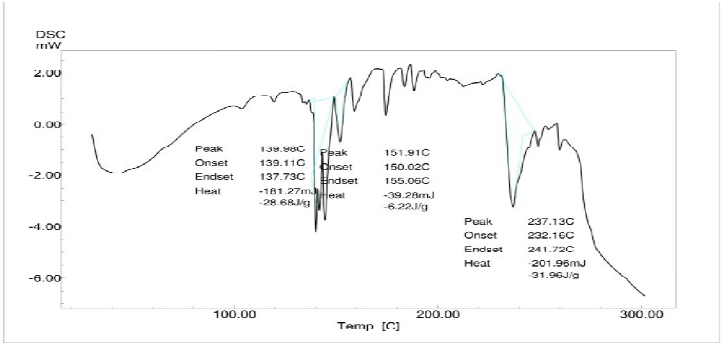
Figure 4. SEM with EDX image of IG3 polymeric NPs.

Differential scanning colorimetry (DSC)

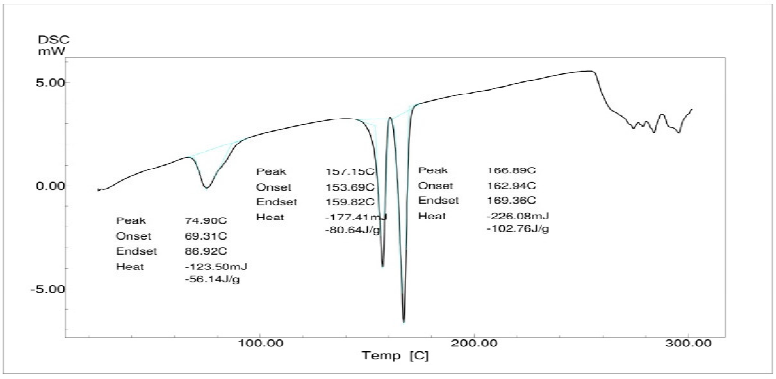
The DSC estimation was carried out for the samples compared with FLS. The nature of FLS found to be amorphous, and polymeric NPs also contained the amorphous nature. The results are shown in Figure 5.



DSC thermogram of Fluvastatin sodium



DSC thermogram of SE2 polymeric NPs

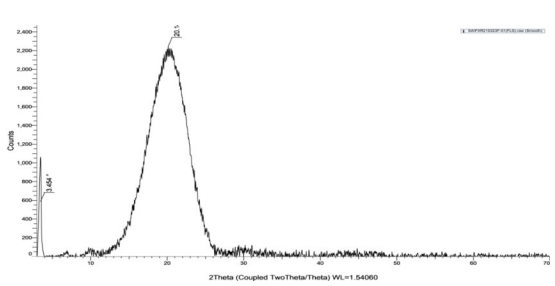


DSC thermogram of IG2 polymeric NPs

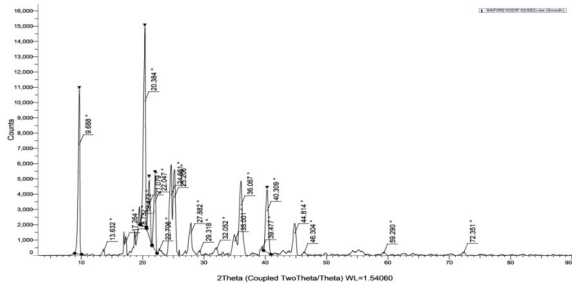
Figure 5. DSC thermograms of pure drug, polymeric NPs from solvent evaporation method and inotropic gelation method.

X-ray diffraction studies (XRD)

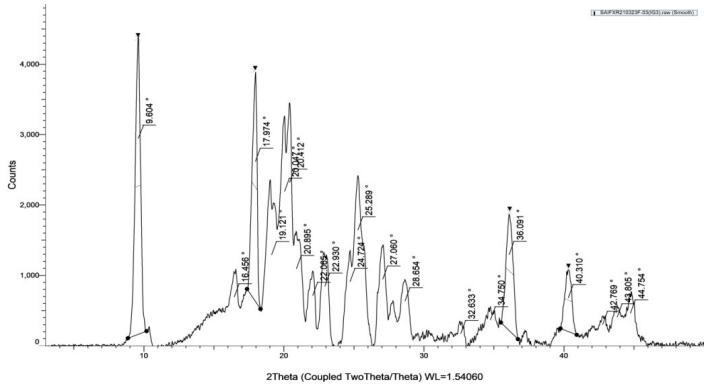
XRD was carried out for these two formulations against the pure drug. Results show that FLS and the prepared formulations are amorphous in nature.



(a) XRD of Fluvastatin sodium



(b) XRD of SE2 NPs



(C) XRD of IG3 NPs

Figure 6. XRD graphs of Fluvastatin sodium and NPs of SE and IG methods.

In vitro drug release studies

In vitro drug release studies were carried out by dialysis method using 1.2 pH buffer at first 2 h and in 7.4 pH buffer up to end. The percentage cumulative drug release of SE1, SE2, and SE3 was found to be 87.13%, 99.02%, and 55.75%, respectively. Meanwhile, IG1, IG2, and IG3 have shown drug release 79.05%, 84.6%, and 73.25%, respectively.

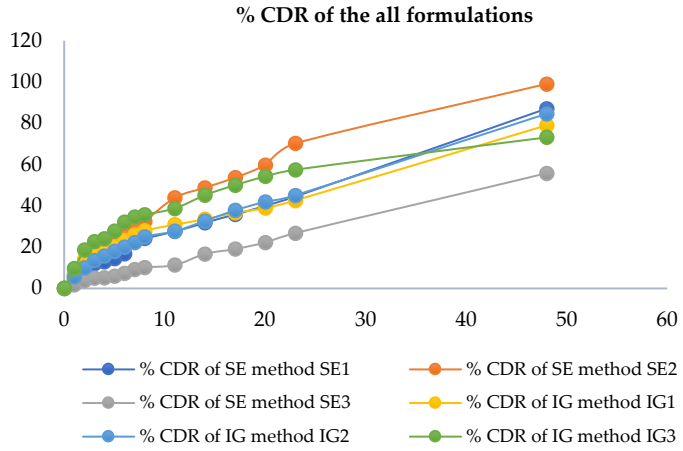


Figure 7. Comparison of in vitro study of SE and IG method.

