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Juglans regia shells as a potential bioresource for extraction and identification of bioactive compounds: A sustainable approach for reduction of agro-food waste contributing to ecological problems worldwide

Rachna Shashikant Jadhav[1*] Niranjan Nandkumar Patil[1] and Bhaskar Saha[2]
[1]Department of Microbiology, Bhavan's College (Andheri), Mumbai, Maharashtra, India
[2]Department of Life sciences and biochemistry, St. Xavier's College, Mumbai, Maharashtra, India
scientist.rachna@gmail.com

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Abstract

Juglans regia shells are considered one of the non-edible parts of the dry fruit, thereby resulting in the addition of shell waste, causing a rise in ecological problems based on waste management. In the fruit and vegetable products industries, a lot of waste occurs during every step of production; when selecting, sorting, and boiling processes are done, tons of dry and wet waste is generated. The non-edible parts of the plants that are discarded are often not known for their bioactive components. The unexplored bioactive activities of the shells and the focus on exploring the possible dietary and therapeutic potentials of such underutilized wastes will reduce the possible environmental waste burden. The objectives of this research were to alert the functionality, chemical composition, and biological properties of Juglans regia shells for their use as food and justification for its medicinal use. In this comprehensive research, the bioactive components in Juglans regia shells were identified, and the total phenolic content, mineral content, flavonoid content, and antioxidant activity were studied as a potential bioresource for the extraction of nutraceuticals and bioactive compounds. Further, their efficient utilization in the development of nutraceutical products, their health benefits, and the value addition of food waste resources have also been discussed for possible dietary and therapeutic potentials of especially underutilized agro-food waste to aid in reducing the possible environmental waste burden.

Keywords: Waste management, Phytoconstituents, GCMS, Antioxidant, Flavonoids, Persian walnut, Nutraceutical, Functional foods.

Introduction

Food waste, a by-product of various industrial, agricultural, household, and other food sector activities, is continuously rising due to an increase in demand for a number of products. According to the Food and Agriculture Organization (FAO), about one-third of the produced food is wasted globally[1]. The lumpsum amounts of agro-food waste not only possess as a challenge for the food processing industries but also are an important issue for both environment and the international economy since they are one of the major landfilling causes[2].

Due to an increasing awareness of waste management of waste obtained from fruit, and vegetable product manufacturing industries, the waste from these industries is usually effectively managed. However, waste such as shells, from dry fruit industries is often neglected. There is a global tendency towards industrial fruit processing and, following such processes, non-edible byproducts are normally discarded. However, these non-edible byproducts contribute to ecological problems such as increased numbers of pests, insects, rodents, increased waste, etc. Thus, studies to explore the benefits of these byproducts as sources of food supplements or medicinal products are a need of the hour.

Different parts of fruit have been used in conventional medications for various ailments. Exploring the possible dietary and therapeutic potentials of especially underutilized agro-food wastes will aid in reducing the possible environmental waste burden. The non-edible parts of the plants are considered so, due to their external characteristics. Often, these non-edible parts too, consist of several bioactive components that prove beneficial in a number of ways. *Ayurveda*, an ancient medicinal science originating from India is a foundation of using the non-edible parts of medicinal plants for various biomedical applications. Scientific literature based on *Ayurvedic* treatment of a wide array of diseases has shown the usage of these non-edible parts[3-6].

Dry fruits are healthier options due to their biological activities. They are healthier and tasty snacks that are extremely beneficial to health and should form an integral part of our diet in the form of raw, cooked forms or as a part of recipes. They provide nutrition that their alternative dairy foods are not sufficiently enough to provide. Dry fruits act as catalysts to process energy building in the human body faster. In ancient medicine, dry fruits' non-edible parts were converted in an efficient manner to use for treating ailments.

Various studies have indicated that different kinds of food wastes obtained from fruits, vegetables, and dry fruit processing industries, can be used as potential sources of bioactive compounds and nutraceuticals which have significant applications in treating various ailments. In *ayurveda*, these non-edible parts were used either in the preparation of topical solutions or converted into edible products for convenient consumption[7]. Not only the edible parts of plants consist of secondary metabolites, but also the non-edible parts. Different secondary metabolites, minerals, and vitamins have been extracted from food wastes, using several extraction approaches[8]. In the next few years, these approaches can possibly provide an innovative approach to increase the production of specific compounds for use as nutraceuticals or as ingredients in the design of functional foods.

