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Phytochemical Analysis of Some Wild Vegetables from Thane Dist, Maharashtra, India

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ABSTRACT

Indian traditional knowledge about the wild edible plants (WEPs) is most important part for the sustainable development. People are addicted to eat hybrid food for the last few decades. This study focuses on the preliminary survey and phytochemical analysis of certain wild edible plants providing valuable insights into their potential benefits. *Hibiscus sabdariffa, Cassia tora, Amaranthus cruentus* (Red leaves), *Amaranthus viridis* (Green leaves), *Holarrhena* spp., these WEPs are tested for analysis of Phytochemicals. During the last few years, several studies have focused on the chemical characterization and the bioactivities of various WEPs, while numerous ethno pharmacological studies have highlighted their contemporary uses in modern dietsand their positive health effects. The results revealed the presence of medicinally active compounds in those five plants. From the phytochemical analysis, it could be seen that Phenolic, Flavonoids and Antioxidant Properties i.e. 2-diphenyl-1-picrylhydrazyl (DPPH) were present in all the plants. Flavonoids were absent only from *Holarrhena spp*. Some plant species are used for nutritional purposes and for enhancing overall health. These WEPs are utilized by tribal communities to effective treatment and manage a variety of diseases and health conditions, including diabetes, malaria, jaundice, stomach disorders, coughs, piles, amebic stool, gastritis, arthritis, and cysts, as well as to promote blood purification. However, these vegetables are growing in wild conditions and have very good nutritional and medicinal properties; they need to be domesticated by proper techniques providing agro climatic practices.

INTRODUCTION

Agriculture plays vital role in the Indian financial system. About 70% of the rural population depends upon agriculture. In India there is long growing season, wide variation in climatic conditions, fertile soil and plain areas (Kekane, 2013). It includes crop plants and the wild edible plants. Wild edible plants (WEPs) are the species those are neither cultivated nor domesticated but growing wild and are however edible (Beluhan and Ranogajec, 2010).

Different wild edible plants have played a significant role in all geographical regions of world throughout human history (Sekeroglu *et al.*, 2006). Poor communities throughout the world are dependent on these wild plants for their food, nutrition, clothing, shelter subsistence needs and improving rural livelihoods as well (Sundriyal *et al.*, 2003; Mishra *et al.*, 2008; Tiwari *et al.*, 2010; Badhani *et al.*, 2011). The role of WEPs in ensuring food and nutritional security to the rural or indigenous communities is now widely recognized. According to history we the humans have utilized over 7000 WEPs (Grivetti *et al.*, 2000).

Thane district, located in Maharashtra, India, is known for its rich biodiversity, including the presence of wild vegetables that thrive in its forested and rural areas. These wild vegetables, often found in regions like Murbad and Jawhar, play a significant role in the lives of local tribal communities such as the Thakars and Katkaris (Leakey *et al.*, 2022; Luo *et al.*, 2022).

Presence and Uses of Wild Vegetables:

Wild vegetables in Thane district include tubers, leafy greens, and fruits that grow naturally in forests and uncultivated lands. These plants are not only a source of nutrition but also hold medicinal value. Tribal communities have traditionally relied on these vegetables for sustenance, especially during monsoons when they are abundant. For example, wild tubers and leafy greens are used in traditional recipes, while some plants are believed to have properties that boost immunity.

Cultivation and Collection:

While these vegetables grow naturally, their collection requires knowledge passed down through generations. Tribal women often lead the effort, gathering vegetables like wild yams, *colocasia*, and other greens. However, due to socio-cultural

changes, the younger generation is losing touch with this traditional knowledge. Efforts are being made to promote the conservation and sustainable collection of these vegetables, with some initiatives encouraging their cultivation in wild states5.

Research has documented the nutritional and medicinal properties of wild vegetables in Thane and other regions. Studies have highlighted their role in food security, especially for marginalized communities. For instance, surveys in Thane have identified various wild edible plants, their local names, and their uses, contributing to a growing database of ethnobotanical knowledge.