Kinetic modelling on drug release study

The release data were subjected to Koresmeyer and Higuchi model using KinetdS3 software. The regression coefficient values obtained have shown in Table 5.

Table 5. Kinetic modelling on drug release study of polymeric NPs.

Formulation code		Zero Order (R^2 value)	Higuchi (R^2 value)	Peppas (R^2 value)	Best Fit Model
SE method	SE1	0.9892	0.771	0.9877	Both zero-order and peppas
	SE2	0.9310	0.8389	0.9902	Peppas
	SE3	0.9968	0.5563	0.9852	Zero-order
IG method	IG1	0.9744	0.9366	0.9743	Both zero-order and peppas
	IG2	0.9895	0.8522	0.9939	Peppas
	IG3	0.8614	0.9636	0.9720	Peppas

In vivo release study

The groups were estimated for total serum Triglyceride levels (TG) and Cholesterol levels (CH) over 14 days. In vivo drug release study showed that on the 0 days, the total serum TG and Ch of the normal group was more than that of the 3rd, 8th, and 14th days. Meanwhile, control and treated groups were too had more TG and Ch groups than on the 14th day. It was due to the random sampling of animals. On the 3rd day, the SE2 group had a slightly increased TG, and IG2 had slightly increased TG levels than standard due to the induction of hyperlipidemia along with the treatment. On the 8th day, the SE2 group decreased the TG level, whereas IG2 showed decreased Ch and TG levels than the standard. On the 14th day, both tested groups showed a significantly lower TG level than the standard. The stability studies showed that the formulation was stable in selected temperature conditions. Results are shown in Figure 8 and 9.

Stability studies

Accelerated stability studies were carried out by placing suspension/ solution of NPs at room temperature and -2 ℃ for 3 months. The % DEE of SE was found to be in the range of 39.2 to 83% at room temperature and 39.50 to 82.33% at -2℃; meanwhile, the IG method resulted in the range of 40.3 to 67% and 39.1 to 67.8%, respectively. The in vitro drug release of the SE method was obtained in the range of 49.99% to 96.23% at room temperature and 56.231 to 95.42% at -2℃, whereas the IG method had the range of 70.2 to 82.1% and 73.81 to 76.23%, respectively.

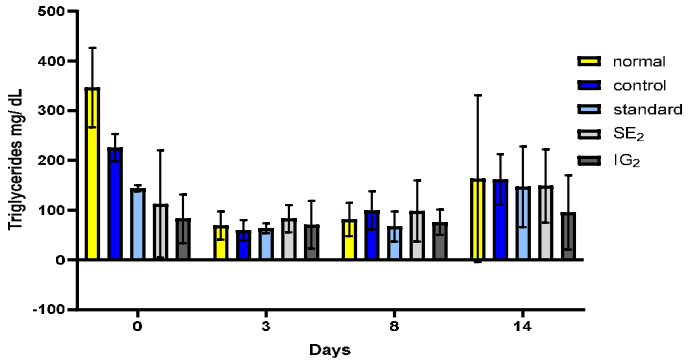


Figure 8. Serum triglyceride of rats group at different time intervals.

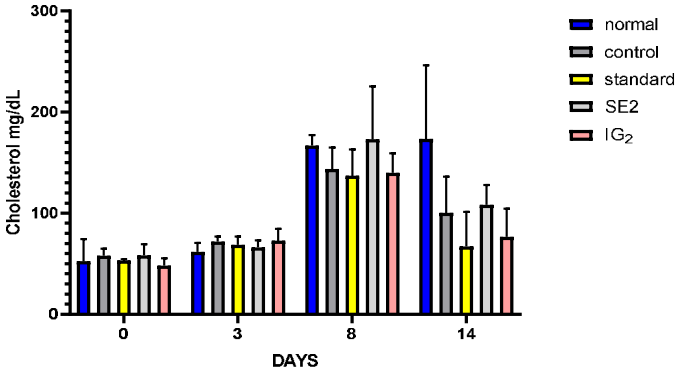


Figure 9. Serum cholesterol of rats group at different time intervals.

Discussion

The primary purpose of this work was to formulate Fluvastatin sodium-loaded polymeric NPs by solvent evaporation and inotropic gelation methods. These two methods were selected to compare the effects of polymeric NPs prepared from polymerization using monomers like inotropes and preformed polymers.

The FTIR peaks of the test samples were compared with a peak of FLS. The peak values present in the FLS graph approximately appeared in SE3. Furthermore, the IG2 showed H bonded N – stretching at 3250 cm^{-1}; C-N Stretching of aromatic amine at 1326 and 1256 cm^{-1} indicates that free amino groups remained while cross-linking as a result of rapid polymerization between chitosan and STPP shown in Figure 1 [22].

The comparison of the drug entrapment capacity of SE polymeric NPs range of 46.8% to 86.1% tells that the drug entrapment was increased by an increase in Drug and PVA and decreasing the Gum rosin concentration. But in the case of the inotropic gelation method, the drug entrapment efficiency found in the range of 44.6% to 70.2% indicates that an increase in the chitosan concentration increases the entrapment of the drug. From the %DEE, it can be predicted that the polymeric concentration increased, and better entrapment was found. Among SE and IG methods SE method exhibited higher entrapment efficiency shown in Figure 2. The value for DEE, particle size, PDI, and Zeta potential is shown in Table 4. The %DEE, particle size, PDI, and Zeta potential values are in the ideal range and hence fitted as the best model for preparing polymeric NPs for FLS [10].

TEM reveals that the polymeric NPs from the SE method were spherical and have mesoporosity, as light can slightly be passed in the section of the particles it was found to be mesoporous (Figure 3). The particles were found like grains and had unidirectional planes and patterns, Confirming that they are in amorphous form (Figure 3). SAED section of a sample was estimated for the diffraction where the d spacing value 0.2, which gives 20 planes which were confirmed to have fewer intensity peaks; hence the nature of SE2 was found to be amorphous (Figure 3) [23].