Due to an increase in awareness of healthy diets among the population of all countries, a number of people follow a healthy, natural diet. The Mediterranean diet has an impact on promoting positive health and longevity. The Mediterranean diet is plant-based food and a monounsaturated fats-rich diet, consisting of nutrition-rich nuts that are filled with fiber, antioxidants, vitamins, protein, unsaturated fat, and omega-3 fatty acids. As a part of this diet, nuts are consumed on a regular basis. Other than being consumed as a snack, they are also processed on a small scale for the production of dry fruit bars or chocolates for added energy and nutrition. A combination of a handful of berries and nuts is used for a bright, mood-boosting healthy option. There is ample evidence that suggests that nuts in the context of the Mediterranean Diet may prevent peripheral artery disease (PAD) and reduce the risk of cardio-metabolic risk factors in obese individuals[9].

The most commonly consumed nuts in the Mediterranean diet are walnuts. Walnuts are not only consumed as a snack but also as an addition to the maximum of the traditional recipes in this diet. Walnuts are cultivated in Europe and Asia and are one of the most common dry fruits that are consumed worldwide on a large scale. However, except for the shells of walnut, research conducted on the leaves, and bark of *Juglans regia* have revealed the presence of antioxidants, phytosterols, antiproliferative compounds, and several other biologically active compounds; among which, phytosterols and antioxidants have attracted considerable attention for their health-promoting activities which have been reported to prevent diseases related to lifestyle; diseases such as obesity, diabetes mellitus, hyperlipidemia, hypertension[10]. Due to the cholesterol-lowering effects of walnuts, they are considered one of the best natural sources as an alternative to synthetic sources or medicines for cardiovascular health maintenance. In the history of mankind, edible and well as non-edible parts of plants have been used for *Ayurvedic* medicinal purposes, in foods and medicines. Several natural products have been extracted and isolated from various parts of plants for the development of new drugs.

Epidemiological studies have consistently shown a significant positive relationship between regular consumption of walnuts and reduced risks of cardiac diseases[11] and some types of cancers[12]. These properties have been attributed to the high content of antioxidants, polyphenols, and other phenolic compounds present in the walnut kernels. Although oxygen is essential for regular survival and for other processes in the human body, it generates free radicals that are potentially harmful due to their ability to damage essential molecules such as DNA and enzymes and eventually affect proper cell functioning. Antioxidants act on these free radicals by scavenging them and converting them back to less reactive forms of the molecules. Oxidative stress is a state when there is unbalance between the generation of reactive oxygen species and the antioxidant defense capacity of the body. It is closely associated with a number of diseases including Parkinson's disease, Alzheimer-type dementia, and Huntington's chorea[13]. And as per the literature review, nuts and fruits have been known to positively affect cognitive development[14].

Scientists have found out that both nuts and dried fruits are rich in quercetin and other polyphenols which have anti-inflammatory, anti-diabetic, and anti-obesity effects. According to research conducted on walnut kernels, and seeds, their antioxidant content and chemo-preventive properties in them have been attributed to their high content of ascorbic acid (vitamin C), tocopherols (vitamin E), b-carotene (provitamin A), anthocyanins and several other polyphenols[15]. Several studies have shown that phenolic compounds are the major bioactive phytochemicals that are beneficial for human health. In fact, many authors have reported a direct relationship between regular consumption of nuts and reduced risk of endometrial, esophagus, pancreatic, lung, oral cavity, pharynx, and colon cancer[16].

The *Juglans* genus (family *Juglandaceae*) is widely distributed throughout the world. Research conducted on walnut leaves, bark, and seeds revealed the presence of antioxidants, phytosterols, antiproliferative compounds, and several other biologically active compounds. Among these biologically active compounds, phytosterols and antioxidants have attracted considerable attention for their health-promoting activities which have been reported to prevent diseases related to lifestyle; diseases such as obesity, diabetes mellitus, hyperlipidemia, and hypertension. Phytosterols have been demonstrated to thus reduce circulating levels of cholesterol by inhibiting the absorption of dietary and endogenously generated cholesterol from intestinal cells. Several effective properties of walnut varieties have resulted in the commercial availability of many dietary supplements and food products fortified with phytosterols, which were considered as alternative sources of cholesterol-lowering drugs[17].

Phytosterols have also been proven to have many other health-promoting effects such as anti-inflammatory, antioxidant, and anticancer activities[18].

