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Food, Ethnomedicinal and Pharmacological Evaluation of *Streblus Asper* Lour. (Fruits): A Minor Nutraceutical of India

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ABSTRACT

A field survey on food, ethnomedicinal and phytochemical investigation was carried out followed by toxicity to *Artemia salina* using three extracts of *Streblus asper* fruit, a minor wild edible fruit of Odisha, followed by thin layer chromatography (TLC) analysis. Results revealed that fruit extracts have diverse secondary metabolites, and methanol extract showed the highest toxicity to *A. salina*. The aqueous, methanol, and ethanol extracts of S. asper fruits contain tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. The aqueous, methanol, and ethanol extracts of *S. asper* fruits exhibit cytotoxicity against brine shrimp nauplii, with death rates ranging from 60 to 100% at concentrations of 25–125 mg/mL. The aqueous extract exhibited the highest cytotoxicity at a concentration of 100 mg/mL, resulting in a 100% death rate. Methanol extract shows the highest cytotoxicity at 125 mg/mL (100% death rate). Ethanol extract shows the highest cytotoxicity at 100 mg/mL (100% death rate). Positive control (Vincristine sulfate) and negative control (3.5% saline) showed expected results. Using the TLC analysis showed clear spots for the aqueous and methanol extracts. Rf values for aqueous extract spots: 0.58, 0.70, and 0.88. Rf values for methanol extract spots: 0.81 and 0.60. These findings suggest that *S. asper* fruit extracts possess bioactive compounds with potential, such as antimicrobial and antioxidant properties, which are attributed to phenolic compounds and flavonoids. The brine shrimp nauplii assay indicates the cytotoxic effects of these fruit extracts on cancer cells. There are potential applications in the pharmaceutical and medicinal industries.

Key words: *Artemia salina*, toxicity, future nutraceutical, secondary metabolites

INTRODUCTION

Odisha, rich in biodiversity, have a plethora of minor nutraceutical fruits that often remain hidden gems in the kingdom of wellness and nutrition. *Streblus asper* Lour., commonly known as the toothbrush tree, which comes under minor nutraceutical fruits of Odisha, is a small to medium sized evergreen tree (Kumar et al., 2012). It is native to various parts of Asia, including India, Sri Lanka, and Southeast Asia, belonging to Moraceae family (Kumar et al., 2022). These minor nutraceutical fruits of Odisha not only intriguing the taste buds also serve as invaluable sources of health-enhancing elements deeply originated in local culture and tradition (Rawat et al., 2018). Several studies on minor wild edible and nutraceutical fruits have been carried out worldwide (Maroyi, 2014) and in comparison, to modern crops, these studies have emphasized their nutritional, ethnomedicinal significance, cultivation but less work has been done on their value additions. With these objectives, an attempt has been made to conduct experimental investigations through phytochemical screening followed by cytotoxicity and TLC analysis. This plant is characterized by its rough, greyish-brown bark and elliptical leaves with serrated edges. This species is found in tropical countries, such as India, Sri Lanka, Malaysia, the Philippines and Thailand (Singh, 2015). Various parts of this plant are used in Ayurveda and other folk medicines for the treatment of different ailments such as filariasis, leprosy, toothache, diarrhea, dysentery and cancer (Rastogi et al., 2006). Research carried out using different in vitro and in vivo techniques of biological evaluation support most of these claims. It is used traditionally in leprosy, piles, diarrhea, dysentery, elephantiasis and cancer (Senthilkumar et al., 2006). It is a rigid shrub or gnarled tree; branchlets tomentose or pubescent. The leaves are 2–4 inch, rigid, elliptic, rhomboid, ovate or obovate, irregularly toothed; petiole 1/12 inch. The male heads globose, solitary or 2-nate, sometimes androgynous; peduncle short scabrid, flowers minute. The female flowers have longer peduncled (Tara, 2011). Fruit pisiform; perianth yellow. It is found in the drier parts of India, from Rohilkund, eastward and southwards to Travancore, Penang and the Andaman Islands (Sivamaruthi et al., 2022). In this study, secondary metabolites, cytotoxicity to

Artemia salina, and TLC analysis along with photographs are presented.

METHODOLOGY

Field Survey

A field survey was carried out during 2022–2024 in Mayurbhanj, Sundargarh and coastal parts of Odisha state for collecting food and medicinal uses through interaction with local communities (Kumar et al., 2012).

Collection of Fruits and Preparation of Extracts

The fruits of *S. asper* (APRFH 085) were collected from the roadsides nearby Khandagiri and Udayagiri of Khordha district, Odisha (Figure 1) in February 2024. The plant was identified by correspondence author (Dr. Sanjeet Kumar). The Soxhlet method of extraction using four solvents (n-hexane, aqueous, methanol, ethanol) was carried out for phytochemical analysis (Das et al., 2019). Detection of nine secondary metabolites were carried out using standard methods (Misra et al., 2012; Kumar et al., 2017; Devi et al., 2023; Parte et al., 2023; Devi et al., 2024). A voucher specimen (APRFH 085) is deposited to the Herbarium unit, Biodiversity and Conservation Lab., APRF, Odisha, India (Figure 2).