SEM reveals that the size of the IG2 was found to be irregular and smooth, and the particles are nonporous (Figure 4). The EDX value has shown in Figure 4. The atomic % and %wt. of C were found to be 57.02% and 49.9%, respectively; meanwhile, for O values were 42.98% and 50.1%, respectively. the cross linking between the chitosan and sodium tripoly phosphate in the inotropic gelation method was found to be rapid [24].

The DSC thermograph of SE2 showed endothermic peaks at 139.98℃, 151.91℃ and 237.13℃ (Figure 5) due to the melting of polymers. However, the peak of the drug corresponding to its melting point of 168℃ was absent, indicating that the NPs nature was amorphous and contained the uniformity of the drug in the polymer. DSC thermograph of IG2 involved a melting point of 74.90℃ nearer to the glass transition temperature, showing that the cross-linking has been taken between polymers and the presence of a peak at 166℃ corresponding to the melting point of the drug outlined that, NPs show amorphous nature but polymer linked to drug caused depression in the melting point and not uniformly dispersed in the linked matrix. However, no interaction was seen [12].

XRD of FLS showed peaks between 2θ of 10° to 30° (Figure 6a). SE2 sample showed peaks between 2θ of 10 to 70° (Figure 6b), and IG3 outlined peaks 2θ of 10° to 40° (Figure 6c) with poor periodicity [12]. Polymeric NPs obtained from both are amorphous in nature.

In vitro, drug release results signified that increased polymer results in slow drug release while more drug loading resulted in maximum drug release at the end of 48hrs. Comparing SE and IG techniques, the SE method fetched better release than IG. Figure 7 showed that the cumulative release for optimized formula SE2, which was found to be 99.02%, was better than IG2 [12]. The best pharmacokinetic fitting model was Kores Meyer and Peppa's model. The R2 values of optimized formulae SE2 and IG2 were found to be 0.9902 and 0.9932, respectively (Table 5) [20]. The in vivo activity showed better action for both methods by reducing cholesterol and triglyceride level. Formulations were stable at the selected conditions.

Conclusion

DEE of NPs was more for the SE method compared to the IG method. The polydispersity index of the SE and IG methods was less than 1. Hence, aggregation within the particles is less. The FT – IR studies indicate that the drug and polymers are compatible. Cross-linking of chitosan and STPP was seen, which was required for inotropic gelation. From SEM and TEM, it was concluded that NPs of the SE method have mesoporous particles, and particles prepared from the IG method are non-porous. By characterizing with XRD and DSC, it was found that NPs are amorphous. In vitro release study concluded that prepared polymeric NPs are a sustained release delivery system. The release kinetics shows that the model follows non – fickian mechanism and thus follows the zero-order release. In vivo release concludes that PNP reduces TG levels compared to Cholesterol. Stability studies showed that there were not many variations in the parameters within 3 months. Future aspects of this work are to approach better bioavailability in humans with less dose frequency.

Ethics approval and consent to participate

The present work was approved by the Institutional Animal Ethical Committee with the reference no: IAEC/HSKCOP/March 2020/PG8.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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GJPB

Research Article

Preliminary investigation of Dioclea reflexa seed gum as a food and potential pharmaceutical excipient

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Abstract

The chemical quality of natural gum determines its functionality and safe use. This study was to characterize some physicochemical properties and microbial load of gum obtained from *Dioclea reflexa* seed (DR), which has a history of folkloric use as a soup thickener in eastern Nigeria. The gum was extracted by aqueous maceration of DR. The microbial load was determined using the pour plate technique. The extract was screened for phytochemical constituents and analyzed for elemental content using atomic absorption spectroscopy (AAS) and scanning electron microscopy (SEM). Also, the morphology was viewed using SEM. The phytochemical screening indicated the presence of carbohydrates, starch, and simple sugars. The total viable aerobic bacterial and fungal counts were 2.0×10^1 and 1.0×10^0 (CFU/mL), respectively. The SEM micrograph showed that the polymer microstructure had dense and smooth surfaces, a property that has been associated with polysaccharides. The AAS elemental analysis showed the presence of several metals in the sample: Fe, Pb, Zn, Cd, Mg, Ca, and Na, in amounts generally within WHO permissible limits, except for Pb and Cd, whose levels were slightly above. The SEM analysis also showed the presence of K, Ca, Mg, Al, P, S, Na, and a preponderance of C and O. The presence of heavy metals could be associated with environmental pollution. DR gum's nature and chemical constituents present it as a potential food and pharmaceutical additive. Further studies should be done to validate the findings.

Keywords: gum; *Dioclea reflexa*; microbial load; phytochemical; elemental analysis

Introduction

The quality and functionality of naturally-sourced biomaterials have been very much attributed to the chemical content of the biomass [1] and the processing conditions [2, 3]. Natural gums are carbohydrate polymers of high molecular weight, composed of monosaccharide units joined by glucosidic bonds [4], constituted of a central unit of D-galactose or D-Galacturonic acid [5]. Plants, animals, or microorganisms usually produce them for structural and physiological functions [6]. Plant gums are:

- mostly exudates of the stem,
- resulting from the cell wall breakdown due to injury or other adverse conditions [1], or
- seed gums obtained from the endosperms of seeds [7,8].

Natural gums have diverse structural compositions and a wide range of physicochemical properties, thus making them useful in dosage forms as manufacturing aids, drug delivery agents, and foods or food additives [5, 1]. They are used as disintegrants, binders, emulsifiers, suspending agents, and release extenders in drug delivery [9]. In the food industry, they are used as binding agents, emulsifiers, stabilizers, and thickeners [10].