Therefore, these reviewed health-promoting effects of phytosterols make walnut varieties as well as walnut products a very attractive natural source of diet due to their nutritional and medicinal values. Walnuts are most commonly consumed in the Mediterranean diet. The by-product of walnut production – the green husk of walnut is formed in large amounts. A recent study on the aqueous extract of green husk that has antimicrobial activities was suggested as a low-cost natural source of phenolic compounds. Not only dry seeds (nuts) are used, but also green walnuts, shells, bark, green husks (epicarps), and leaves are used in the cosmetic and pharmaceutical industries[19]. The health benefits of these nuts are usually due to their chemical composition. Walnuts are good sources of essential fatty acids (linoleic acid is its major fatty acid), tocopherols and tocotrienols, proteins, fibers, melatonin, sterols, folate, tannins and other polyphenols[20]. Among several nut types, walnuts consist the highest content of antioxidants[11]. However, the reason attributed to this antioxidant activity is not yet clear. Other than walnut seeds, walnut leaves are considered as effective as the seeds and have been widely used in *Ayurveda* and several traditional medicine treatments of skin inflammation, ulcers and for its antidiarrheic, anti-helminthic, antiseptic, and astringent properties. In some European and Asian countries, the leaves are also used to prepare infusions[21].

In fruit and vegetable industries, plenty of waste occurs during every step of the processing. When selecting, sorting, and boiling processes are done, tons of dry and wet waste are generated on a daily basis. Solid waste of peels/skin, seeds, stones, etc., and liquid waste of juice, and wash water is generated. Thus, there is a serious waste disposal problem that eventually leads to unhygienic and other types of environmental problems if not managed well in time. Although the quality, quantity, and type of waste are important in the decision to evaluate waste on a large scale, if the problems are managed individually on a small scale, the further serious problems can be evaluated one by one. To eliminate generating waste in dry fruit industries where shells of the dry fruits are thrown away, efforts can be made to study the biological components present in the shells in order to use them in one way or the other instead of throwing them and eventually generating tons of waste.

Apparently, the total waste generated by all the walnut industries in each country is more than 15,000 tonnes/year; where most of the shells have no use in the food industry except for direct combustion in Biofuel industries and blasting purposes as mentioned earlier. The current research was based on waste management of the shell waste obtained from *Juglans regia*, a dry fruit consumed on a large scale in the Asian continent, as a part of the Mediterranean diet. In this research, a comprehensive study of various techniques to identify bioactive components and their activity has been conducted. Further, their efficient utilization in the development of nutraceutical products, their health benefits, and the value addition of food waste resources have also been discussed.

Materials and Methods

Collection and processing of plant material: *Juglans regia* shells were collected from the APMC market of Navi Mumbai, Maharashtra, India. The decayed shells were discarded. The clean and fresh walnut shells were cleaned using distilled water, dried in a hot air oven at 80^{o}C for 48hrs and ground into fine pieces, and milled into fine flour using a miller.

Extraction of components: Soxhlet extraction was performed as per Campos, D. A.[2]. 30gm of the *Juglans regia* shell flour (SF) was placed in a thimble covered with non-absorbent cotton and was placed in an extraction chamber consisting of 100ml HPLC grade methanol in the Soxhlet apparatus and 200ml HPLC grade methanol in the distillation flask. Extraction was carried out at 65^{o}C for 4 hours until the completion of 8 cycles. After completion of extraction, the reflux method was conducted in order to remove excess methanol. 70ml of the methanolic extract consisting of the sample was obtained. The sample was kept for evaporation at 60^{o}C for 30 hrs. The wet powder form of the sample obtained was reconstituted using 30 ml HPLC grade methanol (Sigma-Aldrich). The extract was filtered using Whatman filter paper (Thomas Scientific) to get a clear solution and was stored at 20^{o}C[22].

Identification of components using GCMS: The main focus of the present work was on the identification of the chemical components in the methanol extract of *Juglans regia* shells and confirmation of the presence of bioactive compounds obtained by Jadhav, R. et al.[23] by employing GCMS. Methanolic extracts were prepared using Soxhlet extraction at 60^{o}C. The GC – MS analysis was carried out using a GC JEOL – The Accu TOF. The ion chamber temperature of the instrument was set to 200^{o}C. The inert gas used was Helium with a flow rate of 1 ml/min[24].

