Acta fytotechn zootechn, 25, 2022(2): 165–173
http://www.acta.fapz.uniag.sk

Original Paper

Nutritional prospective of *Sesbania* species: an underutilized wild legume from Northern Western Ghats, Maharashtra, India

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Article Details: Received: 2022-04-25 | Accepted: 2022-06-18 | Available online: 2022-06-30

https://doi.org/10.15414/afz.2022.25.02.165-173

Nutrition plays very important role in longevity of life. Processed food has created various health disorders at a very alarming rate. Wild genotypes have potent nutritive characteristics which are relatively less explored. In the current investigation, two species of *Sesbania* were evaluated for their potent nutritional value. *Sesbania grandiflora* and *Sesbania bispinosa* seeds contained 37.78% and 30.2% crude proteins, 4.24% and 2.06% albumins, 7.20% and 5.16% globulins, 1.75% and 8.34% free amino acids, 34.91% and 59.08% total carbohydrates and 5.2% and 5.02% total lipids respectively. DPPH Radical scavenging assay revealed notable antioxidant potential with 62.27% and 65.45% inhibition respectively. Antinutrient analysis indicated the presence of total free phenols (0.29% and 0.63%), tannins (0.28% and 0.34%), phytic acid (1.70% and 1.59%) and trypsin inhibitor activity (41.8 and 54.4 TIU mg^{-1}) respectively. Both *Sesbania* species had excellent nutritional potential as well as antioxidant activity and a low antinutrient profile. Based on the findings, *Sesbania* species may be investigated further as a potential alternative food source in the near future, particularly in economically disadvantaged areas, and may pave the way toward the wider aim of food security.

Keywords: *Sesbania grandiflora*, *Sesbania bispinosa*, nutritional and antinutrional analysis

1 Introduction

Nutrition is very significant aspect of life considering the current scenario of global health. Nutrient rich food is the need of the hour for the world. Changing lifestyle has created the health-related issues leading to various diseases and disorders. Proteins are one of the prime nutrients and required on daily basis to carry out many important activities. Protein malnutrition is a serious hindrance in the direction of a healthy life especially in developing and underdeveloped nations (Falade et al., 2005). Animal sources of protein are highly priced and may not be eaten due to ethical issues. Legumes are recognized as the second most significant source of proteins for human and animal nutrition (Vietmeyer, 1986). Grain legumes are considered as momentous source of proteins along with other nutrients and are inexpensive compared to animal sources. In addition to be protein rich, leguminous crops also have bone-building fibers, complex carbohydrates, minerals, and vitamins essential for good health (Polak et al., 2015) (Maria et al., 2021). The taxonomic diversity of legumes is huge. It provides significant advantage to humans which consist of food, medicines, and environmental services. India's dietary habits are still predominantly vegetarian, and it relies mainly on vegetal sources to achieve its daily protein requirements. Since India is the world's largest importer of pulses, production of pulse/legume crops has remained stable over time. As a result, the gap between demand and supply is increasing. As the world's population continues to grow, so will the demand for protein in the coming decades, making it critical to find new sources of protein for human consumption. Legumes belong to the family Leguminosae which is the third prevalent family in angiosperms containing around 650 genera and 20,000 species (Doyle, 1994). Wild relatives of plants are the genetically diverse and do possess unique characters.

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Wild and underexploited legumes may have potential to be used as human nutriment for overcoming the malnutrition related problems (Bhat and Karim, 2009). Some Indian tribal pulses have been explored to trace biochemical composition and evaluation of their nutritional potential (Rajaram and Janardhanan, 1991). *Sesbania* species are not frequently used by people all over the world; just the few communities from tropical and subtropical regions, mainly in Southeast Asia, employ them. In the current investigation, two species of *Sesbania*, were evaluated for their nutritive potential.

2 Material and methods

Both the species, *Sesbania grandiflora* (L.) Poir and *Sesbania bispinosa* (Jacq.) steud. Ex Wight were collected from Northern Western Ghats of Nashik district, Maharashtra and identified with the help of flora of Nashik District (Lakshminarsimhan and Sharma, 1991) and flora of Maharashtra (Almeida, 1996). Pods were sun dried and matured, healthy seeds were powdered and used for further analysis. Seeds of both the species were analysed for diffrent nutritional, anti-nutritional and antioxidant activity by following standard protocols.