While synthetic polymers are non-renewable and non-biodegradable, limiting their pharmaceutical and food uses, natural gums, however, are biocompatible, biodegradable, and friendlier to the environment [11]. Gums are hydrocolloids possessing variant physicochemical properties that have resulted in remarkable differences in their functionalities. This important raw material is hard enough to supply to the pharmaceutical and food industries, so it is usually imported due to the local shortfall and the lack of appropriate data on the physicochemical properties which determine the uses. Hence, there is a need for their development to exploit their potential uses.

DR is sometimes referred to as "horse eye" or "sea purse" (due to the resemblance of the latter with the beautiful seeds). The plant *Dioclea reflexa* Hook F. belongs to the family Leguminosae, sub-family Papilionaceae) [2,3,12-14]. It is also referred to as sea beans and "marble vine" [2], probably because of the nature of the seed coat. DR is used locally as food, medicine, and a source of material as an effective larvicide [2,12-14]. In the South eastern part of Nigeria, DR powder, called "ukpo" is commonly used by the Igbos as a thickener in soup preparations.

We have reported the mucoadhesive functionality of DR gum in aminophylline tablet formulation [12] and some of its physicochemical and functional properties [13]. In this study, we aimed to analyse the elemental constituents in order to ascertain the level of heavy metals present in DR gum, using atomic absorption spectroscopy (AAS) and scanning electron microscopy (SEM) techniques, and qualitatively screening the phytochemical constituents as well as determining the microbial load. The study is important because it is a step toward establishing the safety and standardization of DR gum as a food and possible pharmaceutical excipient. DR gum is cheap and easy to find in Nigeria and other parts of the world.

Materials and Methods

Materials

DR, concentrated nitric acid (HNO_3) (Merck KGA, Germany), perchloric acid (H_2O_2) (BDH, England), freshly made deionized water, Ca, Cd, Cr, K, Fe, Mg, Mn, Na, Zn, and Pb salts (Sigma), Muller Hinton agar and broth, Sabouraud dextrose agar and broth, Nutrient agar, and peptone water. Bacterial isolates: *Staphylococcus aureus* (ATCC 25923) and multi-resistant *Staphylococcus aureus* (ATCC 25923); and fungal species: *Candida tropicalis* (ATCC 19092), *Candida albicans* (ATCC 2876), *Trichophyton rubrum* (ATCC 28188), *Microsporum canis*, and *Epidermophyton floccosum* isolates sourced from the Department of Microbiology and Biotechnology, NIPRD, Abuja, Nigeria, were used as test organisms. All the chemicals used were of analytical grade.

Collection and preparation of plant material

DR seeds were sourced from a local market, authenticated (Herbarium Number: NIPRD/H/6413) and extracted as earlier reported [12,13], with slight modifications. The seeds were washed, boiled in distilled water for 3 h to soften the shells, and then cracked to remove the shells. The cotyledons were cut into small-sized pieces and heated in ethanol (95%) at 75 ºC for 1.5 h to remove lipids and denature the proteins they might contain. The treated seed leaf particles were air-dried at 29 ºC for 6 h, and macerated (20% w/v) in distilled water in a flask for 18 h under mechanical shaking at the laboratory temperature (29-30 ºC), and the mucilaginous extract was filtered through a muslin cloth and centrifuged at 4000 rpm for 1.5 h to remove any solid particles. Then, acetone was added to the filtrate to precipitate the gum, which was sieved out using a clean muslin cloth. The wet gum was washed several times in acetone, exposed to air for 3 h for evaporation of the remnant organic solvent, and then

further dried in a hot air oven at 40 ºC for 2 h. The product was then pulverized using a mortar and pestle, weighed and packaged in an airtight screw-capped container till further use.

Phytochemical screening of the extract

The qualitative determination of the presence of phytochemical constituents was done following standard procedures [15,16].

Carbohydrates

To 1.0 gm of the extract in a test, the tube was added 3-4 drops of Molisch's reagent. Then 2.0 ml of concentrated sulphuric acid was carefully added to the mixture from the side of the container and observed. The formation of a purple ring at the interface and a purple precipitate on shaking indicated the presence of carbohydrates.

Starch

To an aqueous dispersion of 1.0 gm of the extract in distilled water was added 1 drop of iodine reagent. A deep blue coloration indicated the presence of starch.

Simple sugars

Equal volumes of Fehling's solutions A and B were added to a dispersion of a 1.0 gm sample of DR gum water extract. The formation of a brick red coloration was an indication of the presence of simple sugar.

Tannins

A few drops of a 10% ferric chloride solution were added to a dispersion of 1.0 gm of DR extract in water.

Alkaloid

The methanol extract of the sample was evaporated to dryness and 1% aqueous hydrochloric acid was added.

Microbial load determination

The test bacteria and fungi were maintained on nutrient agar (NA), and Sabouraud dextrose agar (SDA) slants, respectively. The loopfuls of 48 h surface growth of culture (bacteria and yeast) and spores (dermatophytes) were transferred to 0.9% NaCl solution, vortex-mixed, and the homogenous suspension was used for inoculation. The turbidity was then adjusted to match that of a 0.5 McFarland standard [17]. The DR gum extract was assessed for microbial load using Muller Hinton agar and broth, Sabouraud dextrose agar and broth, Nutrient agar and peptone water. The pour plate technique was used to determine the microbial load. Serial dilutions of the sample were introduced into molten nutrient agar and Sabouraud dextrose agar and allowed to be set in sterile Petri dishes, then incubated at 37 ºC and 25 ºC to estimate the total aerobic bacterial and fungal counts, respectively. The presence of microorganisms was determined based on their morphology on selective media and varied biochemical tests to detect the presence of any pathogenic microorganism.

Morphology

The morphology of the native DR gum was viewed using SEM (SEM, Carl Zeiss, EVO MA/10, Germany). The sample was scanned at a maximum electron accelerating voltage (ETH) of 40 eKV, and the micrograph obtained was recorded.

Elemental analysis by the atomic absorption spectroscopy technique

The elemental content assessment of the DR gum extract was done following the method earlier reported [18].

Apparatus

Atomic Absorption Spectrometer (AAS) (GBC Avanta, Model PM Version 2.2) under standard conditions with air-acetylene gas, Vecstar furnace, oven, desiccators, and glassware. The experimental conditions of the instrument were optimized, as stated in Table 1.