Estimation of Moisture content: 5 gms of the *J. regia* SF was weighed dried, and the dish was covered and kept in the oven for 2 hours. The dish was removed after 2 hrs, weighed, and kept in the oven at half-hour intervals until a constant weight was achieved[25].

The formula used for the calculation of total moisture content was:
Moisture % = W1-W2/W1-W x 100
Where W1 = Weight in grams of the dish with the sample before drying; W2 = Weight in grams of the dish with the sample after drying; W = Weight in grams of the empty dish.

Estimation of total ash content: 5 gms of *J. regia* SF was weighed in a crucible for determination of ash content. The crucible was transferred to a muffle furnace at 550^{o}–600°C and ignition till grey ash was obtained. The crucible was cooled in a desiccator and weighed. The process of heating, cooling, and weighing was conducted at 30 mins intervals till the difference in weight in two consecutive weighing was less than 1 mg[25].

Estimation of Protein content: Estimation of total protein content was done using the determination of organic Nitrogen content by the Kjeldahl method. Kjeldahl digestion flask of 800 mL was fitted with rubber stopper trap to prevent mechanical carryover of Sodium hydroxide (NaOH) during distillation. Concentrated Sulphuric acid, 45% Sodium Hydroxide (NaOH) (HiMedia) reagents were used to dissolve 450gms of Sodium hydroxide (NaOH) pellets in 1000 ml distilled water. 0.1N of Standard Sulphuric acid solution (H_2SO_4) and 0.1N of Standard Sodium Hydroxide solution (NaOH) (HiMedia) were used.

Methyl Red Indicator solution was prepared by dissolving 0.5 gms of methyl red powder in 100 ml of alcohol. Approximately 1-2gms of the *J. regia* SF was weighed and transferred to 800 ml Kjeldahl flask. 0.7 grams of Mercuric oxide (HiMedia), 15 gms of Potassium Sulphate (HiMedia) and 40ml of concentrated Sulphuric acid were added along with 2-3 glass beads. Kjeldahl flask was placed in an inclined position on the stand in the digestion chamber and the sample was allowed to digest by heating the flask gently at low flame until the initial frothing ceased and the mixture boiled at a moderate rate.

The flask was rotated in some time intervals several times. The flask was heated for 1hr and 20 mins until the color of the digest was pale blue. The flask was cooled and 200ml of distilled water was added to it. After the addition of distilled water, a few anti-bump granules and poured down the side of the flask cautiously. 90 ml of Sodium Hydroxide solution (450gm/l) was added to make the contents strongly alkaline before both the acidic and alkaline layers were mixed. The flask was then connected to a distillation apparatus -condenser to fit a delivery tube which was dipped just below the surface of the pipette volume of standard acid in a conical flask receiver. The contents of the digestion flask were mixed homogenously and boiled until 150ml of the mixture was distilled into the receiver. 5 drops of methyl red indicator and titrated against standardized 0.1N Sodium Hydroxide solution. Blank was maintained along with the sample titration reading[26].

Estimation of fat content: 2gms of the *J. regia* SF was added with Anhydrous ether into a thimble. The extraction period was 4 hrs at condensation rates of 5-6 drops/second. The sample extracted was dried at 100^{o}C, cooled, and weighed[25].

Estimation of carbohydrates: Total carbohydrates were calculated after the determination of the percentage of moisture, protein, ash, and fat content[27]. The formula used was:
Percent by mass = 100 - (A + B + C+ D)
Where: A = Mass percentage of moisture; B= Mass percentage of total protein; C = Mass percentage of fat; D = Mass percentage of total ash.

Estimation of energy: Energy content was calculated by using the formula[28]:
Energy = Protein x 4 + Fat x 9 + Carbohydrate x 4.

Estimation of antioxidant activity: Capacity of the *Juglans regia* SF to scavenge the DPPH free radical was estimated using DPPH radical scavenging activity. DPPH (HiMedia) concentration used was 62.13µM with methanol as diluent. Ascorbic acid 1500µM was used as the standard. The range of standards used for measuring the scavenging of DPPH radicals ranged in concentration from 30µg/ml, 60µg/ml, 90µg/ml, 120µg/ml, and 150µg/ml [29].

J. regia SF concentration of 100mg/ml and 1mg/ml was considered. The mixtures of the SF and the standards were vortexed and incubated for 30 mins in dark at room temperature. The reduction of DPPH radicals was measured spectrophotometrically at 517nm. DPPH radical scavenging activity was calculated as the percentage of DPPH discoloration using the formula: [(Absorbance of blank – Absorbance of sample)/Absorbance of blank x 100].