2.1 Crude proteins

Crude proteins were estimated by Micro-Kjeldahl method (Sahrawat et al., 2002). 500 mg of seed powder was put in the digestion flask. 2 gm of potassium sulphate, 90 mg of mercuric oxide and 2 ml conc. H_2SO_4 was added along with boiling chips and allowed to digest till the solution became colourless. It was kept for cooling and diluted with a small quantity of ammonia free water. It was then transferred to the distillation apparatus. Into the 100 ml conical flask, 5 ml boric acid solution along with few drops of the mixed indicator was added and placed below the tip of the condenser. 10 ml of sodium hydroxide-sodium thiosulphate solution was also added. Ammonia was distilled and collected on Boric acid. The Solution was titrated against the standard acid until the violet colour appeared and the reading was noted. Nitrogen content of the sample was calculated from which crude proteins were analyzed using the conversion factor i. e. N X 6.25.

2.2 Proteins fractions (albumins and globulins)

Albumins and globulins were extracted by the method as given by Murray (1979) with slight modifications. 500 mg of seed powder was mixed with 150 mg of polyvinylpolypyrrolidone (PVPP), 8 ml of 5% potassium sulphate and 2 ml of 0.1 M sodium phosphate buffer (pH 7) and kept on a magnetic stirrer for 24 hours and then centrifuged at 20,000 rpm for 20 minutes at 4 °C. Supernatant was kept aside after centrifugation and pellets were preserved. Pellet was again mixed with 150 mg of polyvinylpolypyrrolidone (PVPP) and 10 ml of 0.1 M sodium phosphate buffer and stirred for 30 minutes and then centrifuged at 20,000 for 20 minutes at 4 °C. Now, the supernatant was preserved and was pooled and dialyzed against distilled water for 18 hours. Dialysates were again centrifuged at 20,000 rpm for 20 minutes at 4 °C. Supernatant recovered was designated as albumins and pellet obtained was redissolved in the extraction buffer and designated as globulins. Albumins and globulin extracted were estimated by the method as given by Lowry et al. (1951).

2.3 Total and individual amino acid profiling

Total free amino acids were analyzed by the method as described by Moore and Stein (1948). 100 mg of seed powder was mixed with 5 ml of 80% ethanol, centrifuged and the supernatant was saved. Extraction was repeated twice and entire supernatant collected was used as a sample for analysis. Series of standards was prepared by taking 0.1–1 ml of working standard in separate test tubes. 0.1 ml of sample extract was taken for analysis. 1 ml of Ninhydrin solution was added in all test tubes including a sample test tube and the final volume was made to 2 ml with distilled water. All the test tubes were heated in a boiling water bath for approximately 20 minutes and then 5 ml of the diluent was added. The content was mixed thoroughly and kept for 15 min. The purple colour was developed, the intensity of which was measured by UV visible spectrophotometer (Shimadzu UV-2600) at 570 nm. The amount of total free amino acids was estimated using a standard graph and expressed in %. Individual amino acid profiling was done using RP HPLC method (Bhat et al., 2019).

2.4 Total carbohydrates

Total carbohydrates were measured by the anthrone reagent method (Hedge and Hofreiter, 1962). One hundred mg of the seed powder was taken and was put into a boiling tube. It was hydrolysed by putting it in a water bath for three hours with 5 ml of 2.5 N HCL and then allowed to cool at room temperature. It was neutralized by solid sodium carbonate until the effervescence was stopped. The final volume was made to 100 ml and kept for centrifugation. The supernatant obtained was collected and 0.1 ml of aliquots (sample extract) was taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution. The final volume was made to 1 ml in all test tubes including a sample tube by adding the distilled water. In all test tubes, 4 ml of Anthrone reagent was added. Test tubes were heated for approximately 8 minutes in a boiling water bath. It was allowed to be

cooled and green colour developed was measured by UV visible spectrophotometer (Shimadzu UV-2600) at 630 nm. The standard graph was plotted using the concentration of standard on X-axis versus absorbance on the Y-axis. Readings were taken in triplicate, averaged and amount of carbohydrates were estimated and expressed in %.

