

Pharmaceutical and Nutraceutical Values of *Dioscorea hispida* Dennst. – A Wild Food of Asia and Africa

Sohan Lal¹, Nidhi Mahendru², Chanchal Malhotra³, Jeline Rani J⁴, Poonam Dilip Sable^{5*}, Sanjeet Kumar^{6*} and Miriam Ahunna Ofoeze⁷

¹ Govt. G.S.G. P.G College, Department of Botany, Balod, Chhattisgarh, India

² Guru Nanak Khalsa College Yamuna Nagar, Department of Biotechnology, Haryana, India

³ Baba Mastnath University, Department of Botany, Asthal Bohar, Rohtak, Haryana, India

⁴ Bishop Heber College, Department of Botany, Trichy, Tamil Nadu, India

⁵ Pharate Patil Ayurvedic Medical College, Department of Dravyaguna Vigyana, Mandavgan Pharata, Pune, Maharashtra, India

⁶ Ambika Prasad Research Foundation, Cuttack, Odisha, India

⁷ National Root Crops Research Institute Umudike P.M.B 7006 Umuahia, Nigeria.

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The potential ethnomedicinal plants are considered of great importance in the present day mainly due to the shortcomings of the drugs against antimicrobial resistance (AMR), or, in other words, the ability for cells to mutate with respect to the changing environment. The present study demonstrates the ethnobotanical values of the wild tuberous plant, *Dioscorea hispida*, which is used as medicinal food in Asia and Africa and further performs scientific validation of the ethnomedicinal values claimed by the locals of India and Nigeria, through phytochemical analysis and antibacterial activity. A survey elucidated certain foods and therapeutic uses of the tubers of *D. hispida*. Qualitative analysis of phytochemical compounds revealed the presence of secondary metabolites like tannins, terpenoids, reducing sugar, flavonoids, phenolic compounds and saponins. Subsequent examination of the antibacterial activity of *D. hispida*'s tuber extracts against gram-positive and gram-negative bacteria revealed that the acetone tuber extract had the largest zone of inhibition against *Streptococcus mutans* followed by the methanol tuber extract against *Vibrio cholerae*, the methanol tuber extract against *Shigella flexneri*, the aqueous tuber extract against *S. flexneri*. This scientific confirmation may aid in the identification of possible antibacterial compounds, the confirmation of nutraceutical potential, and the promotion of value additions associated with the consumption of such tuberous plants in Asia and Africa.

Keywords: antibacterial screening, future food & medicines, India, indigenous traditional knowledge, Nigeria, secondary metabolites

1 Introduction

The new world with advance technologies and development of drugs are finding a huge challenge for the potential antimicrobial agents (Sahoo et al., 2021) mostly due to the ability for the emerging microbes to mutate and develop resistance which is also termed as antimicrobial resistance (AMR). Recent studies have estimated about 1.2 million deaths due to resistant bacterial infection or AMR in 2019 (Tang et al., 2023). The improper use of antibiotics has further exacerbated during the global pandemic COVID-19 leading to the shorter lifespan of drugs for antimicrobial agents. It has been estimated that an average of 2-3 antibiotics are launched each year, however, scientist have realized that the lifespan of antibiotics is becoming limited due to AMR (Osborn, 1996; Gigante et al., 2022;

* **Corresponding Author:** Poonam Dilip Sable, Pharate Patil Ayurvedic Medical College, Department of Dravyaguna Vigyana, Mandavgan Pharata, Pune, Maharashtra, India. E-mail: poonam.ayurindia@gmail.com

Devi et al., 2023). Many countries still today continue the traditional therapeutic practices using native medicinal plants. Such practices are common in tribal communities or indigenous communities of a specific region. The traditional system of medicine treats several diseases and disorders including bacterial and fungal infections (Saradar et al., 2024). Most of these wild potential medicinal plants are still unexplored by the scientific community and screening of new antimicrobial agents are highly essential in the present day to fight the AMR and allied challenges of the health care systems (Fischbash & Walsh, 2009). Exploring the knowledge of traditional medicinal system is also important as these knowledges are considered to pass down generation after generation and if not preserve these could slowly vanish in the race for modern world and advance health care system (Saklani et al., 2020). Wild tuberous plants, particularly Yam species are one of the less scientifically unexplored plants with potential food and therapeutics and a rich diversity of around 650 species distributed throughout the world (Mahanti et al., 2018; Karle et al., 2022). They are commonly used in Asian and African countries.