Table 1. Parameters for the spectroscopic determination of metals in the DR gum.

Element	Wavelength (nm)	Sensitivity (μg/ml)	Working Range (μg/ml)	Slid Width (nm)	Gas
Ca	422.7	0.020	1.0-4.0	0.5	Air-acetylene
Cd	228.0	0.009	0.2-1.8	0.5	Air-acetylene
Cr	257.9	0.050	2.0-15.0	0.2	Air-acetylene
Mg	285.2	0.003	0.1-0.4	0.5	Air-acetylene
Na	589.0	0.020	0.18-0.70	0.5	Air-acetylene
Fe	248.3	0.050	2.0-9.0	0.2	Air-acetylene
Pb	217.0	0.060	2.5-20.0	0.2	Air-acetylene
Zn	213.9	0.008	0.4-1.5	0.5	Air-acetylene

Conditions for calibration of the AAS for the elemental analysis

Analytical technique

Standard solutions (1.000 gm/l) and dilutions of the samples and standard reagents were made with deionized water. The working standards of the selected metals were prepared by diluting the standard stock solutions, which were prepared from analytical grade salts of the metals, and the results were corrected from the blanks. The wavelength of analysis, sensitivity, slid width, gas type, and the working range chosen for the analysis were as presented in Table 1. The equipment was calibrated to follow the sensitivity and detection limits of the elements, respectively, using the method of a standard calibration curve, and the results were expressed in μg/gm [18].

Acid digestion and analysis of DR gum

For the acid digestion of the sample, 1.0 gm of powdered DR gum was weighed, placed in a crucible, and heated in a furnace at 550 ºC for 6 h. It was then cooled under desiccators, and 2.5 ml of 6.0 M HNO3 was added to aid in complete dissolution. The reaction mixture was filtered (0.45 μm filter membrane) into a 20 ml volumetric flask and diluted to volume. The solution was then subjected to spectroscopy to quantitatively determine the metal contents in the spectrophotometer [AAS-GBC Avanta: Model PM, version 2.2] using the flame method [18]. Six replicate determinations were done, and the raw data obtained from the sample analysis for Ca, Cd, Cr, Fe, Mg, Mn, Na, Zn, and Pb were processed by calculating the actual concentrations in the samples using equation 1 [18]:

Metal (μg/gm) = (C x V)/W …… (1)

Where C = the concentration obtained from the spectrometer (mg/L); V = the volume of the undiluted sample solution in ml; W = the mass of the sample in grams.

Elemental analysis by scanning electron microscopy (SEM)

This was done using SEM (Carl Zeiss, EVO MA/10, Germany). Calibration was first done using the following standards for 6 iterations: C: $CaCO_3$, O: SiO_2, Na: Albite, Mg: MgO, Al: $Al2O_3$, P: GaP, S: FeS_2, K: MAD-10 Feldspar, Ca: Wollastonite, at various proportions. The specimen chamber was allowed to stay for some minutes for the attainment of vacuum prior to the scanning. The electron accelerating voltage (ETH) was then set at 0-40 eKV, and the instrument ran. The spectrum was processed, omitting no peaks, and all the elements were analysed (normalized) with 6 iterations.

Data handling

Elemental content determinations were done in replicates (n = 6) using aliquots of samples prepared for each metal following the optimized procedure. The data obtained were processed using Excel Microsoft Office, version 2007 and differences between mean values were considered significant at $p < 0.05$.

Results and Discussion

Yield of gum from the extraction

A yield of 10.2 ± 1.5 % gum extract was obtained from the starting material.

Phytochemical screening

Carbohydrates

Results of the phytochemical screening showed the presence of a purple ring at the interface, which formed a purple-coloured precipitate on shaking the mixture. This indicated that the DR gum extract consisted of carbohydrates.

Starch

The test showed the presence of a deep blue colour, indicating the presence of starch particles.

Simple sugar

A brick-red colour was observed, indicating the presence of simple sugars in the sample.

Tannins and alkaloids

The tests indicated the absence of tannins and alkaloids in the extracted DR gum. The overall physicochemical screening tests indicated that the DR gum extract was largely a carbohydrate polymer.

Microbial load

The results of the microbial load test revealed the presence of contamination of the DR gum sample with microorganisms. The microorganisms found were *Bacillus sp., Micrococcus sp., and Mucor sp*. The total viable aerobic bacteria (TVAB) and fungal counts (FC) were 2.0×10^1 (CFU/mL) and 1.0×10^0 (CFU/mL), respectively. However, the bio-burden fell within acceptable limits for *Bacillus sp*. and *Micrococcus* for non-sterile dosage forms as specified in the United States Pharmacopoeia (USP), which stated that *Escherichia coli* and *Salmonella sp*. must not be found in pharmaceutical products, whereas total viable bacterial and yeast counts must not exceed 10^2 CFU/mL [19].

Morphology

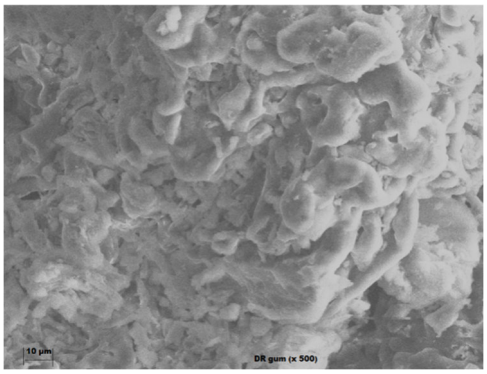
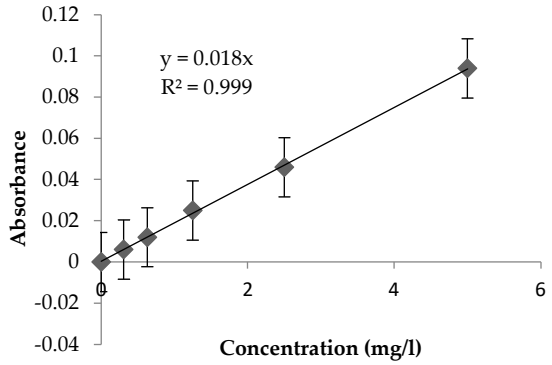


Figure 1. SEM micrographs (x 500) of the native DR gum powder. The smooth and dense surfaces of the hydrogel polymer microstructure might be attributed to polysaccharides.