2.5 Total lipids

Total lipids were estimated by reported method (Folch et al., 1957). Five hundred mg of the seed powder was mixed in mortar and pestle with anhydrous sodium sulphate in the ratio of 1 : 5 (w/w) till the formation of a homogeneous mixture. The mixture of chloroform: methanol (2 : 1 v/v) was added to this homogeneous mixture in the ratio 1 : 20 (w/v). It was intermittently shaken for around 12 hours in an airtight flask. The extract was filtered through a funnel and residue was washed with chloroform: methanol (2 : 1) v/v for complete extraction of the lipids. The combined mixture was poured in a separating funnel and 0.9% of sodium chloride was added (5 : 1 v/v). The separating funnel was kept overnight for complete separation of the chloroform layer. The lower fraction containing lipids in the chloroform-methanol mixture was collected in a round bottom flask. The chloroform-methanol mixture was allowed to evaporate at 78 °C using a rotary evaporator. The difference in the weight of the round bottom flask before and after evaporation was calculated which eventually gave the lipid content which was expressed in %.

2.6 DPPH antioxidant assay

It was analyzed by the method as given by Yamaguchi et al. (1998). One hundred mg of seed powder was mixed with the 10 ml volume of 1% HCl-methanol and centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated with 1% HCl-methanol. The supernatant was collected and evaporated to dryness. The residue was then dissolved in a 5 ml of 1% HCl-methanol and designated as a plant extract. Free radical scavenging activity was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical with slight modification. 0.1 ml of the sample was taken from the extract in the test tube and in that 2.8 ml of methanol was added. The reaction was started by addition of 0.1ml of 1mM DPPH solution. Test tubes were kept to stand for approximately 30 minutes and absorbance was read at 517 nm. inhibition was determined and expressed in %.

2.7 Total free phenols

Among the antinutrients, phenols were measured by the reported method (Malick and Singh, 1980). Five hundred mg of seed powder was mixed with 5 ml of 80% ethanol and centrifuged at 10,000 rpm for 20 min. The supernatant was saved and the residue was re-extracted with 80% ethanol to pool all the extract in the supernatant. Supernatant evaporated so as to make it completely dry. The residue left after evaporation was dissolved in 5 ml of distilled water and used as a sample for analysis. Series of working standards was prepared using a catechin equivalent and 0.2 ml sample was taken for analysis. The final volume was made to 3 ml with distilled water in all test tubes. 0.5 ml of Folin Ciocalteau reagent was added and kept for 3 min. 2 ml of 20% Sodium carbonate solution was added to every test tube and it was mixed rigorously. All test tubes were placed in boiling water for exactly one minute and then cooled. Absorbance was measured using the UV visible spectrophotometer (Shimadzu UV-2600) at 650 nm. Amount of phenol was calculated using a standard graph and expressed in %.

2.8 Tannins

Tannins were estimated by Folin Denis method (Schanderl, 1970). One hundred mg of seed powder was mixed with 7.5 ml of water and heated gently and then boiled for 30 min followed by centrifugation at 2000 rpm for 20 min. The supernatant was collected and taken as a sample. Series of the working standard was prepared using tannic acid of known concentration and poured in a test tube. 0.1 ml of the sample was taken for analysis. 5 ml of Folin Denis reagent and 10 ml of Sodium carbonate were added in all test tubes and diluted to 100 ml with the help of distilled water. All the test tubes were shaken thoroughly and absorbance was recorded at 700 nm in UV visible spectrophotometer (Shimadzu UV-2600) after 30 min. Amount of tannin was calculated using a standard graph and expressed in %.

2.9 Phytic acid

Phytic acid was estimated by reported method (Wheeler et al., 1971). One hundred mg of seed powder was extracted in 50 ml of 3% TCA for 30 minutes and centrifuged for 15 minutes at 2000 rpm. 10 ml aliquot of the supernatant was taken and 4 ml of $FeCl_3$ was added to it. The content was heated in a boiling water bath for 45 minutes and centrifuged for 15 minutes at 2,000 rpm and the supernatant was carefully decanted. The precipitate was washed twice by dispersing it in 25 ml 3% TCA and again heated in boiling water for 10 minutes and centrifuged for 15 minutes at 2000 rpm. The precipitate was dispersed in 3 ml of distilled water and 3 ml of 1.5 N sodium hydroxide was added and thoroughly mixed. Volumes in all test tubes were made to 30 ml with distilled water and heated in boiling water for 30 minutes. It was filtered and precipitate was then washed with 70 ml hot water and filtrate were discarded.

The precipitate was dissolved with 40 ml 3.2 N Nitric acid. Flask was cooled and diluted with 10 ml of water. 5 ml of this aliquot was diluted to 70 ml with the help of distilled water. 20 ml of 1.5M KSCN was added into all test tubes, and colour developed was measured at 480 nm with UV visible spectrophotometer (Shimadzu UV-2600). Iron in gm was measured using a standard curve and phytate was calculated and expressed in %.

