The Pharma Innovation Journal 2023; 12(12): 1196-1201

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 1196-1201

www.thepharmajournal.com
Received: 19-09-2023
Accepted: 26-10-202

Pratiksha Chandrakant Kshirsagar
Research Scholar, Department of Food Engineering, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Patil BM
Assistant Professor, Department of Food Engineering, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Borale SV
Research Scholar, Department of Food Engineering, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Kshirsagar RB
Associate Professor, Department of Food Engineering, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Agarkar BS
Associate Professor, Department of Food Engineering, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Corresponding Author:
Pratiksha Chandrakant Kshirsagar
Research Scholar, Department of Food Engineering, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Comprehensive phytochemical analysis and comparative quantification of bioactive compounds in ashwagandha (*Withania somnifera*) and shatavari (*Asparagus racemosus*) root extracts: A study on health-promoting secondary metabolites and extraction efficiency

Pratiksha Chandrakant Kshirsagar, Patil BM, Borale SV, Kshirsagar RB and Agarkar BS

Abstract
The roots of ashwagandha (*Withania somnifera*) and shatavari (*Asparagus racemosus*) hold significant importance in Ayurveda and traditional medicine due to their health-promoting properties. The roots are the main portion of these plants used in herbal medicine. These remarkable roots are celebrated for their profound therapeutic value and have been cherished for generations in the pursuit of holistic well-being and health. The aim of the study was to investigate the secondary metabolites of various extracts of root of *W. somnifera* and A. racemosus. Phytochemical screenings were performed on extracts from ashwagandha and shatavari roots, uncovering the existence of beneficial compounds like alkaloids, flavonoids, tannins, saponins, carbohydrates, and total phenol. Furthermore, quantitative assessments were performed to measure these active constituents in both ashwagandha and shatavari root extract according to standard procedures. Furthermore, the study examined the yield of ashwagandha and shatavari extract resulted 9±0.90 percent and 11±0.57 percent respectively. Colour attributes of ashwagandha and shatavari extracts were also evaluated.

Keywords: *Withania somnifera*, *Asparagus racemosus*, alkaloids, withanolides, steroidal saponins, soxhlet apparatus

1. Introduction
According to the World Health Organization, traditional medicines are extensively utilized in India, with around 80% of the population in developing countries depending on them as their primary healthcare solution (Allison, 1966; Bhattacharjee, 1998) [2, 6]. Medicinal plants continue to hold a crucial role in the healthcare systems of a significant portion of the global population. These medicinal plants contain various phytochemicals, including vitamins (A, C, E, and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, and minerals, among others. These phytochemicals exhibit antioxidant properties that can be harnessed for treating a range of health conditions.

Withania somnifera, a member of the Solanaceae family, is a well-known medicinal plant in India and goes by the names ashwagandha, ginseng, and winter cherry. The species name, somnifera, is derived from its traditional use as a sedative. Its common name, ashwagandha, originates from the Sanskrit terms “Ashwa” for horse and “Gandha” for smell, giving rise to the common belief that it means “smells like a horse.” For over 3,000 years, it has been a significant herb in both Ayurvedic and indigenous medical systems. This plant typically reaches a height of three to five feet and is commonly found in waste lands, although it is widely cultivated for its various parts, with the root and leaves being the most commonly used for medicinal purposes (Williamson and Hooper, 2002; Kapoor, 1990) [37, 14\]. The plant's roots fall under the category of rasayanas, known for their reputation in enhancing health and longevity by boosting the body's defense against diseases, slowing down the aging process, rejuvenating the body in weakened states, enhancing resilience to adverse environmental factors, and promoting mental well-being (Weiner and Weiner, 1994) [36]. Numerous studies have shown that ashwagandha possesses a range of beneficial properties, including

antioxidant, anti-tumor, anti-stress, anti-inflammatory, immunomodulatory, hematopoietic, anti-aging, anxiolytic, antidepressant, and rejuvenating effects. It also influences various neurotransmitter receptors in the central nervous system (Pattipati *et al*., 2003) [26]. The use of Ashwagandha (WS) is on the rise due to the presence of various chemical constituents that contribute to its health-promoting qualities. Key bioactive components in Ashwagandha root powder include alkaloids, tannins, flavonoids, and phenolic compounds, which play a role in promoting these health benefits (Shah and Seth, 2010; Aiyelaagbe and Paul, 2009).