Researching wild vegetables is crucial for several reasons:

1. Biodiversity Conservation: Understanding and documenting these plants can help preserve them for future generations.
2. Food Security: Wild vegetables are a vital resource for nutrition, especially in rural and tribal areas.
3. Cultural Preservation: Studying their uses helps retain traditional knowledge and practices.
4. Sustainable Agriculture: Exploring their potential for cultivation can offer eco-friendly farming solutions.

This topic not only bridges the gap between traditional knowledge and modern science but also addresses pressing issues like malnutrition, biodiversity loss, and sustainable development.

Material and Method:

Study Area Thane

Thane District forms a part of North Konkan Region. This lies between the Sahyadri hills in the east and the Arabian Sea in the West. It has coastal line of about 113Km. Annual rainfall is more than 25000 mm. This district is a home land of various tribal community followed by different indigenous ethnic groups and subgroups. More than 1.5 million people living here are tribal such as Varali, Kokana, Mahadev Kohli, etc. of which Varali tribe are well known for their paintings throughout the world. Geographically Thane district cover an area 9,33,700 hectares of which 3,30,300 hectare covered with various common and endangered plant species including wild edible plants. These wild vegetables are not only a part of the local cuisine but also play a significant role in the traditional medicine (Stangeland, 2009; Aregheore, 2012) and cultural practices of the communities in Thane. They are often foraged during the monsoon season and are known for their nutritional and medicinal benefits.

Information about wild edible plants in Maharashtra was sourced from various research articles, books, and reviews found on Springer, Scopus, and Google Scholar. Due to the limited number of available papers, a specific time frame was not set for the literature search on wild edible plants in Maharashtra. Additionally, the Plant List website was utilized to verify synonyms, updated binomial names, and the authority of the reported wild edible plants. The plant materials were taxonomically identified and authenticated with the help of The Flora of Presidency of Bombay by T. Cooke (1958) Vol. I, II & III.

The list of wild vegetable species taken for study with their photographs is given below respectively. **(Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig. 5.)**

Hibiscus sabdariffa, Cassia tora, Amaranthus cruentus (Red leaves), *Amaranthus viridis* (Green leaves) and *Holarrhena* sp.

Fig. 1- *Hibiscus sabdariffa*

Fig. 2- *Cassia tora*

Fig. 3- *Amaranthus cruentus*

Fig. 4- *Amaranthus viridis*

Fig. 5- *Holarrhena sp.*

Data Collection & Analysis:

Since April 2022 till the date survey is being carried out. Initial stage of survey i.e collection of information related to wild edible plants and fruits is been noted from the various sources like local markets, retail fruits-vegetable seller, some local peoples and the tribal peoples living the areas around (i.e. Ambarnath, Belapur, Ulhasnagar, Kalyan and Shahapur). Various surveys were been carried out with the information noted down and proper entries of plants was done. Identification was completed (Cooke, 1967; Singh *et al.*, 2001).

Fresh five different selected wild vegetable plants such as *Hibiscus sabdariffa, Cassia tora, Amaranthus cruentus* (Red leaves), *Amaranthus viridis* (Green leaves), *Holarrhena* spp **(Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig. 5.)**. Plants along with leaves and stem were collected from different regions like Belapur, Dombivli, Thane local market, Ambarnath and Kalyan regions of Thane District. The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight polythene bags with proper labeling for future use.

Ultra-Sonication Method of extraction:

This method uses ultrasound waves to enhance the extraction of compounds from plant material into a solvent. (Freitas de Oliveira C. *et. al.*, 2016). Here's a detailed explanation of each step:

Sample Preparation: Plant Material: 10 grams of dried and powdered plant material is recommended. Drying is crucial to remove water, which can interfere with extraction and promote degradation of some compounds. Powdering increases the surface area exposed to the solvent, improving extraction efficiency. The specific plant part used (stem, leaves, bark, etc.) are used specified and consistent for comparative studies.

Solvent:

100 ml Water (Aq. Sol.) and methanol (Altemimi *et al.*, 2016; Gimbun *et al.*, Jakobe *et al.*, 2015 and Kim *et al.*, 2019) is used in this case. Water and Methanol are common solvent for extracting a wide range of plant compounds, including phenolics, alkaloids and flavonoids. The choice of solvent depends on the target compounds. Other suitable solvents include ethanol, water, acetone or mixtures of these. The solvent's purity is important (e.g., analytical grade). The ratio of plant material to solvent (1:10 w/v in this case) can be optimized depending on the plant material and target compounds.

