Research

Rohan Sarkar*, Biraj Bandhu Basak and Manish Das

Bioprospection of above ground biomass from ashwagandha plant: A waste valorisation approach

The medicinal plant, *Withania somnifera*, commonly known as ashwagandha is mainly grown for the roots having several health promoting effects that is in conformity to the traditional knowledge of Indian system of medicine. But once the roots are harvested, the large amount of residual plant biomass (above ground part) remains unused. Studies suggest presence of various bioactive compounds in this plant biomass that can be transformed to several value-added products. This article primarily focuses on important bioactive compounds like plant secondary metabolites and dietary fibres, their content and how these compounds can be utilized further as an effective biorefinery that would be of immense useful for the stakeholders i.e., industry as well as farmers. Fabrication of efficient technologies are the prime factor to utilize the residual biomass in an economical way that can be beneficial to industries and this will ultimately help to provide an assured market to the farmers to sell their produce. This article provides an insight regarding utilization of the residual biomass of *Withania somnifera* for its value addition towards higher economic return.

ASHWAGANDHA or Indian Ginseng (*Withania somnifera*; Solanaceae family) is a very popular as well as important medicinal plant used in Indian system of medicine. For millenia, this medicinal herb is being used as Rasayana (tonic) and/or churna (powder) having specific health benefits like improving memory, enhancing functions of nervous system, maintaining sexual and reproductive balance, increasing resilience of body towards stress, inducing immunomodulatory effect, antioxidant activity etc. The major secondary metabolites reported to be present are withanolides (triterpenic lactones like withaferin A, withanolide A, withanolide B, withastromonolide, withanone), alkaloids (tropine, causcohygrine), phenolics, saponins etc. that possess specific biological activities inside body. Although the root powder is primarily used for commercial use, the other plant parts or residual biomass (leaf, stem) are mentioned to be used in Indian Ayurvedic system. As this plant is mainly grown for the purpose of roots, a large amount of plant biomass remains left unused that can be considered for recycling in terms of waste reduction as well as valorisation purposes. The harvest index of *W. somnifera* is 29.24-43.61 that indicates more than 50% of the plant biomass (above ground part) remain unutilized after harvesting of the root. Annual root production of this crop is estimated to be approximately 1500 tonnes in India that in turn generate a large amount of waste (residual biomass) that can be a serious concern if not managed rationally. There is every scope for utilization or valorisation of this residual biomass based on the chemical constituents (bioactive compounds) present, their cellular component, surface morphology etc. that facilitate the recycling of the biomass into different value-added products. This requires a comprehensive study towards biochemical characterization of these materials that can provide a wholistic approach regarding utilization of this plant biomass.

Residual plant biomass

W. somnifera comes under medium to small shrub category with an average height of 30-150 cm. The whole *W. somnifera* plant along with different plant parts separately is depicted in Fig. 1. The plant is erect, branching perennial, greyish, that is almost completely surrounded and covered with very short, branched and fine silver-grey coloured hairs (Fig. 1a). The stems of the plant are erect, dark coloured with lesser leaves at lower part of the stem sometimes (Fig. 1a and 1c). The leaves are narrow with slightly waved, oblong or ovate shaped having length of 2.9-8.0 cm and width of 2.1-5.0 cm, arranged in an alternate manner i.e., opposite to the flowering shoots (Fig. 1b). Flowers are small, greenish, axillary, generally bell shaped with bisexual or monoecious in nature. Fruits are small, berry like, orange-red in colour that holds many reniform, discoid and yellow seeds (Fig. 1d). The roots are generally unbranched having height of 10-17.5 cm and width of 0.6-1.2 cm that are fleshy, long, woody and tuberous in nature. Talking about the biomass partitioning of this plant, a large amount of biomass exists in above ground part compared to root.