Asparagus racemosus (AR), a member of the Asparagaceae family, is a plant commonly referred to as shatavari, satawar, or satmuli, and it can be found throughout India. The name "Shatavari" translates to "one who possesses a hundred husbands or is acceptable to many," suggesting its reputation for enhancing fertility and vitality (Bopana and Saxena, 2007) [8]. The roots of this plant are elongated and tuberous, with a brownish color and tapered tips on both ends. It typically reaches a height of 25-90 cm and has a thickness of 1-2 cm, appearing silver-white either internally or externally. In Ayurveda, the dried root of *Asparagus racemosus* (AR) is employed as a tonic, galactogogue, aphrodisiac, rejuvenator, antispasmodic, antiulcer, and anti-inflammatory agent. Modern research has revealed that it exhibits estrogenic and galactagogic effects in animals, along with anti-fungal, anti-diarrheal, antioxidant, and anti-cancer properties *in vitro* (Sharma *et al*., 1996; Pandey *et al*., 2005; Onlom *et al*., 2014; Venkatesan *et al*., 2005; Kongkaneramit *et al*., 2011; Bhutani *et al*., 2010) [31, 24, 23, 33, 18, 7]. The therapeutic significance of the *Asparagus* genus is attributed to the existence of a range of phytochemicals, including steroidal saponins and sapogenins (Gurudeva, 2001) [11]. Various parts of the plant contain tannins, alkaloids, flavonoids, terpenoids, phenols, and steroid glycosides.

The medicinal benefits of these plants are attributed to their phytochemical constituents, which exert specific physiological effects on the human body. Plants can produce diverse secondary metabolites that have effects on other organisms (Zwenger and Basu, 2008) [38]. The current study aims to evaluate the phytochemical properties of the roots of *Withania somnifera* as well as *Asparagus racemosus* which may contribute to its diverse medicinal properties.

2. Materials and Methods

2.1 Selection and Collection of Plant Materials

The required materials i.e roots of *W. somnifera* and *A. racemosus* for the present investigation were collected from the local markets of the Parbhani.

2.2 Preparation of *Withania somnifera* root extract

The obtained dried roots of *Withania somnifera* were first meticulously cleaned to eliminate any impurities like dirt, dust, chaff, and other debris using a clean cloth. After this cleaning process, the roots were cut into small pieces and further ground into a powder using a mixer grinder. This powdered material was subjected to extraction using a Soxhlet apparatus, employing ethanol as the solvent, at a temperature range of 70-80 °C to yield the extract. To concentrate the extracts, a rotary evaporator was employed under reduced pressure, maintaining the temperature below 40 °C. Once the extraction and concentration were completed, the resulting extract was weighed and transferred into clean and sterilized bottles, subsequently stored at room temperature for both analysis and the production of herbal syrup based on jaggery.