Total phenolic content estimation: Phenolic compounds in the *J. regia* SF extract were estimated using Folinciocalteau reagent (HiMedia). The phenols in the standard and the sample reduce the phosphomolybdic-phospho tungstic acid in 10% FC reagent to form molybdenum which is a blue-colored complex. 25 mM Gallic acid equivalent (GAE) (HiMedia) in 95% methanol was used as a standard from the range of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml along with 70mM Sodium carbonate. The phenolic content was measured spectrophotometrically at 765 nm[30].

Estimation of flavonoids: Standard used was quercetin. Quercetin (HiMedia) was prepared by mixing 5mg in 50 ml of 95% methanol. 10% Aluminium chloride ($AlCl_3$) was prepared by mixing 10gms powder in 100ml distilled water. Another reagent used was 1% Potassium acetate. The standards varied in a concentration range of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml. The flavonoid content was measured spectrophotometrically at 415nm[31].

Estimation of minerals: Ash preparation for mineral estimation: The weight of ash obtained from the 10gms SF was 0.057gm. The ash sample obtained was dissolved in 1:1 HCl in a volumetric flask and the volume was made up to 100ml [32].

Estimation of phosphorous content: 2.5% NH_4 molybdate was prepared by weighing 2.5gm of NH_4 molybdate in 100ml of distilled water. ANSA reagent was prepared by mixing 0.2gm of 1-amino-2-naphthol-5-sulphonic acid (ANSA) and 1.3gm of sodium sulfite to form a fine powder. Working standard phosphorus solution (31µg/ml) – Potassium dihydrogen phosphate (HiMedia) was prepared by dissolving 1.36g of the chemical in 100 ml of distilled water. The Standard used was Di-potassium phosphate in the range of 3.1µg/ml, 6.2µg/ml, 12.4µg/ml, 18.6µg/ml, and 31.0µg/ml. The tubes were vortexed after the addition of the chemicals and measured spectrophotometrically at 660 nm[33].

Estimation of Iron content: Saturated Potassium dichromate ($K_2S_2O_8$) solution was prepared by adding 50ml of distilled water in ($K_2S_2O_8$) (HiMedia) till the mixture remained undissolved in the solution. 3N Potassium cyanite (KCNS) was made by dissolving 30gms of KCNS (HiMedia) in 100ml of distilled water. The Standard used was Ferric chloride ($FeCl_3$) (HiMedia) in the range of 0.005µg/ml to 0.025µg/ml. The tubes were vortexed after the addition of the chemicals and incubated at room temperature for 20 mins. Absorbance for iron content in the sample was measured spectrophotometrically at 510 nm[34].

Results and Discussion

Identification of compounds using GCMS: Methanolic extracts of shells of *Juglans regia* were prepared using Soxhlet extraction at 60°C. The GC – MS analysis was carried out using a GC JEOL – The Accu TOF. The ion chamber temperature of the instrument was set to 200°C. The inert gas used was Helium with a flow rate of 1ml/min. The compounds identified in the methanolic extract of *Juglans regia* shells are illustrated in Table-1.

Table-1: Bioactive components identified in the *Juglans regia* shell flour.

Retention time	Compound
4.72 mins	Tridecanoic acid
5.41 mins	Acetoxyacetic acid, nonyl ester
12.09 mins	2-hexenal, 2-ethyl
15.1 mins	Eicosanoic acid, phenylmethyl ester
16.23 mins	Undecane
21.19 mins	Benzeneacetic acid decyl ester
21.49 mins	(1-pentyl-allyoxymethoxy-methyl)-benzene)
23.27 mins	9,12-octadecadienoic acid(Z,Z), phenylmethyl ester
24.92 mins	Benzyl oxytridecanoic acid
25.41 mins	6,9,12- octadecatrienoic acid, phenylmethyl ester (Z,Z)
26.82 mins	9- octadecanoic acid (Z), phenylmethyl ester
30.64 mins	9,12,15- octadecatrienoic acid, Z [(trimethyl (sil)oxy, 1 – trimethyl (sily)oxy] ethyl ester (Z,Z,Z)

Table-2: Proximate analysis of *Juglans regia* shell flour.