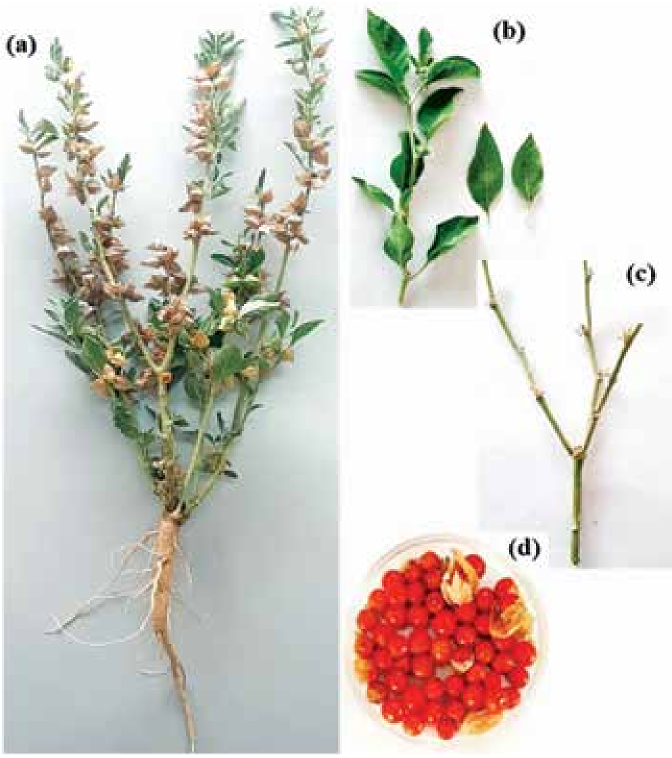

Fig. 1. Whole plant of *W. somnifera* (a) with different parts of above ground part i.e., leaf (b), stem (c) and fruit (d)

There are reports regarding presence of important therapeutic properties like anticarcinogenic and neuroprotective activities in alcoholic and water extracts of *W. somnifera* leaves. Diverse group of phytochemicals such as flavonoids, alkaloids, steroids, tannins, anthraquinones, saponins are present in leaf and stem extracts of this plant that exhibit antiproliferative effect and cytotoxicity towards tumour cells. Presence of these chemical compounds are also responsible for antimicrobial activity of different solvent extracts (ethyl acetate, acetone, methanol, ethanol, water) of *W. somnifera* leaf having both antibacterial and antifungal property against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus* sp., *Candida* sp. etc. By incorporating *W. somnifera* leaf powder in different bakery and cereal products, their nutritional value can be improved. These functional foods are prominent source of bioactive compounds that helps in improving overall health. These findings indicate the possibilities of using this residual plant biomass for preparation of phytochemical enriched extracts having significant biological activities. Moreover, there are chances for converting the residual plant biomass into different value-added products such as bioethanol, compost, biochar, biosorbent due to presence of lignocellulosic materials in plant cell. The feasibility of such product development largely depends on quantity of cellulose, hemicellulose and lignin present in the matrix. Altogether these residual plant biomass of *W. somnifera* has diverse possibilities to get utilized into several form as suitable.

Approaches of biorefinery

The conversion of the plant biomasses into suitable form largely depends on chemical nature of the matrix, their availability, amount of material generated etc. Based on suitability and feasibility, the residual biomasses have diverse scope to get utilized into different value-added products. Here the bioactive components i.e., phytochemicals and dietary fibres are of interest for their use into value addition process. Phytochemicals include secondary metabolites having specific bioactivity. Although a large variation among bioactive compounds is present, the bioactivities of this plant are primarily adhered with withanolides. On the other hand, the dietary fibre includes cellulose, hemicellulose and lignin. The bioactive components have been discussed in the succeeding paragraphs.