Withania somnifera roots (100 g)
↓
Cleaning
↓
Cutting
↓
Grinding
↓
Powdered root
↓
Solvent extraction with ethanol 95% (Ashwagandha powder and solvent =1:5)
↓
Removal of solvent
(Using rotary vacuum evaporator)

Flow sheet 1: Preparation of *Withania somnifera* extract

2.3 Preparation of *Asparagus racemosus* root extract

The acquired dried roots of *Asparagus racemosus* underwent a thorough cleaning process to eliminate any contaminants like dirt, dust, chaff, and other debris, utilizing a clean cloth. Following this cleaning procedure, the roots were then cut into small pieces and further pulverized using a mixer grinder to obtain a powdered form. Subsequently, extraction was carried out with the assistance of a Soxhlet apparatus, employing ethanol as the solvent, at a temperature range of 70-80 °C in order to obtain the extract. To concentrate the extracts, a rotary evaporator was utilized under reduced pressure, ensuring that the temperature remained below 40°C. After completion of the extraction and concentration, the resulting extract was weighed and transferred into sanitized bottles, which were stored at room temperature for both analysis and the production of herbal syrup based on jaggery.

Asparagus racemosus roots (100 g)
↓
Cleaning
↓
Cutting
↓
Grinding
↓
Powdered root
↓
Solvent extraction with ethanol 95% (Shatavari powder and solvent =1:5)
↓
Removal of solvent
(Using rotary vacuum evaporator)

Flow sheet 2: Preparation of *Asparagus racemosus* extract

2.4 Percent yield of extracts

Percent yield of extracts from root powders was calculated by formula,

$$\% \text{ yield} = \frac{\text{obtained weight of extract}}{\text{Weight of powder}} \times 100$$

2.5 Color analysis

Color is a visual attribute, and the assessment of color in the samples followed the procedure established by Rajiv *et al*.,

2015 [39]. This evaluation employed a Hunter Lab Colorimeter, specifically the Colour Flex EZ model, which was located in the Department of Horticulture at the College of Horticulture, VNMKV, Parbhani. To ensure accuracy, the instrument was calibrated using a standard reference tile with a light-yellow color, characterized by L* = 77.14, a* = 1.52, and b* = 21.88. The colorimeter was configured with 10° observers and a $45^0/0°$ geometry. Measurements of L* (Where 0 indicates black and 100 signifies white), a* (Positive values denoting red and negative values indicating green), and b* (Positive values representing yellow and negative values suggesting blue) were taken using a glass cell containing the sample. This cell was placed above the light source and covered with a white plate. The color index provided information about the sample's lightness, redness, and yellowness. The Hunter Lab colorimeter quantified the color through values for L*, a*, and b*, offering insights into chroma (C) and hue (h) by referencing a standard white tile or board during the instrument's setup with the illuminant.

2.6 Phytochemical Analysis

2.6.1 Preparation of extract

The powdered ashwagandha and shatavari root samples (50 g/250 mL) were extracted successively with ethanol and water using soxhlet apparatus at 55-85 ^{0}C for 8- 10 h in order to extract the polar and non-polar compounds (Elgorashi and Staden, 2004) [10]. The solvents of the respective extracts were reduced under room temperature and stored at 4^0C for further use.

The freshly prepared ethanolic and aqueous extracts of *Withania somnifera* and *Asparagus racemosus* were qualitatively analysed for the presence of phytochemical constituents using the following standard protocol.

2.6.2 Test for Phenols

2.6.2.1 Ferric Chloride Test

Take 2 ml of plant extract was taken in a test tube and then add 2 ml of ferric chloride (1%). The appearance of dark green or bluish green color indicated the presence of phenol (Kar, 2004) [15].

2.6.3 Test for Tannins

2.6.3.1 Lead Acetate Test

Add few drops of lead acetate solution in a test tube with 2 ml of filtrate. Yellowish coloration was indication of positive result.

2.6.4 Test for Saponin

2.6.4.1 Froth Formation Test

1 ml of extract was taken in test tube add 20 ml of distilled water. Take 10 ml of filtrate was taken in a graduated cylinder. Add 5 ml of distilled water and shake vigorously. Formation of persistent froth indicates the presence of saponins (Kokate *et al*., 1994) [17].

2.6.5 Test for Flavonoids

2.6.5.1 Lead Acetate Test

The extract was treated with a few drops of ten percent lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids. Orange to crimson colour shows the presence of flavonoids.