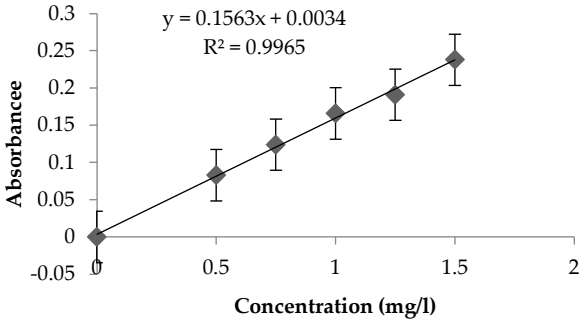
Figure 1 shows the SEM photomicrograph obtained of the native DR gum powder. The micrograph showed that the hydrogel polymer microstructure had dense and smooth surfaces, which might be attributed to polysaccharides [20]. This might predispose the gum to versatile uses as a pharmaceutical excipient. As a hydrogel, it is hydrophilic and can absorb large amounts of water molecules and swell in an aqueous environment. As such, it may function effectively as a disintegrant or drug-carrier matrix in solid dosage formulations. The amorphous nature makes it especially suited for efficient drug loading, possessing enough surface areas for drug particle adhesions and adsorptions. Moreover, the gummy nature empowers the polymer for use as a binder in solid dosage formulations and as a viscosity enhancer in liquid and semisolid formulations. We have reported its functional similarities to guar gum [13] and its mucoadhesive functionality in formulations for aminophylline tablets [12].

Elemental analysis by AAS

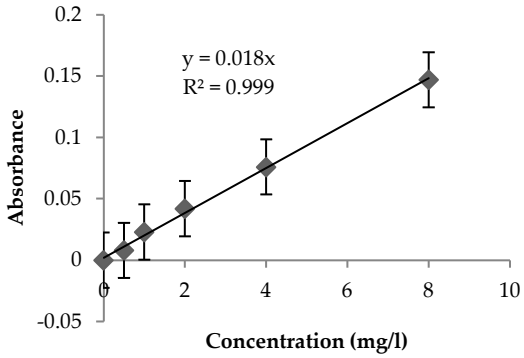
Figure 2 (a-i) shows the working curves obtained for the AAS analysis. Figure 3 shows the plots of the concentrations (mg/g) of the various elements obtained in the studied sample. The ascending order of the levels of the minerals (elements) in the sample was: Fe > Pb > Zn > Cd > Mg > Ca > Na, while Cr and Mn were below the detection limit of the instrument used. Various roles played by these elements were highlighted and discussed below.



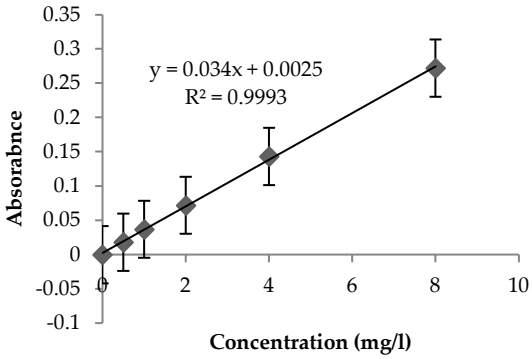
(a) Sodium (Na)



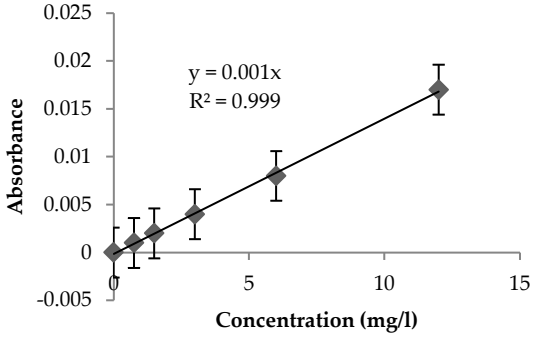
(b) Zinc (Zn)



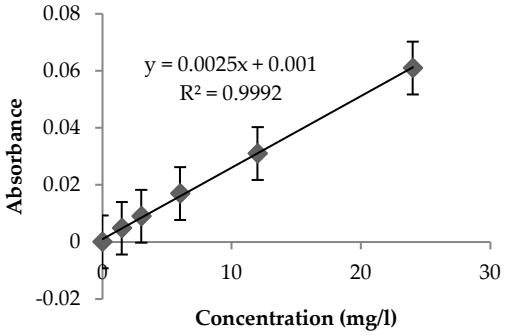
(c) Chromium (Cr)



(d) Manganese (Mn)



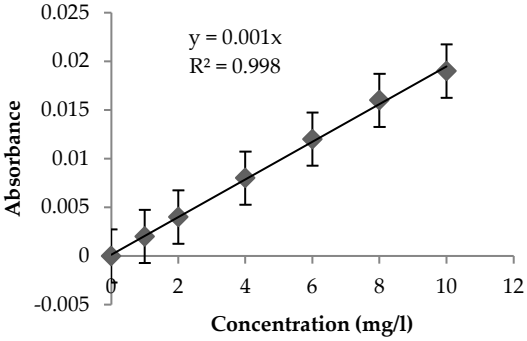
(e) Lead (Pb)



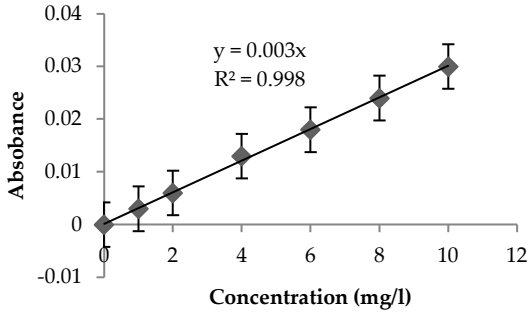
(f) Iron (Fe)

Calcium (Ca) at a specific concentration is known for its role in developing bones, teeth, muscle systems, and heart functions [21]. Its level in the seed under study was 0.70 µg/g (Figure 3), indicating a satisfactory accumulation level.