Among the yam species, *Dioscorea hispida* Dennst. is also considered the oldest food consumed by ethnic groups or indigenous communities in specific patches of the tropical and subtropical parts of the world including West Africa, some parts of Central America, Southeast Asia, Caribbean, Pacific islands, and some parts of temperate regions (Dutta 2015; Sinnadorai et al., 2022). Certain utilization of *D. hispida* has been reported by different tribes of Africa and Asia. Dutta (2015) documented 16 *Dioscorea* species with traditional food and medicinal values. Several scientists and researchers have realized the potential of *Dioscorea* species and initiated some scientific findings of phytochemical compounds and bioassays. Studies on the antioxidant activity of *D. pentaphylla*, *D. bulbifera*, *D. deltoidea*, *D. triphylla* and *D. versicolor* have been reported (Adoménienė & Venskutonis, 2022). Many phytochemical compounds like phenolic compounds, flavonoids, saponins, anthocyanins, carotenoids, etc. have been recorded from different *Dioscorea* species which attribute to certain pharmacological activities like anticancer, anti-inflammatory and antidiabetic activities (Bhandari et al., 2003; Kumar et al., 2017). Earlier studies have isolated specific bioactive compounds namely dioscin, protodioscin, diosgenin, gracillin, rutin, quercetin, etc. responsible for many pharmacological activities (Wang et al., 2023). Therefore, keeping the problems of AMR and need to document the traditional therapeutic knowledge, an attempt has been made to document the ethnomedicinal uses of *Dioscorea hispida* from India and Nigeria. The research team considering its ethnic food and medicinal values tried to scientifically validate its therapeutic claims by the various tribes of India and Nigeria through evaluation of phytochemical analysis and antibacterial activity for future nutraceuticals and pharmaceuticals.

2 Material and methods

The survey for ethnobotanical data collection was carried out in India (Fig. 1) and Nigeria in Africa for tuberous species during 2017–2023 and 2023–2024, respectively. In India, Odisha state [Sundargarh (2 tribal areas: Bonai and Rourkela), Mayurbhanj (1 tribal areas: Badampahar), Cuttack (1 tribal areas-Mahanadi areas of Athagarh), Kalahandi (1 tribal areas of Karlapat), Dhenkanal (1 tribal areas of Kapilash) districts] and in Nigeria, South East and North Central (Anambra and Benue states) are selected (6 localities) as study areas for ethnobotanical surveys on *Dioscorea hispida*. The survey incorporated standard techniques and frameworks of exploration. Interviews of the different tribal communities were done using semi-structured questionnaires (Martin, 1995). Informants an average age between 35–50 years were considered for interview. Further group discussions and analysis of the collected data was done (Cotton, 1996; Alexiades, 1996; Vogl & Lukasser, 2004). Different plant parts of about 10 plants of *Dioscorea hispida* (leaves, stem and tubers) were collected, cleaned, and dried in shade (Fig. 2). The plant parts were then grinded into a course fine texture using an electric grinder. Maceration method of extraction was incorporated for phytochemical analysis using four solvents (n-hexane, acetone, methanol and aqueous; Kumar et al., 2013). For the analysis of antibacterial activity, Soxhlet method of extraction was carried out using different (n-hexane, acetone, methanol and aqueous) solvents (Devi et al., 2023). Triplicates of experiments were taken and results were analyzed.

2.1 Phytochemical analysis

Phytochemical analysis for secondary metabolites were carried out on different solvent extracts (n-hexane, acetone, methanol and aqueous) of *D. hispida* (leaves, stem and tubers) using standard procedure to identify the bioactive compounds (Harborne, 1973; Trease and Evans, 1989; Kumar et al., 2013; Devi et al., 2023). Replicates of experiments were carried out.

Test of tannin

2 mL of filtrate (plant extract) was taken in a test tube and 3–5 drops of 0.1% ferric chloride solution were added. The brownish green or blue-black colouration indicated the presence of tannins.

Test for saponin

2 mL of filtrate (plant extract) was mixed with 2 mL of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponins.