2.6.6 Alkaline Reagent Test

To the 1 ml of extract in a test tube add few drops of Sodium hydroxide solution (ten percent). Formation of an intense yellow colour, which turns colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids (Harbourne, 1973) [12].

2.6.7 Test for Alkaloids

2.6.7.1 Dragendorff's Test

In 2 ml of filtrate add 1 ml of 1% hydrochloric acid and steam heated the solution for 2 min. filtered the solution and take 1 ml of filtrate. Add six drops of Dragendorff's reagent. The change in color of precipitate to orange red/ brownish red showed the presence of alkaloids.

3. Result and Discussion

3.1 Yield of ashwagandha and shatavari root extracts

W. somnifera and *A. racemosus* root extract was prepared by using solvent extractor by using ethanol as solvent. The results for percent yield of extracts are presented in table 1

Table 1: Yield of the extracts of ashwagandha and shatavari root

Material	Yield (%) *Ethanolic extract*
Ashwagandha root powder	9 ±0.90
Shatavari root powder	11±0.57

The data presented in table 4.9 revealed that yield of the ethanolic extract of ashwagandha and shatavari root powder. It is found that yield of ashwagandha extract is 9 ±0.90 percent which is similar to findings with Dhanani *et al*., (2017) [9] that shows the solvent extraction method by using ethanol as solvent having yield (%) of 9.08 and Jain *et al*., (2010) [13] reported percentage yield of extracts by Soxhlet is 7.52. The yield of shatavari extract was estimated as 11±0.57 percent which has similar reporting with Nagamani *et al*., (2012) [20] that shows percentage yield of *A. racemosus* using ethyl alcohol as solvent results in 13.03 percent yield of extract.

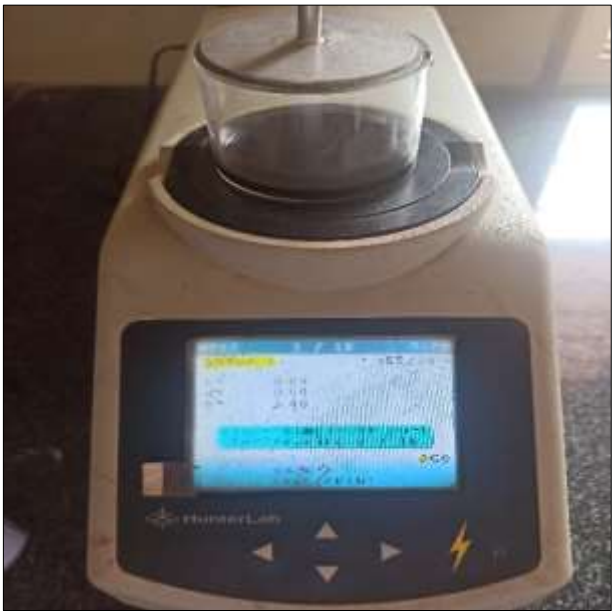
3.2 Colour analysis of ashwagandha and shatavari extract

Color index indicates the lightness, redness and yellowness of ingredients. L* indicates lightness, L*= 0 indicates black color, L*= 100 pure whiteness, a* positive (+) indicates red colour and a* (-) negative indicates green colour. b* positive (+) indicates yellow colour, b* (-) negative indicates blue colour. C* chroma, h* Hue angle Pankaj *et al.*, (2013) [25].

Table 2: Colour characteristics of ashwagandha and shatavari extract

Colour Parameter	Ashwagandha Extract	Shatavari Extract
L*	0.88±0.09	0.86±0.12
a*	0.99±0.13	0.86±0.10
b*	1.42±0.09	1.64±0.12
Hue (h*)	56.37±0.81	57.82±1.17
Chroma (C*)	1.56±0.11	1.54±0.11

Table 2 has indicated the values of L*, a*, b*, C*, h* for ashwagandha extract and shatavari extract by using Hunter Lab colorimeter. The readings for ashwagandha extract were L* lightness value was 0.89, a* value was 0.99 and b* value was 1.40 while Chroma (C*) and Hue value (h*) were 1.53 and 56.19 respectively. The readings for shatavari extract were L* lightness value was 0.87, a* value was 0.95 and b* value was 1.63 while Chroma (C*) and Hue value (h*) were 1.56 and 56.19 respectively.