2.10 Trypsin inhibitor activity

Trypsin inhibitor activity was analyzed by the method as given by Kakade et al. (1974). Five hundred mg of seed powder was extracted with 25 ml of water in pre chilled mortar and pestle and centrifuged at 12,000 rpm for 20 minutes at 4 °C. 1 ml of supernatant was taken and diluted to 10 ml with distilled water and used as a trypsin inhibitor (TI) source. 0.2, 0.4, 0.6, 0.8 and 1 ml of TI source was taken and final volume was made to 1 ml with distilled water.1 ml of trypsin solution (containing 20 µg trypsin) was added in each test tube. All test tubes were incubated in a water bath at 37 °C for 30 minutes. 2.5 ml of substrate (Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride i.e. BAPNA) was added to each tube all the test tubes were kept for 60 minutes to allow the reaction to proceed. Reaction was stopped by adding 0.5 ml of 30% glacial acetic acid. Absorbance was measured at 410 nm in UV visible spectrophotometer (Shimadzu UV 2600). Protein content in the extract was determined by the method of Lowry et al. (1951) and expressed the trypsin inhibitor activity in trypsin inhibitor units or TIU mg^{-1} protein. One trypsin unit was expressed as an increase of 0.01 absorbance unit per 10 ml of reaction mixture at 410 nm.

2.11 Statistical analysis

Triplicate set of experiments (otherwise mentioned) were set up and data collected was subjected to statistical analysis using GraphPad Prism 8 Version 8.4.1(676) software and values were expressed as mean ± standard deviation (n = 3). Data analysis was done using one way ANOVA and Tukey's Multiple Comparison test to compare the means and to trace any significant difference between them at significance level, 0.05.

3 Results and discussion

Obtained results show significant content of nutritional component and antioxidant activity. Crude protein content in both the evaluated species *S. grandiflora* (37.78 ±0.28%) and *S. bispinosa* (30.2 ±0.72%) are higher than the *Phaseolus angularis* (25.2%), *Phaseolus calcaraus* (26.5%), *Dolichos lablab* (24.9%) (Chau et al., 1998), *Paracalyx scariosus* (Roxb.) (20.56%) (Murthy and Rao, 2009), *Cajanus albicans* (wight & arn) van der maesan (21.35%) (Murthy, 2011).

In case of protein fractions i.e., albumins and globulins, *Sesbania grandiflora* contain 4.24 ±0.18% albumin and 7.20 ±0.08% globulin which is higher than the as *Sesbania bispinosa* (2.06 ±0.10% and 5.16 ±0.05% respectively). In comparison with wild legumes *Neonotonia wightii* (Wight & Arn.) Lackey (5.1% and 7.8%) (Viswanathan et al., 2001), *Rhynchosia hirta* (Andr.) Meikle and Verdc. (5.12% and 6.75%) (Murthy et al., 2007), obtained values are more. While in comparison with cultivated legumes *Vigna mungo* L. Hepper (Vamban-1) (5.20% and 11.36%) (Tresina et al., 2010) and *Vigna radiata* L. Wilczek (Tresina et al., 2014), it was found to be in less amount.

Total free amino acids were found to be significant in *S. bispinosa* (8.34 ±1.04%) than the *S. grandiflora* (1.75 ±0.28%). Reported content of *S. bispinosa* are higher than commonly consumed pulses *Phaseolus vulgaris* L. (4.8%), *Lens culinaris* L. (0.52%) and *Pisum sativum* L. (1.1%) (Kuo et al., 2004).

Amino acid profie obtained by RP-HPLC revealed the presence of essential amino acids in considerable amount. *S. grandiflora* exhibit methionine (1.37 µg mg^{-1}), valine (5.41 µg mg^{-1}), and threonine (3.46 µg mg^{-1}),

Table 1 Nutritional Assay

Sr. no.	Nutritional analysis	*Sesbania grandiflora* (L.) Poir (g 100 g^{-1})	*Sesbania bispinosa* (Jacq.) steud. ex Wight (g 100 g^{-1})
1	crude proteins*	37.78 ±0.28^a	30.2 ±0.72^b
2	albumins*	4.24 ±0.18^a	2.06 ±0.10^b
3	globulins*	7.20 ±0.08^a	5.16 ±0.05^b
4	total free amino acids*	1.75 ±0.28^a	8.34 ±1.04^b
5	total carbohydrates*	34.91 ±0.76^a	59.08 ±0.76^b
6	total lipids*	5.2 ±0.30^a	5.02 ±0.45^a