Tests	Results
Moisture	6.88 % gm/gm
Ash	0.62 % gm/gm
Protein	7.2 % gm/gm
Fat	2.7 % gm/gm
Carbohydrate	82.6% gm/gm
Energy	383.6 kcal/100gm

Antioxidant activity: Antioxidant activity of the *J. regia* SF was estimated using DPPH radical scavenging assay, using ascorbic acid as a standard. The standard curve representing Standards of 30µg/ml, 60µg/ml, 90µg/ml, 120µg/ml, and 150µg/ml were maintained to calculate the antioxidant activity of the *J. regia* SF. 84% Antioxidant activity was observed in *J. regia* SF of concentration 100 mg/ml. Thus, 100 mg/ml, i.e. 225 µg/ml of *J. regia* SF can achieve 84% antioxidant activity. DPPH radical scavenging activity of the sample is attributed to the presence of eicosanoic acid, undecane, benzeneacetic acid, 9,12-octadecadienoic acid (Z,Z), phenylmethyl ester, benzyl oxytridecanoic acid and tridecanoic acid present in the sample extract that possess antioxidant activities. According to research conducted on leaves of *Jatropha gossypifolia*, antioxidant activity of the extract was reported[35]. Antioxidant activity of *Scapania verrucose* was reported due to the presence of benzeneacetic acid.

Other than antioxidant activity of the two compounds mentioned above, DPPH radical scavenging activity of *Mollugocerviana* plant was also exerted due to tridecanoic acid as a biological component present in the plant. DPPH activity of *J. regia* SF is attributed to the presence of eicosanoic acid, undecane, 12-octadecadienoic acid (Z,Z), phenylmethyl ester, benzyl oxytridecanoic acid and tridecanoic acid present in the sample.

Total phenolic content: The total phenolic content found in the standards and the sample was estimated spectrophotometrically using Folin Ciocalteau assay. The total phenolic content of the standards was found to increase with increase in the standard concentration. The total phenolic content concentration in *J. regia* SF was found to be 121µg/ml in 100mg/ml. The phenolic content in the standards and the sample is represented graphically in Figure-2. This total phenolic content of the sample is attributed to the presence of 9,12-octadecadienoic acid (Z,Z), phenylmethyl ester[36].

Estimation of Flavonoids: The Flavonoid content present in the methanolic extract of *J. regia* SF was estimated and measured graphically. The total flavonoid content in the SF was found to be 90µg/ml in the 100mg/ml sample. Flavonoids are polyphenolic plant compounds abundantly available in natural foods such as fruits and vegetables. They influence digestion, absorption, metabolic rate, and biotransformation.

In several research studies conducted to date, it is found that flavonoids exhibit anti-inflammatory, antidiabetic, anti-thrombogenic, anticancer, and neuroprotective activities through different mechanisms of action. The flavonoid content estimated from the walnut shell flour was extremely low. Despite low concentrations of flavonoids in walnut shells, they can be used along with another natural food rich in flavonoid content to enhance the overall effect on the human body.

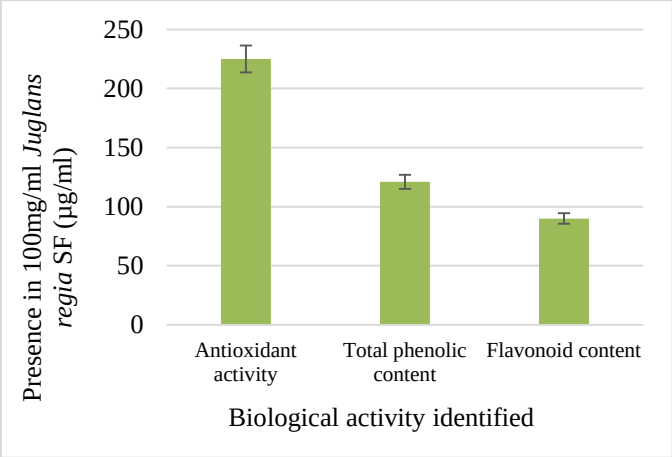

Figure-1: Graphical representation of bioactive compounds.