Test of flavonoids

2 mL of 2% sodium hydroxide solution was added to portion of the aqueous filtrate of plant extract followed by addition of concentrated sulphuric acid. A yellow colouration indicated the presence of flavonoids.

Test of terpenoids

2 mL of filtrate was mixed in few drops of chloroform and then 4–5 drops of H₂SO₄ acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.

Test of glycosides

0.5 g of extract was treated with 1% ferric chloride solution and was put into water bath for 5 minutes at 100 °C. The mixture was cooled and equal volume of benzene was added. The benzene layer was separated and 5 mL of ammonia solution was added. Formation of rose-pink colour indicated the presence of glycosides.

Test of phenolic compounds

2 ml of the filtrate was taken and treated with 3–5 drops of 1% ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

Test for reducing sugar

2 ml of the filtrate was boiled with 2 drops of Fehling's solution A and B for 5 minutes. An orange-red precipitate was obtained indicated the presence of reducing sugar.

Test for steroids

2 mL of plant extract was dissolved in 2 mL chloroform and then 2 mL of concentrated sulphuric acid was added. Formation of 2 phases (upper red and lower yellow with green fluorescence) indicated the presence of steroids.

Test for alkaloids

2 ml of the filtrate was mixed with 2 mL of 1% aqueous HCl on water bath and then filtered. 2–5 drops of dragendorff's reagent were added in the filtrate. The occurrence of orange-red precipitate indicated the presence of alkaloids in the sample extract.

2.2 Analysis of antibacterial activity

For analysis of antibacterial activity, two gram-positive *Streptococcus mutans* (MTCC 497) and *S. pyogenes* (MTCC 1926) and three gram-negative organisms *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252) were taken as test organisms. Antibacterial activity was done using slight modification of standard methods of Agar Well Diffusion assay and Broth dilution assay. Different concentrated extracts were diluted with 10% DMSO to make a concentration of (400, 500, 1000 and 2000) µg/ml. Nutrient agar plates were used as substrate for bacterial growth. 24-hour fresh bacterial cultures were taken for the antimicrobial susceptibility test. Different bacterial cultures were spread on fresh sterile nutrient agar plates using spread plate method. Agar wells of 6 mm were then made using sterile borer. 100 µl of the different concentrations of the plant extracts were added into the agar wells. It is repeated for all the solvent plant extracts and for the concentration range of (400, 500, 1000 and 2000) µg/ml. The same experiment set is repeated thrice. Agar plates were then incubated at 35 ± 2°C for 18-24 h. Kanamycin and Ampicillin served as standard antibiotics control. For each replicates the readings (diameter of zone of inhibition in cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded (Allen et al., 1991). Broth dilution assay was carried out for finding the minimum inhibitory concentration (MIC). The minimum concentration with good inhibitory zone was taken. Serial dilutions were prepared using sterile nutrient broth and the plant extract below and above the selected concentration (Rai et al., 2010). Fresh

cultures from nutrient broth were inoculated in the different serial dilutions of the plant extracts and incubated at $35 \pm 2^\circ\text{C}$ for 18–24 h. Kanamycin and Ampicilin served as standard antibiotics control. After 24 hours incubation, clear tube among the inoculated serial dilution was taken as a MIC (Gonelimali et al., 2018; Balouiri et al., 2016; Kumar et al., 2013; Devi et al., 2023).



Fig. 1 Discussion with local communities of India and forest officials about the uses of *Dioscorea hispida*



Fig. 2 Stored tubers of *Dioscorea hispida* along with other wild medicinal foods

3 Results and discussion

The ethnobotanical survey among different tribal communities of India and Nigeria with respect to the *Dioscorea hispida* revealed that mostly Ho, Kolho, Kharia and Bathudi tribes were familiar in India and Igbo, Igala, Ibira, Igede and Idoma of South and middle belts of Nigeria. However, Igbo, Idoma, Ho and Bathudi tribes had better ethnobotanical uses of *D. hispida*. It was known by different local names such as “Bayan sanga” and “Hasar sanga”. They normally harvest it around August to November and available at the weekly local markets (Haat) from October to December. Ho and Bathudi tribal communities mentioned that tubers of *D. hispida* were roasted and consumed as snacks, successively

boiled, and cooked with other vegetables and tamarind pulp. Raw tuber pastes mix with little water and applied externally to treat skin infections. Tuber juice along with water and pinch of salt is given for the treatment of stomach worms. The detailed ethnobotanical information is given in Table 1.