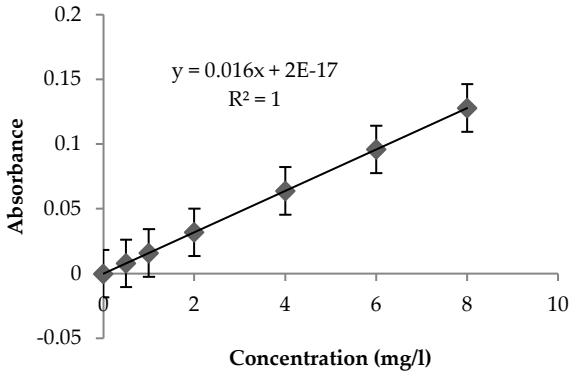
Chromium (Cr) has been recommended by the National Academy of Sciences of the US for a daily intake of 50–200 μg for adults because it is good for vision [22]. However, its critical level (5.30 ppm) could probably cause a yield reduction.



(g) Magnesium (Mg)



(h) Calcium (Ca)



(i) Cadmium (Cd)

Figure 2. Typical calibration curves obtained for some of the elements (a = Pb; b = Zn; C = Cr; d = Mn; e = Cd; f = Fe; g = Mg; h = Ca; I = Cd). All the plots showed high linearity ($R^2 > 0.99$).

When Cr is taken in large amounts, it can cause skin rashes, nasal irritation, bleeding, stomach upset, kidney and liver damage, itching in the nose, and even lung cancer. A deficiency of Cr causes disturbances in the metabolism of glucose, lipids, and proteins [23]. In this study, the Cr level was less than the instrument's detection limit. So, people who use this seed as a spice in a soup might not have to worry about health problems that can come from getting too much Cr.

Mg is an essential mineral for good health. It is required for over 300 natural biochemical processes in the body. Amongst the numerous health functions of Mg in the body is the transmission of nerve impulses, temperature regulation, energy production, detoxification, and the formation of healthy teeth and bones [24]. The Mg supplement dose associated with diarrhoea ranges from 1,000–5,000 mg, with a tolerable upper limit of 350 mg/day for individuals 9 years and older [25]. The results of the study indicated that the Mg content of the DR seed gum was lower than the upper limit, which means it is safe for consumption.

Mn sometimes referred to as the humble trace element, is essential to health, as it facilitates the utilization of vitamins C, B1, biotin, and Cl and neutralizes free radicals. Mn deficiency may cause increased problems with blood glucose levels, congenital disabilities, poor growth, decreased fertility, deafness, blindness, and paralysis. However, the symptoms of Mn deficiency have not been well-established except for limited data on demineralization and inadequate growth in children and a few others. The recommended dietary allowance is 2 mg per day [25]. The level of Mn in the studied sample fell short of the limit of detection, indicating, most probably, that the content was below its critical

concentration in the DR gum. For consumption of the seed as a spice, the sample is safe in terms of the critical level of Mn.

Fe is essential for humans and animals, a significant constituent of haemoglobin. It plays an essential role in the metabolism of major food components, such as carbohydrates, proteins, and fats, by facilitating their oxidation in the body, thereby controlling weight, which is a risk factor in diabetes [26]. The results (Figure 3) showed a high amount of Fe in the DR gum. This might be due to the absorption of traces of the element from the environment. The recommended amount of Fe in food is not more than 10-60 mg daily, and intake of less than the prescribed daily dose in diets may result in nose bleeds, myocardial infarction, and increased chances of infection in the gastrointestinal tract [26]. The results of this study have indicated that the Fe content of the DR gum sample is within the required dietary limit; therefore, its consumption in moderate amounts can provide body requirements per day.

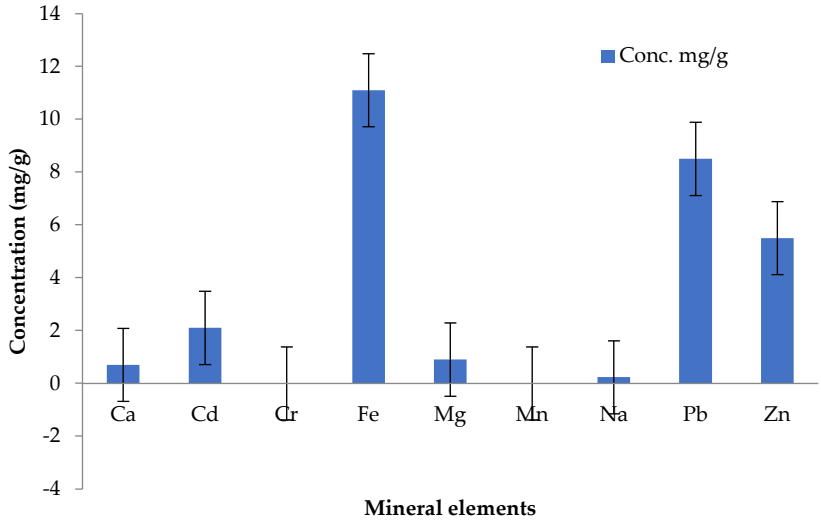


Figure 3. The Plot of the concentrations of the various elements in the sample as determined by AAS. The DR gum sample had the highest concentration of Fe compared to other elements. The concentrations of Pb and Cd in the gum material are above WHO limits of 10 ppm in herbal medicine and 1 ppm in foods, respectively, which raises some concerns.