* values are expressed as means of triplicate determination ± standard deviation; * values are expressed in % or g 100 g^{-1}; * values in the column with different alphabet superscript are significantly different from each other (P <0.05); according to Tukey's multiple comparison test

Table 2 Antioxidant assay

Sr. no.	Antioxidant activity	*Sesbania grandiflora* (L.) Poir	*Sesbania bispinosa* (Jacq.) steud. ex Wight
1	DPPH assay**	62.27	65.45

** values are expressed as % inhibition @10 mg ml^{-1}; (single determination)

Table 3 Antinnutritional assay

Sr. no	Antinutritional analysis	*Sesbania grandiflora* (L.) Poir* (g 100 g^{-1})	*Sesbania bispinosa* (Jacq.) steud. ex Wight *(g 100 g^{-1})
1	total free phenols*	0.29 ±0.02[a]	0.63 ±0.02[a]
2	tannins*	0.28 ±0.08[a]	0.34 ± 0.05[a]
3	phytic acid*	1.70 ±0.03[a]	1.59 ±0.05[a]
4	trypsin inhibitor activity**	41.8[a]	54.4[a]

* values are expressed as means of triplicate determination ±standard deviation; * values are expressed in % or g 100 g^{-1}; * values in the column with same alphabet superscript are not significantly different from each other (P >0.05) according to Tukeys multiple comparison test; ** values are expressed TIU mg^{-1} protein (single determination)

While *S. bispinosa* contain isoleucine (5.91 µg mg^{-1}), phenylalanine (5.39 µg mg^{-1}) in notable extent. Ample quantity of non-essential amino acids was found in both the species. Among the non essential amino acids, Aspartic acid (5.87 and 7.83 µg mg^{-1}), glutamic acid (3.78 and 17.02 µg mg^{-1}), serine (1.44 and 2.25 µg mg^{-1}), arginine (7.92 and 8.15 µg mg^{-1}), alanine (2.22 and 3.25 µg mg^{-1}), tyrosine (76.12 and 9.08 µg mg^{-1}) were present in considerable amount respectively in *S. grandiflora* and *S. bispinosa*. Glycine (0.16 µg mg^{-1}) was exclusively detected in *S. grandiflora*. Cosidering the results it can be said that both the species were found to have good amino acid profile which could be one of the benchmark in assigning it as the alternative source of food.

In *Sesbania bispinosa* 59.08% carbohydrate was noted, While in *S. grandiflora* is was 34.91%. Reported content found to be in consonance with earlier reports on other wild legumes *Canavalia ensiformis* (46.1%), *Canavalia gladiata* (45.1%), and *Canavalia maritima* (44.9%) (Bressani et al., 1987) and *Alysicarpus rugosus* (51.1%) (Siddhuraju et al., 1992) and *Entada scandens* (45.99%) (Vijayakumari et al., 1993). Lipid content was found to less in both the species i.e. *S. grandiflora* (5.2%) and *S. bispinosa* (5.02%).