Phytochemicals or secondary metabolites

Phytochemicals are generally extracted from plant matrices by solvent extraction method. Various solvent based extraction techniques are there such as, refluxing, Soxhlet extraction, ultrasound assisted extraction, microwave assisted extraction, supercritical fluid extraction and many more. Here conventional refluxing method was followed while extracting the chemical constituents with different solvents like hexane, ethyl acetate, acetone, methanol and water. The polar solvents i.e., methanol and water provided highest extraction yield compared to other solvents. To get clearer picture regarding effect of different solvent extracts over yield of bioactive compounds, we used different combinations of methanol-water (75:25; 50:50 and 25:75) apart from sole solvents. As withanolides (polyoxygenated steroidal lactones) are the prime chemical constituents of this plant, the concentration of these compounds in each extract indicates the quality of the extract that could exhibit specific bioactivity.

Among different withanolides present, withaferin A (WA), 12-deoxywithastromonolide (WD) and withanolide A (WLA) claims the major fraction. That's why our discussion primarily focusses on these three withanolides. Table 1 exhibits the concentration of WA, WD and WLA in different hydro-methanolic solvents extracts of above ground part and root. The solvent system of methanol-water, 25:75 extracted highest amount of withanolides from both above ground part (in terms of WA) as well as root (in terms of WLA) followed by methanol-water, 50:50 and methanol-water, 75:25 i.e., 0.065, 0.052 and 0.028% respectively for above aground part and 0.079, 0.064 and 0.038% respectively for root. Among the individual components, WA content was higher in above ground part compared to root, whereas, WLA content was higher in root than the other plant parts across different solvents tested. In the above ground part, the WA content was significantly higher (up to 14.5 times) than compared to root.

Further, when the WA content was estimated in each of the component of the above ground part (Fig. 2), maximum contribution was from leaf followed by stem i.e., 51.76 and 40.26%, in total 92% approximately. Thus, it implies that the residual biomass of *W. somnifera* particularly the leaf and stem portion can be utilized as a good source for WA. At a very meagre dose, WA provides neuropharmacological properties promoting outgrowth and synaptic reconstruction that is of academic and research interest. The *in-silico* studies demonstrate

Table 1. Total withanolide content in terms of withaferin A (WA), 12-deoxywithastromonolide (WD) and withanolide A (WLA) in different solvent extracts (n=3) of above ground part and root of *W. somnifera*

Solvent system	Above ground part				Root			
	WA (%)	WD (%)	WLA (%)	Total (%)	WA (%)	WD (%)	WLA (%)	Total (%)
Methanol	0.013	0.003	0.007	0.023	N.Q.	0.002	0.031	0.033
Methanol-Water (75:25)	0.028	0.000	0.000	0.028	0.009	0.002	0.027	0.038
Methanol-Water (50:50)	0.046	0.006	0.000	0.052	0.017	0.008	0.039	0.064
Methanol-Water (25:75)	0.062	0.003	0.000	0.065	0.004	0.006	0.069	0.079
Water	0.018	0.000	0.000	0.018	0.001	0.006	0.016	0.023

the modulatory effect of WA over multiple targets such as, inflammatory cytokines, transcriptional factor, enzymes, suggesting a potential candidate for treatment of neurological disorder and cancer. Different other bioactivities like antifibrotic, antiherpetic, profibrinolytic, antileishmanial and wound healing effects are associated with this compound. Therefore, WA has profound applications in pharmaceutical as well as nutraceutical industry that can be made available through residual biomass of *W. somnifera*. As the root portion is used profusely in various ayurvedic preparations for presence of WLA primarily, the above ground part or residual biomass can be used as a potential source for WA. Appropriate solvent extracts may be prepared optimizing extraction parameters that would be rich in WA, will be useful for preparation of ayurvedic formulations, development of potential bioactive drugs, functional foods and others that can accomplish the waste to wealth approach.