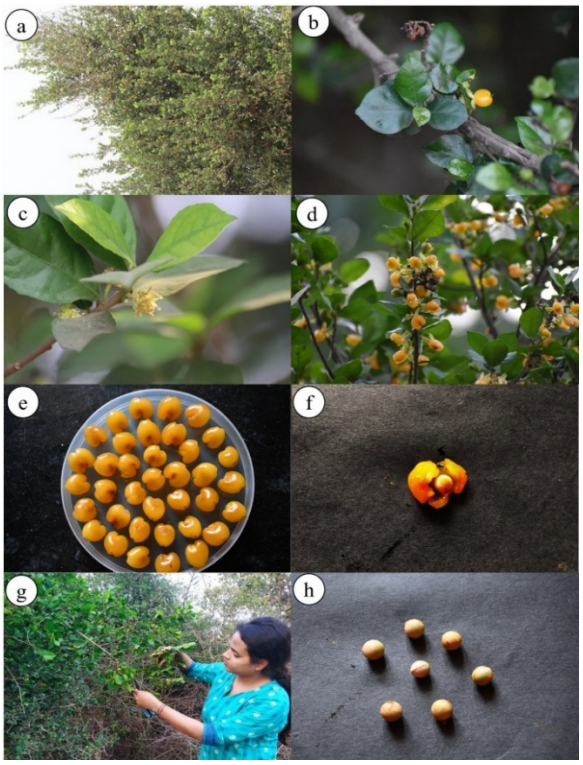

Fig. 1. Plant parts of *S. asper* (**a**) whole plant, (**b**) stem with leaves, (**c**) flower, (**d**) fruits, (**e**) Collected fruits, (**f**) fruit pulp, (**g**) collection of fruits in the field and (**h**) seeds.

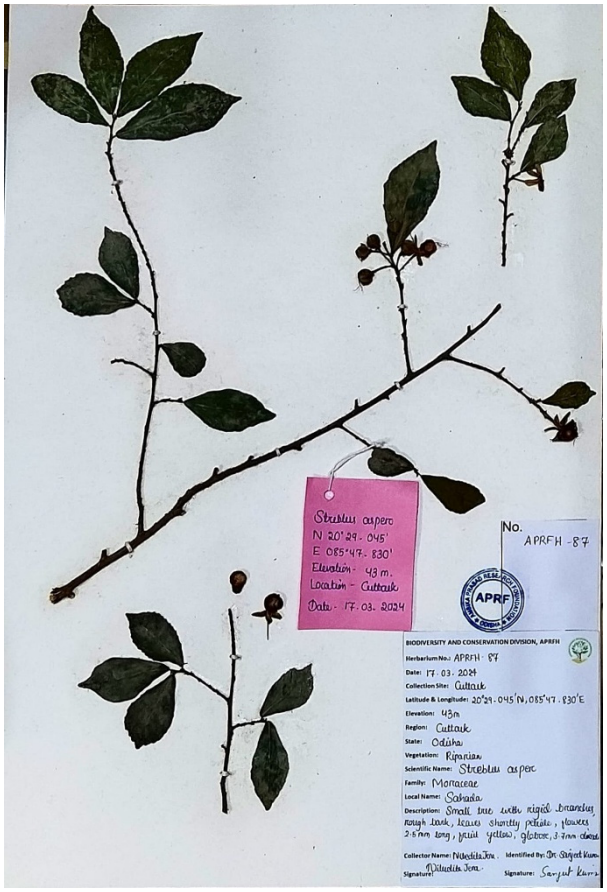

Fig. 2. Herbarium specimen of *S. asper*.

Cytotoxicity Analysis

The cytotoxicity test was initiated by hatching Brine shrimp (*Artemia salina*) cysts (MAF Peqon Artemia). To optimize hatching conditions, standardization was performed at 2.0, 3.5, and 5.0% saline concentrations (Kumar et al., 2012; Dash et al., 2022). The cysts were incubated in 3.5% saline water with proper aeration, room temperature (28–35 °C), and light for 48 h. Successful hatching was observed within 24–48 h, depending on cyst quality, aeration, and light. To evaluate cytotoxicity, different concentrations (25–125 mg/mL) of *S. asper* fruit extracts were prepared in 3.5% saline water. The extracts were dissolved in 1% DMSO (Dimethyl Sulfoxide). Ten nauplii were introduced into five test tubes containing the extracts. Positive and negative controls were prepared using vincristine sulphate (5 mg/mL) and 3.5% saline water respectively. The number of surviving nauplii was counted, and the percentage of death was analyzed (Nayak et al., 2021).