Hunter Lab Colorimeter

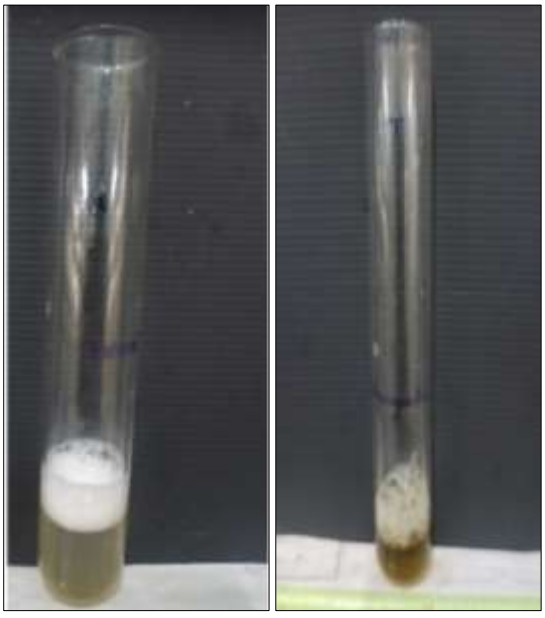
3.3 Qualitative analysis of phytochemicals from ashwagandha and shatavari root extract

Qualitative analysis of ashwagandha and shatavari root extracts was carried out to determine the presence of phytochemical constituents. This analysis aimed to identify the secondary metabolites present in the ashwagandha and shatavari root powder extracts of ethanol and aqueous. The following results had been made for qualitative phytochemical analysis are summarized in table 3

Table 3: Qualitative analysis of phytochemicals from ashwagandha and shatavari root extract

Phytochemical constituents	Extracts				Name of the Test
	Ashwagandha		Shatavari		
	Aqueous	Ethanolic	Aqueous	Ethanolic	
Alkaloid	+	+	-	-	Dragendorff's reagent
Flavonoid	-	+	-	+	Shinoda test, Lead acetate test
Saponin	+	+	+	+	Foam test
Tannins	-	+	+	+	Lead Acetate Test
Total phenol	+	+	+	+	Ferric chloride test
Carbohydrate	+	+	+	+	Fehling's test
Where, + = Present and - = Absent					

Through phytochemical testing, it was noted that different extracts, including ethanolic and aqueous ones, displayed the presence or absence of various phytochemical constituents. Specifically, the ethanolic extract of ashwagandha root powder was found to contain alkaloid, flavonoid, saponin, tannins and total phenol. The aqueous extract, on the other hand, tested positive for alkaloid, saponins and total phenol. These outcomes are consistent with the research conducted by (Veer *et al*., 2019; Bargale *et al*., 2020) [32, 3], In the phytochemical screening of shatavari root powder, the ethanolic extract was found to contain flavonoids, saponins, tannins and total phenols, while the aqueous extract showed positive results for saponins, tannins and total phenols. These results parallel the findings reported by (Selvaraj, 2019; Roy *et al*., 2014) [29, 28], who identified flavonoids, phytosterols, tannins/phenolic substances, and glycosides in the ethanolic extract of shatavari root powder. The presence of these phytochemical constituents in the extracts signifies their potential nutraceutical properties. Each of these phytochemicals is associated with different protective and therapeutic effects.