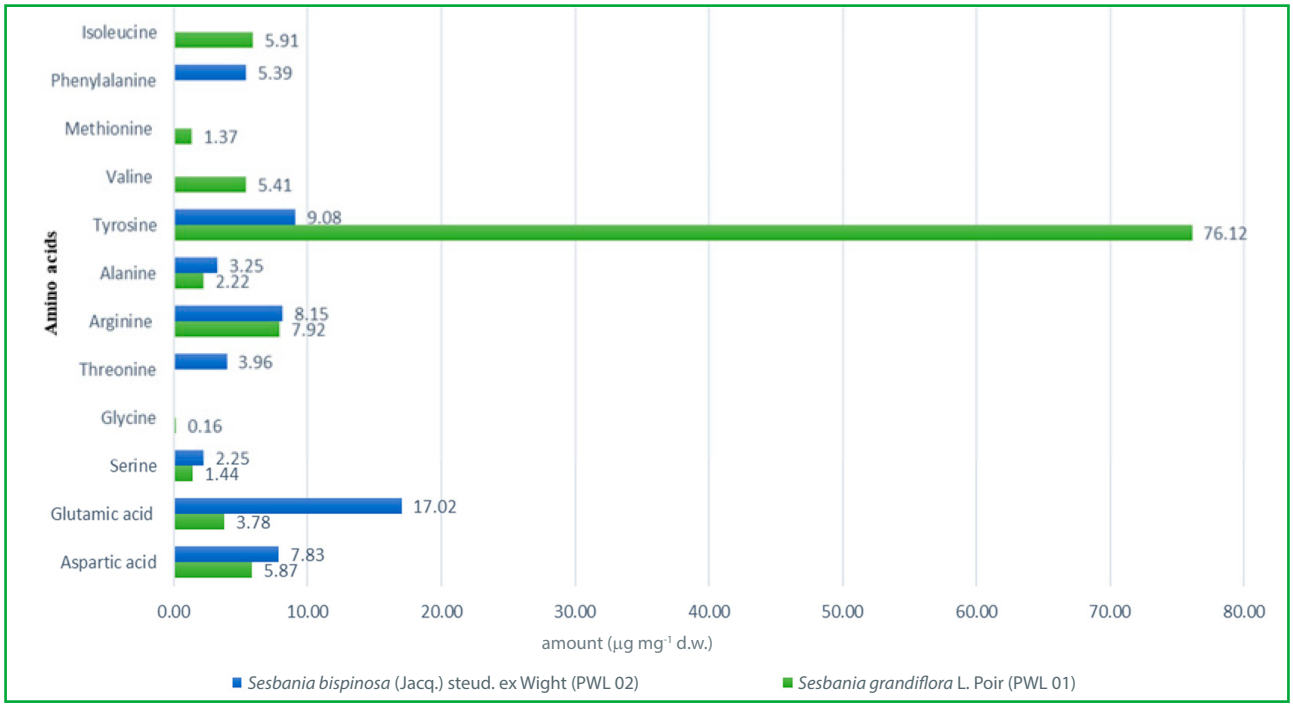

Figure 1 Individual amino acid profile

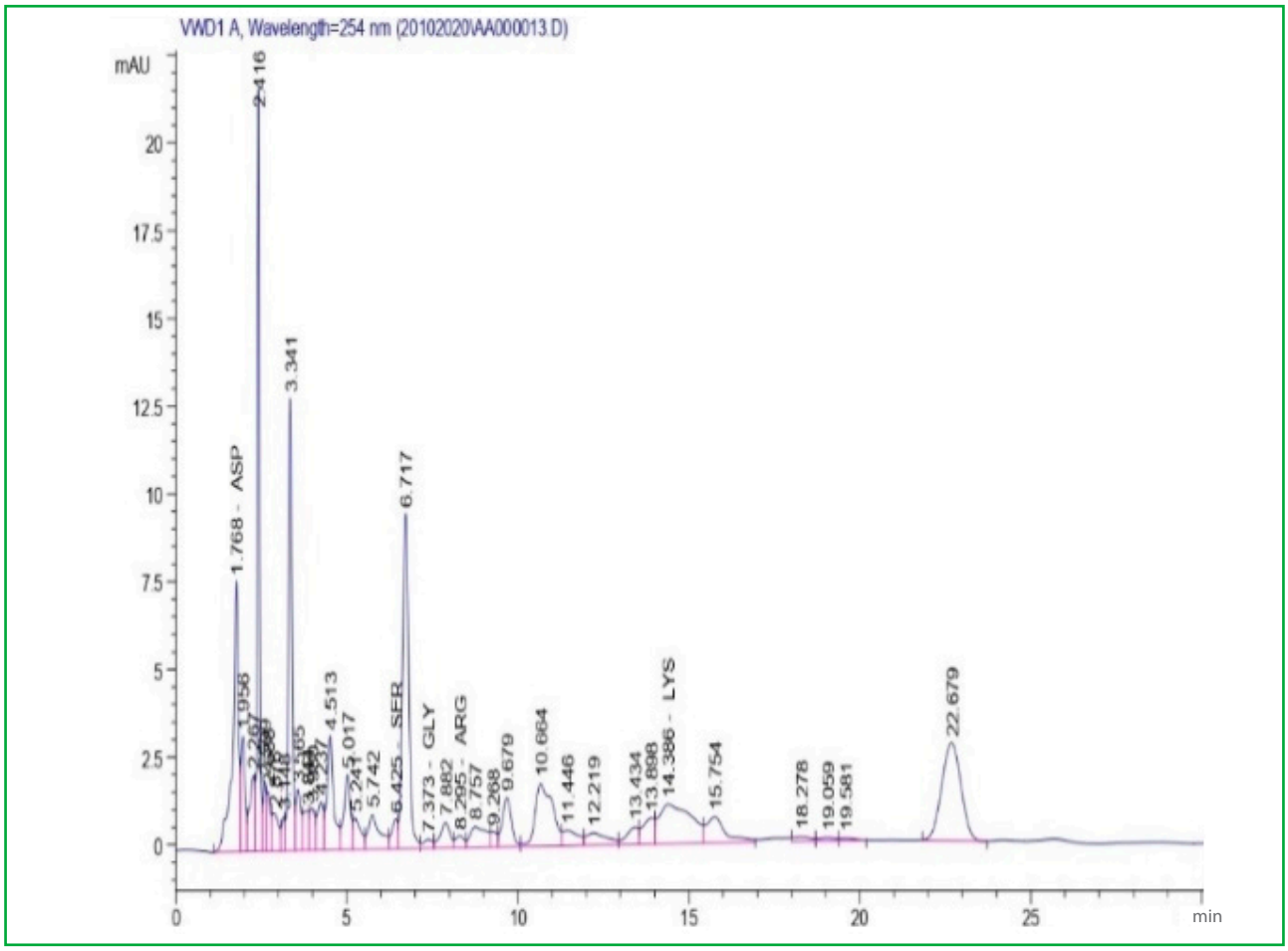

Figure 2 Chromatogram: individual amino acid profile of *Sesbania grandiflora*

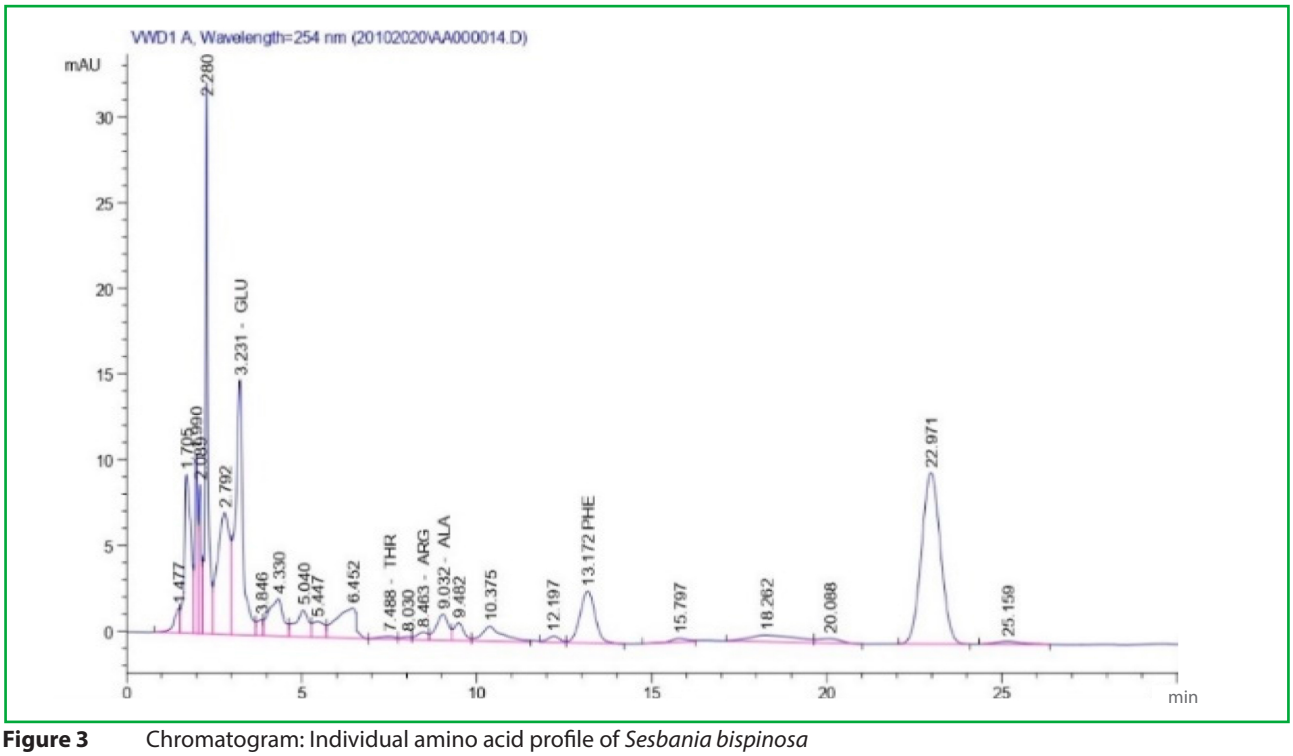

Figure 3 Chromatogram: Individual amino acid profile of *Sesbania bispinosa*

Obtained results of analysis of antinutritional compounds such as tannins and total phenols were reported to be significantly low (Table 3). Total free phenols investigated in both species, *S. grandiflora* (0.29%) and *S. bispinosa* (0.63%) were found to be in consonance with the previously reported value on cultivated legumes *Cajanus cajan* (0.33%) (Kaushal et al., 2012), *Vigna mungo* L. (0.41%) (Tresina et al., 2010) *Vigna radiata* L. (CO4) (0.27%) (Tresina et al., 2014). It was found to be lower than other wild legume *Neonotonia wightii* (Wight & Arn.) Lackey (2.6%) (Viswanathan, 2001), *Canavalia cathartica* (1.5%), *Canavalia maritima* (1.4%) (Arun et al., 2003), *Rhynchosia hirta* (Andr.) Meikle and Verdc (2.25%) (Murthy et al., 2007).

Amount of tannin in both the species was found to be lower than commonly consumed pulses *Cajanus cajan* (1.60%) and *Vigna unguiculata* L. (3.42%) (Onwuka, 2006). Obtained results are in agreement with previous reports on *Dolichos trilobus* (0.58%), *Entada rheedii* (0.63%), *Rhynchosia cana* (0.44%), *Rhynchosia suaveolens* (0.58%), *Teraminus labialis* (0.21%) (Arinathan et al., 2009).