Estimation of minerals: Estimation of phosphorous content**:** The phosphorous content in per gram of the walnut shell sample was estimated and found to be 16.7µg. The main function of phosphorus in the human body is the formation of bones and teeth by using carbohydrates and fats. It also produces proteins for the growth, maintenance, and repair of cells and tissues. The phosphorous content present in the walnut kernels consumed worldwide is 97mg per serving, i.e. 1 kernel (2 halves). Most of the phosphorus in walnuts is stored as phytic acid, which is not digestible by humans. However, soaking the nuts helps in increasing the digestibility of the walnut kernels. Phosphorous in nuts works in association with vitamin B and helps with regular kidney function, and muscle contractions regularizes heartbeat, and is a cause of proper nerve signaling. As the phosphorus content estimated from the walnut shell flour is 16.7µg/gm of the flour, it can be used along with the kernels which can benefit the human body when combined with diet. Not only it will benefit the muscular system, but also the cardiovascular system without the side effects caused by chemically derived phosphorus rice supplements.

Estimation of iron content**:** The iron content estimated in 1 gm of the *J. regia* SF was 0.017mg (17µg/gm). Iron is an essential component of an erythrocyte protein – hemoglobin; that transfers oxygen from the lungs to the tissues of organs in the human body. Iron supports the process of its metabolism and is necessary for growth and development, normal cellular functioning, and synthesis of some hormones and connective tissues. Although the amount of iron estimated from the walnut shell flour is less as compared to what other dietary supplements rich in iron provide, it can be used along with other iron-rich foods in order to avoid the consumption of dietary supplements available in the market. Not only it is an effective natural source for increasing the consumption of diet in the human body, but also it can be easily consumed by anemic people.

Discussion: *Juglans regia* shells were found to possess a higher antioxidant activity of 225µg/ml in 100mg/ml of SF and total phenolic content of 121µg/ml in 100mg/ml SF. As the flavonoid content estimated from the *J. regia* SF was estimated to be 90 µg/ml in 100mg/ml SF, which is not as high as other naturally available foods rich in flavonoids, *J. regia* shells can be incorporated into other foods rich in flavonoids. Minerals like phosphorous and iron content of 16.7µg/gm and 17µg/gm respectively, in *J. regia* shells indicate that they can also be used along with other foods rich in phosphorous and iron. Considering the variety of bioactive compounds identified in this research and their antioxidant activity, total phenolic content, flavonoid content, and mineral content, *Juglans regia* shells can be successfully used to produce nutraceutical or dietary supplements, thereby reducing the industrial food agro-waste generated, and help in effective waste management.

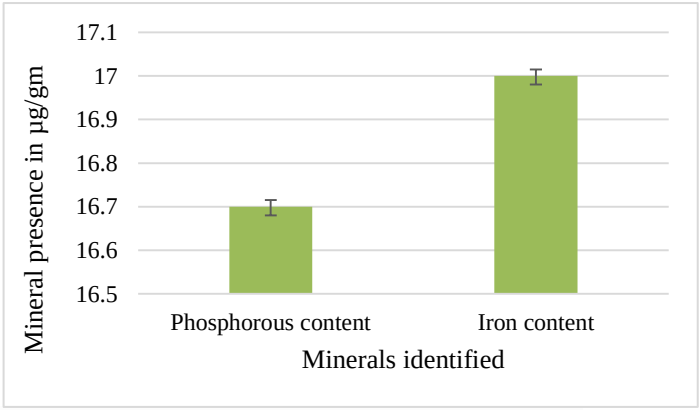

Figure-2: Graphical representation of mineral content.

Conclusion

To date, a diverse array of bioactive compounds from specific food residues and the availability of advanced scientific techniques for bioactive compounds identification have provided a great opportunity to quantify metabolites in a range of food waste materials. Based on the identification of specific bioactive components, food waste or a by-product could be utilized on the basis of the bioactive components extracted from them. The development of a nutraceutical from food waste with better efficiency of bioactive component recovery will not only add value to the food waste but also be useful in reducing the cost of chemically formulated products. With the increasing setup of food processing industries, an increasing amount of food and agriculture waste is available. Thus, its utilization as a source of bioactive compounds will decrease the burden of waste management.

Moreover, in India alone, the discarded portion of industrial waste is very high, which creates a serious waste disposal problem. Organic wastes generated from industries are hazardous to the environment owing to the presence of bioactive compounds in them. These organic wastes can be used as a potential bioresource for a supplementary food material or a nutraceutical product. The present research ascertains how *Juglans regia* shells are a rich source of a variety of bioactive compounds and can result in the production of nutraceuticals and dietary supplements. Ensuring that every food waste is studied for its compounds will help in the complete utilization of industrial food waste, thereby providing extra compensation to the industries by the sale of residues, which will also help in eradicating environmental pollution caused by the production of industrial food waste.

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