Test for Saponins Aqueous Extract (Left: Shatavari, right: Ashwagandha)

3.4 Quantitative estimation of phytochemical component of ashwagandha and shatavari root extract

The analysis of phytochemicals holds great significance in the discovery of new sources of medically and industrially valuable compounds found in medicinal plants through chemical investigation. Research on quantifying ashwagandha and shatavari extracts revealed the existence of alkaloids, flavonoids, saponins, and phenolics. Phytonutrients, which are secondary metabolites of plant materials, are known for their medicinal properties (Viswesari *et al*., 2013) [35].

Table 4: Phytochemical composition of ashwagandha and shatavari extract

Parameters	Ashwagandha extract	Shatavari extract
Total Phenolic content (GAE mg/100 g)	9299±2	10522±1
Flavanoid content (QE mg/100 g)	8746±1	9412±2
Total alkaloid content mg/100 g	786±1	-
Saponins (Foaming Index)	>100	>100

Plant alkaloids, whether derived from plants or synthesized, play a crucial role as essential medicinal agents, offering benefits such as alleviating pain, inducing muscle relaxation, and exerting antibacterial effects (Okwu, 2004) [21]. Saponins, classified as glycosides, possess hypocholesterolemic effects that alleviate the strain on the liver's metabolism. They also contribute to the body's defense against fungal, microbial, and viral threats, with a notable impact on specific tumor cells, particularly in cases of lung and blood cancer (Olivebever, 1986) [22]. Flavonoids showcase antioxidant properties, aiding in the reduction of cellular stress. Plant phenolic compounds, which encompass flavonoids, are acknowledged for their potent antioxidant abilities and their potential to hinder mutations and impede the progression of cancer (Middleton and Kandaswami, 1994) [19].

The data presented in table 4 revealed that the total phenolic content in ethanolic extract of ashwagandha showed the level as 9299±2 (GAE mg/100 g), flavonoid content as 8746±1 (QE mg/100 g) and total alkaloid content 786±1 as mg/100 g. (Dhanani *et al*., 2017) [9] reported the similar findings for total phenolic content i.e., 35.93 GAE mg/g. (Vinotha *et al*., 2015) [34] reported that total alkaloids 0.81±0.01 and total flavonoids 14.43±0.40 present in *W. somnifera* root**. (**Kherde *et al*., 2020) [16] reported that the total phenolic content (mg/g gallic acid equivalent) 90.325 ±4.15, total alkaloid content (percent w/w in plant material) 0.20±0.001, total flavonoid content (mg/g rutin equivalent) 80.23 ±5.93 of ashwagandha.

The total phenolic content in ethanolic extract of shatavari showed the level as 10522±1 (GAE mg/100 g) and flavanoid content as 9412±2 (QE mg/100 g). Roy *et al*., (2014) reported similar findings total phenol (mg/gm, gallic acid equivalent) and flavonoid (mg/gm, quercetin equivalent) contents of the ethanol fraction of shatavari as 165.22 ± 8.26 and 188.33 ±7.33 respectively. (Behera, 2018) [4] revealed that the total phenolic (mg of GAE/g) and flavonoid (mg of RUE/g) content and total antioxidant activity (mg of AAE/g) of methanolic extract of shatavari as 12.90 ± 0.002, 0. 80 ± 0.001 and 132.53 ± 0.12 respectively. (Prabakaran *et al*., 2015) [27] reported similar findings of total tannins, total flavanoid and total phenolic content present in ethanol extracts of shatavari 19.34±0.33 µg/mg 34.44 ±0.62 µg/mg 73.78±0.53 µg/mg respectively.

4. Conclusion

It can be concluded from the study that the ashwagandha and shatavari root powder extracts exhibit diverse range of phytochemical compositions including alkaloids, flavonoids, saponins, tannins and phenolics. Root powders of ashwagandha and shatavari are reservoirs of various, abundant sources of diverse secondary metabolites. Secondary metabolites were detected in good proportion in ethanolic extract. These biologically active components are responsible for various pharmacological effects. Such preliminary phytochemical screening of these drugs as well as quantification of phytochemical components underscores their importance in traditional medicine and their potential applications in developing health-enhancing products.