Pb and Cd are non-essential trace elements having functions neither in the human body nor in plants. At low doses, they induce various toxic effects in humans. The typical symptoms of Pb poisoning are colic, anaemia, headache, convulsions, chronic nephritis of the kidneys, brain damage, and central nervous system disorders. Cd accumulates in the human body and damages mainly the kidneys and liver. The World Health Organization (WHO) prescribed limit for Pb content in herbal medicine is 10 ppm, while the dietary intake limit is 3 mg/week [27]. The lowest level of Cd, which can cause yield reduction, is 5-30 ppm, while the maximum acceptable concentration for foodstuffs is around 1 ppm [27]. The results of this study, as shown in Figure 3, indicated that the sample's Pb and Cd content exceeded the WHO and dietary limits for herbal products and foods. Therefore, sample consumption should be stopped or taken with caution after a rigorous confirmatory determination has been carried out to ascertain exactly where the source of the contamination is. This is because continual intake of traces of Pb and Cd can lead to long-term accumulation in the body system, which could lead to grave health implications. The presence of Pb and Cd might be due to contamination of the plant's soil. This needs further investigation.

Zn is an essential trace element for plant growth and also plays a vital role in various cellular processes, including normal growth, brain development, behavioural response, bone formation, and wound healing in man. Routine supplementation with zinc is highly recommended in managing diarrhoea, especially in infants and young children [28]. People with diabetes who are zinc deficient do not improve their perception abilities and also lose their sense of touch and smell [26]. Daily adult

dietary upper limit for zinc is 40 mg [29]. The results (Figure 3) indicated that the Zn content of the sample falls below the dietary requirement limit per day. This indicates that taking the sample only once per day will not be able to meet the zinc requirement of the body.

Elemental analysis by SEM

The results of elemental analysis by SEM showed the presence of nine elements, namely, C, K, Ca, O, Mg, Al, P, S, and Na, with C and O constituting 54.50 and 41.29 % of the atoms, respectively (Figure 4 and Table 2). The proportions of C and O were significantly ($p < 0.05$) higher than those of the other elements in the DR gum sample. The preponderance of C and O points to the likelihood of the gum being composed of mainly cellulosic polysaccharides $(C_6H_{10}O_5)_n$ [30].

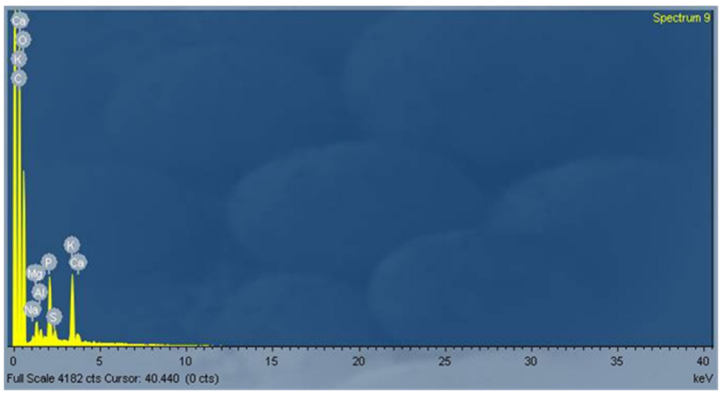


Figure 4. SEM showing the various elements present in the DR gum sample. This indicates that SEM is also useful in elemental analysis.

Table 2. Results of elemental analysis by SEM

Element	Apparent Conc	Weight (%)	Atomic (%)
C	106.20	45.04	54.50 ± 2.5*
O	67.89	45.44	41.29 ± 2.2*
Na	0.80	0.37	0.24 ± 2.3
Mg	1.74	0.86	0.51 ± 1.0
Al	0.92	0.40	0.22 ± 1.5
P	10.58	2.89	1.36 ± 1.2
S	1.20	0.46	0.21 ± 2.4
K	12.05	4.14	1.54 ± 2.2
Ca	1.06	0.40	0.14 ± 2.2
Total		100.00	

*Proportions of C and O were significantly ($p < 0.05$) more than the other elements in the DR gum sample.

The previous results of the study have provided helpful information on the chemical constituents of DR seed gum in an attempt to answer, though inconclusively, the research question about its safety as a food and potential pharmaceutical material. While the AAS analysis indicated the presence of Cd, Cr, Fe, Pb, and Zn, the SEM result did not. However, both test techniques showed the presence of Ca, Na, Mg, and Mn. The differences were attributed to the varying test conditions of the instruments. Even though AAS is usually used to do elemental analysis, the results of this study show how useful SEM is for this investigation.

The future perspective is to carry out further work on the material to establish more characteristics that will enable its informed acceptance and registration as a generally safe biomaterial for various pharmaceutical uses. The plan in our subsequent research is to expand the investigation on samples of DR seed to be collected from various locations to find out the possible influence the soil type could have on the constituents and other physicochemical qualities of the gum. We recently reported the comparable performance of DR gum to tragacanth powder as a suspending agent in metronidazole suspension [31]. In addition to the already established functionalities, we are currently exploring other possible pharmaceutical applications of the biomaterial.

Conclusion

According to the study's findings, DR gum's phytochemical contents primarily consisted of various types of polysaccharides and mineral elements. The elements found in the DR gum investigated using AAS, and SEM analysis included K, Ca, Mg, Al, P, S, Na, Mn, Zn, Fe, C, and O, with the last two constituting 54.50 ± 2.5 and 41.29 % atoms, respectively. The DR gum also had high levels of some heavy metals, such as Pb and Cd, slightly above WHO permissible limits. The sample had permissible bioburden. Notwithstanding the long-time folkloric use, it is essential to conduct quality checks on food and pharmaceutical materials to save consumers from toxicity. The study's results present DR gum as a potential food and pharmaceutical additive. We recommend improving DR gum processing for purification purposes before its use as a food or pharmaceutical additive. Further studies are necessary to establish the sources of the high Pb and Cd content in the sample and validate the findings.

Key findings

The investigation has provided preliminary information on DR gum's phytochemical constituents, morphology, elemental content, and microbial load. The new information about the biomaterial is relevant for its characterization and standardization for possible acceptance and use as a generally safe pharmaceutical and food excipient. Scientists who work on developing raw materials in the food and drug industries will be interested in the report.

Acknowledgements

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Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

Financial support

None

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