Phytic acid content was observed to be on higher in both the species with 1.70 ±0.03% and 1.59 ±0.05% respectively. It is higher than earlier reports on *Cassia laevigata* Willd (0.78%), *Tamarindus indica* L. (0.27%) (Siddhuraju et al., 1995b), and commonly used pulses *Vigna radiata* L. (0.67%) (Grewel and Jood, 2006), *Vigna mungo* L. (CO5) (0.33%) (Tresina et al., 2010), *Cajanus cajan* (0.10%) (Kaushal et al., 2012). Though phytic acid content is higher, it can be correlated with significant antioxidant activity. All of the antioxidant properties of phytic acid are likely to be derived from its relatively high binding affinity for iron (Graf and Eaton, 1990).

S. grandiflora (41.8 TIU mg^{-1}) and *S. bispinosa* (54.4 TIU mg^{-1}) were found to have notable trypsin inhibitor activity. It was found to be in accordance with the previous reports on other wild species *Cassia laevigata* Willd (35 TIU mg^{-1}), *Tamarindus indica* L. (26 TIU mg^{-1}) (Siddhuraju et al., 1995b), *Sesbania bispinosa* (47.18 TIU mg^{-1}) (Siddhuraju et al., 1995a).

Antioxidants are the compounds which prevent the oxidation reaction and are of paramount importance that have the ability to safeguard the body from damage due to oxidative stress induced by free radicals (Ozsoy et al., 2008). There is a rising interest in herbal antioxidants found in medicinal and nutritional plants, which may assist the body to fight against oxidative damage (Silva et al., 2005). In this regard, both the species analyzed exhibited