Ultra-Sonication:

Conical Flask: The mixture was placed in a conical flask. This is a standard laboratory vessel, but other vessels suitable for sonication can be used.

Bath Ultra-Sonicator: The flask is placed in a bath ultra-sonicator. These devices generate ultrasound waves that are transmitted through the water bath to the sample.

Sonication Time: 30 minutes is the duration of sonication. This time can be optimized. Too short a time may result in incomplete extraction, while too long can lead to degradation of some compounds due to heat generated by the ultrasound (Buddin *et al.*, 2018; M. Fuad & M. Don, 2016; Tian *et al.*, 2013; Yang *et al.*, 2017).

Amplitude/Frequency: The sonicator is set at working amplitude of 60 Hz. (Gonzalez-Centeno *et al.*, 2014). This seems unusually low. Typical ultrasonic baths operate in the kHz range (e.g., 20-100 kHz). 60 kHz is more likely the intended setting. The amplitude (or power) of the sonication is a crucial parameter. Higher amplitudes generally lead to better extraction. So, the optimized amplitude and frequency are used for the extraction (Zhou *et al.*, 2017).

Mechanism: Ultrasound creates cavitation (the formation and collapse of bubbles) in the solvent. This cavitation disrupts cell walls, releasing the compounds of interest into the solvent. It also enhances the diffusion of the solvent into the plant material (Ofori-Boateng & Lee, 2013).

Filtration:

Whatman No. 1 Filter Paper: After sonication, the extract is filtered through Whatman No. 1 filter paper. This removes solid plant material from the liquid extract. Other filter papers with different pore sizes can be used depending on the fineness of the plant material.

Centrifugation:

Centrifuge: The filtrate (the liquid that passed through the filter paper) is centrifuged at 7000rpm for 15 minutes. This further removes any fine particles that might have passed through the filter paper, resulting in a clearer supernatant.

Supernatant: The supernatant (the liquid at the top after centrifugation) is the extracted solution containing the desired compounds. This is then typically used for further analysis or processing.

The analysis of both aqueous (water-based) and methanolic (alcohol-based) extracts was performed using a sophisticated instrument called the Multiskan Sky High series 1530-00496C spectrophotometer. This device measures how much light a sample absorbs at different wavelengths, which can help identify and quantify various compounds.

- **Phenolics**: These are a group of chemical substances found in plants, known for their antioxidant properties. They were measured at a wavelength of 560nm.
- **Flavonoids**: These are another group of plant compounds that have various health benefits, including antioxidant effects. They were measured at a wavelength of 367nm.
- **Antioxidants**: These are substances that can prevent or slow damage to cells caused by free radicals. They were measured at a wavelength of 517nm.

By comparing the results obtained at these specific wavelengths for both aqueous and methanolic extracts, we could observe differences in the concentration and effectiveness of the phenolics, flavonoids, and antioxidants in each extract. This comparative study helps in understanding which type of extract may be more beneficial or potent for specific applications.

Result and Discussion:

The analysis that was conducted on five different plant samples: *Hibiscus sabdariffa, Cassia tora, Amaranthus cruentus* (Red leaves), *Amaranthus viridis* (Green leaves), *Holarrhena* spp. For each plant, both aqueous and methanolic extracts were prepared and analyzed for their Phenolic, Flavonoids and Antioxidant Properties (DPPH) content. Five wild edible plants tested are summarized in the **Table No. 1, Table No. 2 and Table No. 3.** given below. The results revealed the presence of medically active compounds in the five plants studied. From the table, it could be seen that, Phenolic, Flavonoids and Antioxidant Properties (DPPH) were present in all the plants. Flavonoids were absent only from *Holarrhena* sp.

Phenolic content analysis:

The phytochemical analysis focused on quantifying the phenolic content in various plants extracts using a spectrophotometric method. The content of phenolics was calculated from the regression equation of the calibration curve (R^2 = 0.998, y = 0.002x + 0.069), the results are presented in micrograms per milliliter (µg/ml) and micrograms per 100 milligrams (µg/100mg) of dry plant material.