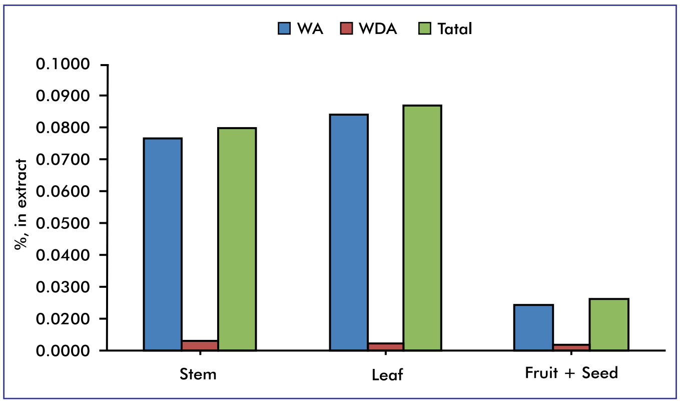

Fig. 2. Withanolide content in different parts (stem, leaf and fruit with seed) of above ground biomass of *W. somnifera*

Apart from withanolides, different types of phenolic compounds and flavonoids are present that provide antioxidant capacity to the plant. Total phenol and total flavonoid content of the above ground part was measured in each respective extracts of different solvent systems as mentioned earlier and methanol-water, 50:50 exhibited highest content as 50.74 mg GAE/g of dry matter and 32.03 mg QE/g of dry matter respectively. The IC_{50} value (concentration for 50% inhibition of free radicals) of 129.93 µg/mL denotes good antioxidant potential of the material. Therefore, this residual plant biomass can also be utilized as a prominent antioxidant agent by using appropriate solvent extracts. Apart from WA, these materials can be treated as a source for phenolics as well as flavonoids having diverse array of applications in nutraceutical as well as pharmaceutical sector.

Dietary fibres

The cellulose, hemicellulose and lignin content in the residual plant biomass of *W. somnifera* is 38.62, 27.03 and 11.94% respectively. The values indicate that this material comes under medium to high cellulose with high hemicellulose and low lignin content group. The relative distribution of dietary fibres is of utmost importance to find the feasibility of the materials to be converted into suitable value-added products like bioethanol, biochar, compost, growth medium etc. Basically, cellulose remain in crystalline form in cell while hemicellulose in branched form with low molecular weight while both are shielded by a matrix of lignin that creates hindrance for bioethanol production The predominant sugars that are most suitable for fermentation are reducing sugars like glucose, xylose and arabinose that are derived from cellulose and hemicellulose mainly. Therefore, the materials with cellulose, hemicellulose and lignin content of 30-38%, 14-27% and 9-18% respectively are useful as feedstock for bioethanol production. On the other hand, degradation/ breaking of cellulose and hemicellulose are relatively faster than lignin during pyrolysis process. So, higher lignin content is often inferred to have better thermal stability, a desirable property of substrate one can expect during preparation of biochar. The values of dietary fibre content of the residual plant biomass clearly show its suitability for bioethanol production opposite to biochar where it seems not suitable.

Similarly, composting should be relied on such plant biomasses having less lignin content as the cellulose and hemicellulose are easy to degrade in biomass and lignin is hardest to degrade/decompose by microbes. Such kind of materials are also useful for preparation of growth medium for mushroom. It implies that the residual plant biomass is suitable for preparation of compost as well as matrix for mushroom cultivation. Besides pharmaceutical and/or nutraceutical applications, this material can be utilized in preparation of different value-added products having wide industrial applications; ultimately facilitating the waste valorisation/recycling process. Different value-added products than can be prepared by utilizing the residual biomass are depicted in Fig. 3.

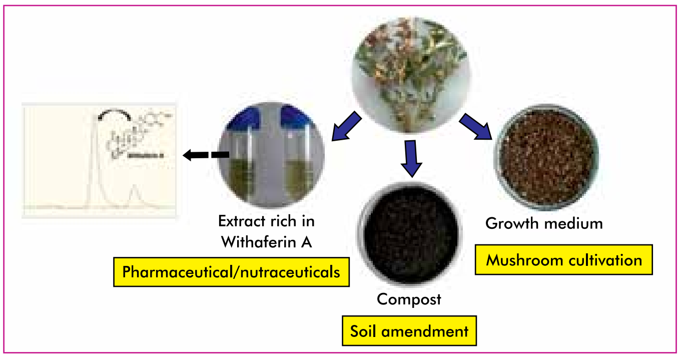

Fig. 3. Different value-added products prepared from residual biomass of *W. somnifera* having diverse applications