significant antioxidant activity with 62.27% and 65.45% in *S. grandiflora* and *S. bispinosa* respectively. It is higher than the % Inhibition reported in *Vigna radiata* (25%) (Randhir and Shetty, 2004), soybean (44%), kidney bean (62%) (Boateng et al., 2008), *Mucuna pruriens* (50%) (Randhir et al., 2009). (*Vigna subterranea* L. Verdc) (53.7%) (Nyau et al., 2015). The significant inhibition % is found among both the species. This significant finding proves their antioxidant potential. Overall, both the species were found to have remarkable nutritional value along with significant antioxidant activity. Antinutrients analysed were also found to be in tolerable limit.

4 Conclusions

Due to presence of high protein content, the presence of albumin and globulin fractions, and a diverse profile of essential amino acids, both *S. grandiflora* and *S. bispinosa* can be used as a potential protein source. Significant thing is both species have low levels of antinutritional compounds such tannin, total phenols, and trypsin inhibitors, which could help to improve protein digestion and availability. Because of these, it can be utilised as a protein supplement for persons of all socioeconomic levels.

Acknowledgments

Authors are thankful to Hon. Principal, KKHA Arts, SMGL Commerce and SPHJ Science College Chandwad and Hon. Principal, HPT Arts and RYK Science College, Nashik for support and neccessary laboratory facilities.

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