Among the aqueous extracts, *Holarrhena* sp. exhibited the highest phenolic content with a concentration of 111.6508 µg/ml and 55.8254 µg/100mg. *Hibiscus sabdariffa* showed moderate phenolic levels at 51.1746 µg/ml and 25.5873 µg/100mg. *Cassia tora* and *Amaranthus* spp. (both red and green) displayed lower phenolic content compared to *Holarrhena* sp. and *Hibiscus sabdariffa*. In the methanolic extracts, *Holarrhena* sp. continued to show the highest phenolic content, reaching 100.6984 µg/ml and 50.34921 µg/100mg. *Hibiscus sabdariffa* maintained moderate levels at 59.90476 µg/ml and 29.95238 µg/100mg. *Cassia tora* and *Amaranthus* sp. (both red and green) exhibited lower phenolic content compared to *Holarrhena* sp. and *Hibiscus sabdariffa* **(Table No. 1)**.

Flavonoid content analysis:

The results of a flavonoid phytochemical analysis conducted on various plant extracts. The analysis was performed using both aqueous (Aq) and methanolic extraction methods. The content of phenolics was calculated from the regression equation of the calibration curve (R^2 = 0.991, y = 0.008x + 0.595), the results are presented in terms of average flavonoid content (in µg/ml and µg/100mg) for each plant sample.

Hibiscus sabdariffa: This plant showed the highest flavonoid content in the aqueous extract, with an average of 35.835 µg/ml and *Holarrhena* sp. showed a negative flavonoid content of -27.378 µg/ml. *Hibiscus sabdariffa* showed the highest Flavonoid content, with an average of 119.691 µg/ml amongst the all five plants. And *Holarrhena* sp. had an lowest Flavonoid content of 3.305 µg/ml **(Table No. 2)**.

Result of Antioxidant Properties (% inhibition):

The phytochemical analysis was conducted to investigate the presence of various bioactive compounds in different plant extracts using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. This assay measures the ability of the extracts to scavenge free radicals, indicating their antioxidant potential. The results showed varying levels of antioxidant activity among the tested plant extracts.

Among the aqueous extracts, *Hibiscus sabdariffa* exhibited the highest antioxidant activity with a DPPH inhibition of 21.188%, suggesting the presence of potent antioxidant compounds. *Cassia tora* also showed moderate antioxidant activity with a DPPH inhibition value of 12.956%. *Amaranthus* sp. (red) and *Holarrhena* sp. exhibited moderate to low antioxidant activity. In the methanolic extracts, *Hibiscus sabdariffa* maintained its strong antioxidant activity with a DPPH inhibition of 23.372%. *Cassia tora* also showed moderate antioxidant activity with a DPPH inhibition value of 17.609%. *Amaranthus* sp. (red) and *Holarrhena* sp. exhibited moderate to low antioxidant activity **(Table No. 3)**.

These results suggest that *Hibiscus sabdariffa* and *Cassia tora* extracts may be potential sources of natural antioxidants. The higher antioxidant activity in the methanolic extracts compared to the aqueous extracts for some plants indicates that certain compounds may be more effectively extracted using methanol as a solvent.

Table No. 1. Total Phenolic Content in plant Samples:

	Sample No	1	2	3	Average	ug/ ml	ug/100mg
Aq. Sol	*Hibiscus sadbariffa*	0.17	0.179	0.181	0.176667	51.1746	25.5873

	Cassia tora	0.139	0.143	0.141	0.141	34.19048	**17.09524**
	Amaranthus viridis (Green leaves)	0.208	0.211	0.219	0.212667	68.31746	34.15873
	Amaranthus cruentus (Red leaves)	0.179	0.183	0.182	0.181333	53.39683	26.69841
	Holarrhena sp.	0.297	0.306	0.308	0.303667	111.6508	**55.8254**
Methanolic	*Hibiscus sadbariffa*	0.19	0.198	0.197	0.195	59.90476	29.95238
	Cassia tora	0.152	0.162	0.166	0.16	43.2381	21.61905
	Amaranthus cruentus (Red leaves)	0.137	0.142	0.139	0.139333	33.39683	**16.69841**
	Amaranthus viridis (Green leaves)	0.133	0.142	0.147	0.140667	34.03175	17.01587
	Holarrhena sp.	0.268	0.292	0.282	0.280667	100.6984	**50.34921**