Screening for presence of secondary metabolites acts as a first step towards validating the therapeutic claims made by the tribal communities. The qualitative phytochemical analysis of the different parts (leaves, stem and tubers) was carried out using different solvents exhibited a wide range of bioactive compounds. Tannins, terpenoids, reducing sugar, flavonoids, phenolic compounds, and saponins are mostly present in different solvents like acetone, methanol, and aqueous extracts (Table 2).

The antibacterial activity of *D. hispida* against two gram-positive *Streptococcus mutans* (MTCC 497) and *S. pyogenes* (MTCC 1926) and three gram-negative organisms *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252) found that the highest zone of inhibition was shown by acetone tuber extract against *S. mutans* (1.68 ± 0.12) followed by methanol tuber extract against *V. cholerae* (1.46 ± 0.20), methanol tuber extract against *S. flexneri* (1.40 ± 0.20), methanol tuber extract against *S. mutans* (1.40 ± 0.05), aqueous tuber extract against *S. flexneri* (1.40 ± 0.05), and so on. Detail information is provided in Table 3 and Fig. 3. Earlier studies demonstrated that aqueous extract of tubers of *D. bulbifera* was susceptible against *Proteus vulgaris* and *Pseudomonas aeruginosa* (Adeosun et al., 2016) while other study have demonstrated a significant antibacterial and antifungal activity of tubers of *D. bulbifera* against multidrug resistance bacteria like *Escherichia coli*, *Salmonella paratyphi*, *Acinetobacter* spp. and *Candida albicans* (Dahiya, 2017). Kumar and his team reported phytochemical and antibacterial activity of *D. pentaphylla* (Kumar et al., 2013). MIC of acetone and methanol tuber extract were observed at 2000 µl/ml.

This study elucidates the validation of certain therapeutic claims against skin infection and stomach worms. However, further analysis of the detected phytochemical compounds needs to be work on for its characterization at molecular level and re-analyse the bioassay for confirmation. Such level of research could confirm the advantages of consuming the study plant for its specific therapeutic uses without side effects or could be a potential source of antimicrobial agents in future drug development (Fig. 4) in both countries.

Table 1 Ethnobotanical data collected from tribal communities on *Dioscorea hispida* from India and Africa

Tribe(s)	Ethnobotanical use	Mode of uses	Location
Ho	Fish Poison	Fresh mature tubers pieces used for catching fish. It helps to paralyze fish and made easy for catching.	India
Ho	Skin infections	Tuber pastes mixed with water and applied externally on infected skin as an antiseptic for 2–3 days.	India
Bathudi	Stomach worms	Tuber juice mix with water with a pinch of salt taken twice a day for 5 days helps to get relieve from stomach worms.	India
Bathudi	Vegetables	Tubers were boiled successively after which it was cooked with other vegetables and tamarind pulp.	India
Ho and Bathudi	Vegetables	Tubers were stored in dry place and later cooked during food scarcity and thin slices of tubers are dried in sunlight to be consumed as chips.	India
Ho and Bathudi	Snacks	Tubers are roasted and consumed as snacks.	India
Wollof tribe	Food	Tubers consumed as food.	West Africa
Igbo, Igala, Ibira, Igede and Idoma	Medicinal food	the locals use it for food and medicinal purposes after carefully processing to reduce what they believe is toxic.	South and the middle belt of Nigeria

Table 2 Phytochemical analysis for secondary metabolites from different plant parts of *Dioscorea hispida*

Plant parts	Solvent Used	Secondary metabolites
Leaves	n-Hexane	Terpenoids
	Acetone	Tannins, terpenoids, and reducing sugars
	Methanol	Tannins, saponins, terpenoids, steroids and reducing sugars
	Aqueous	Tannins, saponins, flavonoids, terpenoids and reducing sugars
Stem	n-Hexane	Not detected
	Acetone	Reducing sugars and steroids
	Methanol	Tannins, flavonoids, phenolic compounds, saponins and reducing sugars
	Aqueous	Flavonoids, alkaloids, saponins, terpenoids, reducing sugars and glycosides
Tuber	n-Hexane	Terpenoids
	Toluene	Glycosides
	Petroleum ether	Terpenoids
	Chloroform	Not detected
	Acetone	Tannins, flavonoids, reducing sugars and glycosides
	Acetone water (1:1)	Tannins, flavonoids, phenolic compounds, saponins, reducing sugars and glycosides
	Methanol	Tannins, phenolic compounds, and steroids
	Methanol:Water (1:1)	Tannins, phenolic compounds, saponins, terpenoids, reducing sugars, glycosides, and steroids
	Ethanol	Tannins
Aqueous	Saponins, terpenoids, reducing sugars and glycosides	