5. References

1. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in Mangifera indica leaves from Ibadan, Oyo State. Plant Sci Res. 2009;2(1):11-13.
2. Allison P. Global survey of marine and estuarine species used for traditional medicine and tonic foods. WHO Rep. McGill Univ, Quebee, Canada; 1966.
3. Bargale S, Tripathy TB, Shashirekha H. Phyto physico-chemical profile of Ashwagandha (*Withania somnifera* Dunal). J Ayurveda Integr Med Sci. 2020;5(6):120-129.
4. Behera SK. Phytochemical screening and antioxidant properties of methanolic extract of root of Asparagus racemosus Linn. Int J Food Prop. 2018;21(1):2681-2688.
5. Berhane M, Singh V. Effect of feeding indigenous galactopoietic feed supplements on milk production in crossbred buffalos. Indian J Anim Sci. 2000;72(7):609-611.
6. Bhattacharjee SK. Handbook of medicinal plants. Jaipur: Pointer Publishers; 1998.
7. Bhutani K, Paul A, Fayad W, Linder S. Apoptosis inducing activity of steroidal constituents from Solanum xanthocarpum and Asparagus racemosus. Phytomedicine. 2010;17:789-793.
8. Bopana N, Saxena S. Asparagus racemosus - Ethnopharmacological evaluation and conservation needs. J Ethnopharmacol. 2007;110:1-15.
9. Dhanani T, Shah S, Gajbhiye N, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. Arab J Chem. 2017;10(01):1193-1199.
10. Elgorashi EE, Staden VJ. Pharmacological screening of six Amaryllidaceae species. J Ethnopharmacol. 2004;90:27–32.
11. Gurudeva MR. Botanical and vernacular names of south Indian plants. Divyachandra Prakashana; 2001.
12. Harbourne JB. Phytochemical analysis. London: Chapman and Hall Company Ltd; 1973.
13. Jain H, Parial SD, Jarald E, Daud A, Ahmad S. Extraction of Ashwagandha by conventional extraction methods and evaluation of its anti-stress activity. Pharmacognosy J. 2010;4(3):183-185.
14. Kapoor LD. CRC Handbook of Ayurvedic Medicinal Plants. Boca Raton, FL: CRC Press; 1990.
15. Kar A. Pharmacognosy and Pharmabiotechnology. New Delhi: New Age Publishers & Distributors; 2004.
16. Kherdea S, Parmara K, Tawar M, Prasada S, Itankar P. Study on impact of different climatic zones on