Table No. 2. Total Flavonoid Content in plant Samples:

	Sample No	**1**	**2**	**3**	**Average**	**ug/ml**	**ug/100mg**
Aq	*Hibiscus sadbariffa*	0.883	0.883	0.914	0.893333	35.83534	17.91767
	Cassia tora	0.649	0.65	0.658	0.652333	6.799197	3.399598
	Amaranthus cruentus (Red leaves)	1.154	1.177	1.199	1.176667	69.97189	**34.98594**
	Amaranthus viridis (Green leaves)	0.773	0.781	0.803	0.785667	22.86345	11.43173
	Holarrhena sp.	0.369	0.365	0.372	0.368667	-27.3775	**-13.6888**
Methanolic	*Hibiscus sadbariffa*	1.467	1.635	1.666	1.589333	119.6908	**59.84538**
	Cassia tora	1.257	1.356	1.351	1.321333	87.40161	43.7008
	Amaranthus cruentus (Red leaves)	1.179	1.279	1.296	1.251333	78.96787	39.48394
	Amaranthus viridis (Green leaves)	0.935	1.024	1.021	0.993333	47.88353	23.94177
	Holarrhena sp.	0.615	0.623	0.632	0.623333	3.305221	**1.65261**

Table No. 3. Total Antioxidant Properties (% inhibition) in plant Samples:

Aq	**1**	**2**	**3**	**Average**	**ug/ml**	**% inhibition**
Hibiscus sadbariffa	0.856	0.734	0.612	0.734	0.931	21.188
Cassia tora	0.833	0.83	0.769	0.811	0.931	**12.956**
Amaranthus cruentus (Red leaves)	0.808	0.727	0.784	0.773	0.931	17.001
Amaranthus viridis (Green leaves)	0.816	0.764	0.847	0.809	0.931	13.135
Holarrhena sp.	0.537	0.478	0.591	0.535	0.931	**42.520**
MeoH						
Hibiscus sadbariffa	0.653	0.703	0.785	0.714	0.931	23.372
Cassia tora	0.755	0.817	0.73	0.767	0.931	17.609
Amaranthus cruentus (Red leaves)	0.811	0.771	0.782	0.788	0.931	15.390
Amaranthus viridis (Green leaves)	0.835	0.835	0.871	0.847	0.931	**9.055**
Holarrhena sp.	0.493	0.547	0.557	0.532	0.931	**42.842**
Control	**0.941**	**0.902**	**0.951**	**0.931**	**0.931**	**0.000**

All these plants some of them are very essential for nutrition's purpose and enhancement of health. Among these plants species most of the plants are used for medicine purposes, like, Diabetics, Malaria, Jaundice, Stomach disorder, Cough, Piles, Amebic stool, Gastritis, Arthritis, blood purification, Cyst, Worm, etc. Different dishes prepared by them having medicinal properties (Nikalje *et al.*, 2018).

CONCLUSION

We can conclude that exploring and documentation of wild edible plants is more important. With that enhancing the knowledge of tribal is needed. Least use of hybrid varieties, WEPs should be increase and it should be commercialized. Overall we can say that these findings indicate that *Holarrhena* sp. is a rich source of phenolic compounds, followed by *Hibiscus sabdariffa*. The higher phenolic content observed in the methanolic extracts compared to the aqueous extracts. For some plants we can suggest that methanol may be a more efficient solvent for extracting these compounds.

Generally, the methanolic extracts showed higher flavonoid content compared to the aqueous extracts, suggesting that methanol is a more effective solvent for extracting flavonoids from these plant samples. The flavonoid content varied significantly among the different plant species, indicating that the presence and concentration of flavonoids are influenced by the plant's genetic makeup and chemical composition. The antioxidant activity was assessed using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, which measures the ability of the extracts to scavenge free radicals. The results suggest that *Hibiscus sabdariffa* and *Cassia tora* extracts may have potential health benefits due to their antioxidant properties.

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