Table 3 Antibacterial activity of *Dioscorea hispida* tuber extracts

Test organisms	Zone of inhibition (ZI) (cm)				Extracts
	400 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	
<i>V. cholerae</i>	ZI ≤ 0.70	0.73 ± 0.02	0.93 ± 0.02	1.46 ± 0.20	Methanol
<i>S. typhi</i>	ZI ≤ 0.70	0.71 ± 0.02	0.85 ± 0.05	1.26 ± 0.05	
<i>S. flexneri</i>	ZI ≤ 0.70	0.78 ± 0.02	0.93 ± 0.02	1.40 ± 0.20	
<i>S. pyogenes</i>	ZI ≤ 0.70	0.73 ± 0.07	0.93 ± 0.02	1.38 ± 0.12	
<i>S. mutans</i>	ZI ≤ 0.70	0.70 ± 0.08	0.93 ± 0.02	1.40 ± 0.05	
<i>V. cholerae</i>	ZI ≤ 0.70	0.76 ± 0.07	1.01 ± 0.07	1.25 ± 0.05	Acetone
<i>S. typhi</i>	ZI ≤ 0.70	0.83 ± 0.02	1.06 ± 0.15	1.33 ± 0.12	
<i>S. mutans</i>	ZI ≤ 0.70	0.86 ± 0.02	1.05 ± 0.22	1.68 ± 0.12	
<i>S. pyogenes</i>	ZI ≤ 0.70	0.86 ± 0.02	1.16 ± 0.15	1.33 ± 0.10	
<i>S. mutans</i>	ZI ≤ 0.70	0.85 ± 0.08	1.1 ± 0.20	1.25 ± 0.15	
<i>V. cholerae</i>	ZI ≤ 0.70	0.73 ± 0.02	0.88 ± 0.10	0.90 ± 0.05	Aqueous
<i>S. typhi</i>	ZI ≤ 0.70	0.76 ± 0.07	0.83 ± 0.02	1.26 ± 0.07	
<i>S. flexneri</i>	ZI ≤ 0.70	0.7 ± 0.05	0.86 ± 0.07	1.40 ± 0.05	
<i>S. pyogenes</i>	ZI ≤ 0.70	0.71 ± 0.05	0.85 ± 0.05	0.91 ± 0.02	
<i>S. mutans</i>	ZI ≤ 0.70	0.71 ± 0.07	0.90 ± 0.08	0.90 ± 0.05	

(ZI < 0.70: zone of inhibition is less than 0.70 cm; ZI ≤ 0.70: zone of inhibition is less than or equal to 0.70; NI: no inhibition, AWD assay, mean ± SD, n=3) (*Vibrio cholerae* MTCC 3906, *Salmonella typhi* MTCC 1252, *Shigella flexneri* MTCC 1457, *Streptococcus pyogenes* MTCC 1926, *Streptococcus mutans* MTCC 497)