physicochemical and phytochemical profile of *Withania somnifera* (L.) Dunal. Indian J Tradit Knowl. 2020;19(3):486-493.
17. Kokate CK, Purohit AP, Gokhale SB. Practical pharmacognosy. Pune: Nirali Prakashan; 1994. p. 54-60.
18. Kongkaneramit L, Witoonsaridsilp W, Peungvicha P, Ingkaninan K, Waranuch N, Sarisuta N. Antioxidant activity and antiapoptotic effect of Asparagus racemosus root extracts in human lung epithelial H460 cells. Exp Ther Med. 2011;2:143-148.
19. Middleton EJ, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. London: Chapman and Hall; 1994. p. 619-652.
20. Nagamani, Suresh J, Ahuja J, Reddy V. Comparative phytochemical screening of Vatashunga, Shatavari and Shatapushpa claimed for Prajasthapana activity. Ann Biol Res. 2012;3(3):1294-1304.
21. Okwu DE. Phytochemicals and vitamins content of indigenous spices of south eastern Nigeria. J Sustain Agric Environ. 2004;6(1):30-37.
22. Olivebever B. Medicinal plants in tropical West Africa. Cambridge: Cambridge University Press; 1986. p. 123-125.
23. Onlom C, Khanthawong S, Waranuch N, Ingkaninan K. *In vitro* anti-malassezia activity and potential use in anti-dandruff formulation of Asparagus racemosus. Int J Cosmet Sci. 2014;36:74-78.
24. Pandey SK, Sahay A, Pandey RS, Tripathi YB. Effect of Asparagus racemosus rhizome (Shatavari) on mammary gland and genital organs of pregnant rat. Phytother Res. 2005;19:721-724.
25. Pankaj B, Pathare L, Opera, Al-Said A. A review of colour measurement and analysis in fresh and processed foods. Food Bioprocess Technol. 2013;6(1):36-60.
26. Pattipati S, Amanpreet S, Shrinivas K. Effect of *Withania somnifera* root extract on Haloperidol-induced Orofacial Dyskinesia: Possible mechanism of action. J Med Food. 2003;6(2):107-114.
27. Prabakaran DK, Vadivu R, Jayshree N. Preliminary Phytochemical and *In vitro* Cytotoxic Activity Of The Leaves Of Asparagus Racemosus Willd., (Liliaceae). Int J Pharma Sci Res. 2015;6(4):743-748.
28. Roy S, Pradhan S, Mandal S, Das K, Patra A, Samanta A, Sinha B, Kar S, Nandi D. Phytochemical analysis, antimicrobial activity and assessment of potential compounds by thin layer chromatography of ethanol fraction of Asparagus racemosus roots. Int J Pharm Pharm Sci. 2014;6(8):367-370.
29. Selvaraj K, Sivakumar G, Pillai A, Veeraraghavan V, Bolla S, Veeraraghavan G, Rengasamy G, Joseph J, Janardhana P. Phytochemical Screening, HPTLC Fingerprinting and *In vitro* Antioxidant Activity of Root Extract of Asparagus racemosus. 2019;11(4):818-823.
30. Shah B, Seth A. Textbook of pharmacognosy and phytochemistry. India: Elsevier; 2010.
31. Sharma S, Ramji S, Kumari S, Bapna J. Randomized controlled trial of Asparagus racemosus (shatavari) as a lactogogue in lactational inadequacy. Indian Pediatr. 1996;33:675-677.
32. Veer S, Sawate A, Kshirsagar R, Agarkar B, Patil B. Studies on quality assessment of ashwagandha root (*Withania somnifera*) powder. Int J Chem Stud. 2019;7(2):556-559.
33. Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Vijaya Kumar SG, Rajarajan T, Perianayagam JB. Anti-diarrhoeal potential of Asparagus racemosus wild root extracts in laboratory animals. J Pharm Pharm Sci. 2005;8:39-46.
34. Vinotha S, Thabrew I, Sri Ranjani S. Phytochemical Screening of Various Extracts of Root of *Withania somnifera* (L) Dunal. Arch Bus Res. 2015;3(2):179.
35. Viswesari G, Christopher R, Rajendra W. Phytochemical screening of active secondary metabolites present in *Withania somnifera* root: role in traditional medicine. Int J Pharm Sci Res. 2013;4(7):2770-2776.
36. Weiner MA, Weiner J. Ashwagandha (India ginseng). In: Herbs that. Quantum Book, Mill Valley, CA; 1994. p. 70-72.
37. Williamson EM, Hooper M. Major Herbs of Ayurveda. Edinburgh: Churchill Livingstone; 2002.
38. Zwenger S, Basu C. Plant terpenoids: applications and potentials. Biotechnol Mol Biol Rev. 2008;3:001-007.
39. Kang YB, Sodunke TR, Lamontagne J, Cirillo J, Rajiv C, Bouchard MJ, *et al*. Liver sinusoid on a chip: Long-term layered co-culture of primary rat hepatocytes and endothelial cells in microfluidic platforms. Biotechnology and bioengineering. 2015 Dec;112(12):2571-2582.