Farmers' perspective

The value addition process will be useful to the farmers in terms of utilizing their produce completely and increase in profitability. When a farmer grows this crop only for root purpose, their utility get restricted as the residual material is treated like waste. But the grower can take added benefit by using these materials by various means. The utility of biorefinery concept lies here. If the important bioactive compounds can be converted into suitable value-added products, the demand of these residual materials will increase among industrial sector. As for example, the major withanolide present in the leaf and stem i.e., withaferin A has profuse scope of application in Ayurveda, pharmaceutical and nutraceutical sector due to diverse health benefit effects. Through proper technological intervention, this chemical compound can be isolated in pure form or enriched extract can be prepared for development of suitable formulations. For that purpose, supply of raw materials to the production site is of prime importance where the growers or farmers can take the lead. Similarly, these materials are also suitable for bioethanol production, composting and development of mushroom growing media. Farmers can supply the residual materials to industry according to the demand and need, provided appropriate technology is available with respective end users. This implies that by application of appropriate biorefinery, it is possible to create a niche market for this residual biomass of *W. somnifera* which will be useful to the farmers to sell their produced material and get extra income rather leaving unutilized.

SUMMARY

The use of *W. somnifera* roots is an age-old practice. But some studies clearly suggest the usefulness of utilizing the above ground portion of this plant. If appropriate and user-friendly technology can be developed for conversion of this plant material into suitable high value products having commercial importance, the acceptability of this materials will boost up among the industries or manufacturers. Eventually the demand will be increased and farmers may be motivated to sell their produce at good price. Besides selling the roots, they can have extra income from these residual materials. The diverse array of applications of this material has potential to create different opportunities that would be beneficial to industry, farmers and end users as well. The application of suitable techniques plays the key role towards getting the full benefit from this residual material that can be accomplished through effective waste valorisation approaches.

For further interaction, please write to:
Dr Rohan Sarkar, Scientist, (Agricultural Chemicals) ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat 387 310. *Corresponding author: rohan.sarkar@icar.gov.in

Infestation of lesser grain borer on roasted makhana seeds under storage

The internal stored grain insect (lesser grain borer), *Rhizopertha dominica* (Coleoptera: Bostrichidae) was found infesting on the roasted makhana seeds of 3 standard size grades, i.e. 7 mm, 9 mm and 11 mm. It was recorded that insects preferred to feed on 11 mm size seeds, followed by 9 mm and 7 mm, respectively. Both grub and adult stages were able to cause substantial damage. The adult laid the eggs on the seeds by entering inside the kernels through the apical natural opening. The average temperature and relative humidity for *R. dominica* development was maintained as 32.5 ± 1°C and 70 ± 5°C, respectively. It took 35–50 days for completing its life cycle, which included four stages: egg, larva, pupa and adult. Females laid about 200–500 eggs in their lifetime, singly. Incubation period lasted for 5±0.3–9±0.4 days, while larval and pupal period took 30±5 and 8±2 days, respectively. Mean longevity of adult male and female was 26 and 17 weeks, respectively. The damage potential was assessed using the artificial infestation (purposive samples) with different numbers of tested insect. The study indicated that significant loss of roasted makhana seeds during 15 days of storage with 40±1.24% losses, caused by 10 adults per 100g seeds. The total quantitative losses observed for 6 months storage period was 64±1.16% in the samples with 10 adults per 100g of roasted makhana seeds. The initial losses were very high and became slow after 20 days. Presently available method of fumigation by aluminium phosphide was practised and found feasible for the insect control.

Internal makhana feeding

Lesser grain borer (adult and grub)

Source: ICAR Annual Report 2022-23