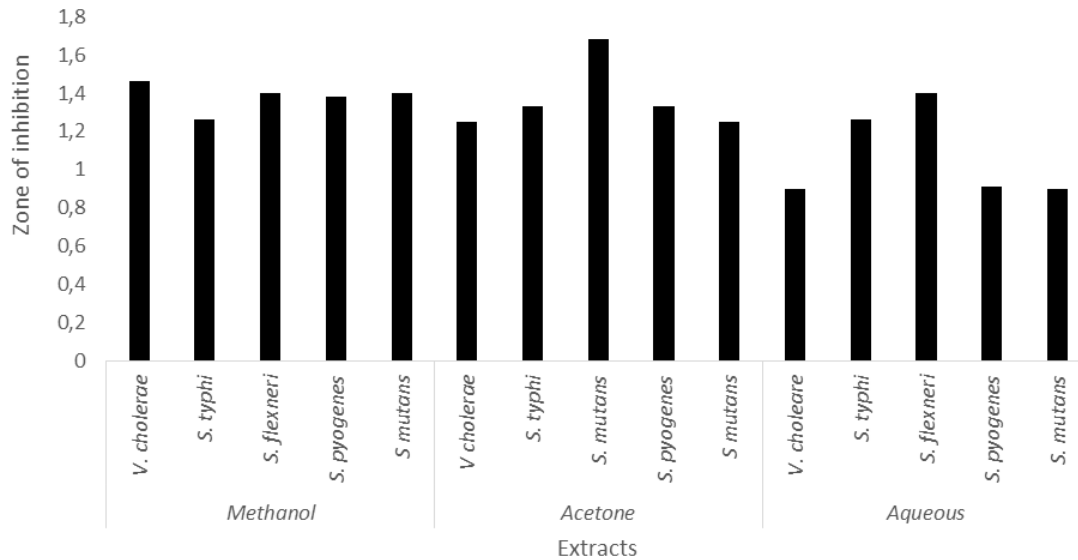


Fig. 3 Antimicrobial activity of *Dioscorea hispida* tuber extracts

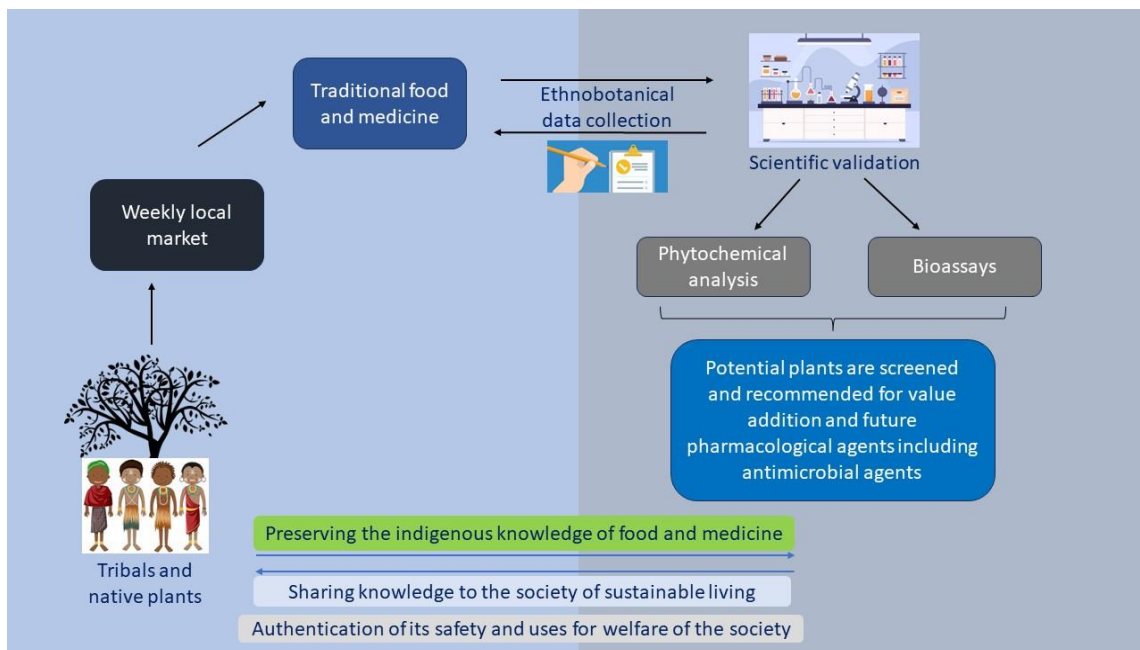


Fig. 4 Overall bridge between the indigenous traditional knowledge of native plants to scientific validation of less unexplored plants for future sustainable development and welfare of the society

4 Conclusions

Present study concludes that tuber of *Dioscorea hispida* is used as traditional food after indigenous practices for removing anti-nutritional factors in India and Nigeria. In both places, it is also used as medicinal agent. Study also showed that tubers have diverse bioactive compounds which have antibacterial activities. Therefore, it could be a nutraceutical for future generation which could minimise the problems of food and antimicrobial resistance in Asia and Africa.

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