TLC Analysis

TLC plates were prepared on 6 cm glass slides coated with silica gel powder (SILICA GEL G, CAS 112926-00-8, Spectrochem Pvt. Ltd., Mumbai, Maharashtra, India). The slides were cleaned with a clinical laboratory detergent, dried, and then wiped with ethyl acetate to remove surface adherents. A slurry

was prepared by mixing 3 g of silica gel with 20 mL of distilled water, stirring constantly. The slurry was then poured onto the slides, which were left undisturbed until the silica layer dried (Kumar et al., 2013). The slides were activated at 50 °C for 20 minutes before use (Mishra and Bhatnagar, 2024). Methanol: chloroform (3:2; Volume: Volume) was used as the mobile phase. After running the TLC, spots were visualized, and the retention factor (Rf) values were recorded (Kumar and Jena, 2014).

RESULTS AND DISCUSSION

Field survey revealed that fruits of *Streblus asper* are edible and used occasionally. It comes under minor wild edible fruit of Odisha state. In food aspects, twigs of contain a rennin-like milk-clotting protease, offering potential applications in cheese production (Senthilkumar et al., 2006). Additionally, the leaves of *S. asper* are a nutritious feed supplement for cattle, providing greater benefits than green grass. Furthermore, the fruit juice of *S. asper* has been reported to have a cooling effect when administered orally to ruminant animals, such as cows and goats, suggesting its potential use as a natural refrigerant (Aziz et al., 2018). In present study, the ecological services are observed and found that fruits are consumed by birds and most of the tribal people in other parts of the state. This fruit is not much highlighted for its food and medicinal values. Minor fruits grown in India, wildly or cultivated locally and for home garden but currently having low export and local market values due to increase urbanization followed by several factors. During field survey it was noticed that leaf is used to cure fever whereas root decoction is employed to treat ulcers and sinuses, while the latex serves as an antiseptic and astringent agent, effective in treating sore heels and glandular swellings. Additionally, the seeds are used to treat piles and diarrhea, and the twigs are used as toothbrushes, which can be chewed to clean teeth and cure pyorrhoea (Sivamaruthi et al., 2022). Therefore, the phytochemical analysis, cytotoxicity to *Artemia salina* and TLC analysis of *S. asper* fruits were carried out. It was observed that the n-hexane, aqueous, methanol and ethanol all these fruits extracts showed several secondary metabolites (Table 1; Figure 3). It was noticed that tannin, flavonoids, phenols and alkaloids are detected in all three extracts. These secondary metabolites having reported antimicrobial, antioxidant (Kumar et al., 2012), antifilarial, anticancer (Kumar et al., 2022), anti-allergic (Senthilkumar et al., 2006) and antidepressant activity (Singh, 2015) and other pharmacological potentials. The presence of secondary metabolites with phenolic groups in the fruit extracts of *S. asper* led us to investigate their potential cytotoxic activity.

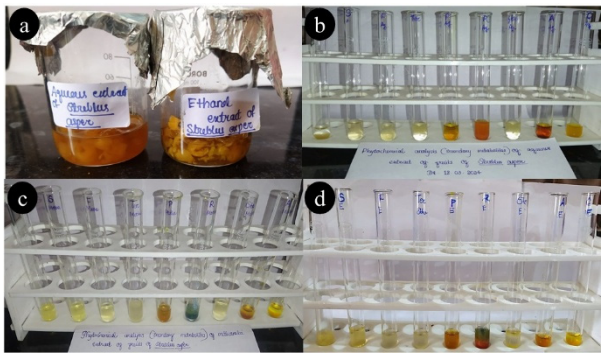

Fig. 3. Phytochemical analysis of *S. asper,* (**a**) maceration, (**b**) aqueous extract, (**c**) methanolic extract, (**d**) ethanolic extract.

Table 1. Qualitative phytochemical analysis of *S. asper* fruit extracts.

Bioactive compounds	Solvents			
	n-hexane	Aqueous	Methanol	Ethanol
Tannin	−ve	+ve	+ve	+ve
Saponin	−ve	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve
Terpenoids	−ve	−ve	−ve	−ve
Phenolic compounds	−ve	+ve	+ve	+ve
Reducing sugars	−ve	+ve	+ve	+ve
Steroids	−ve	−ve	−ve	−ve
Alkaloids	−ve	+ve	+ve	+ve
Carbonyl compounds	−ve	−ve	−ve	−ve

To this end, a cytotoxicity test was conducted using *Artemia salina* nauplii. The results showed that the methanol extract of the fruits exhibited the highest activity at a concentration of 25 mg/mL after 3 h, followed by ethanol, aqueous and n-hexane extracts (Table 2; Figure 4; Figure 5). These findings suggest that the fruit extracts of *S. asper* have potential as effective anticancer agents.

Table 2. Cytotoxicity analysis of *S. asper* fruit extracts.

Extracts	Concentration (in mg/mL)	Initial number of nauplii	Number of deaths of nauplii (after in hours)				Death rates (after 3 h) (%)
			1	2	3	4	
Aqueous	25	10	2	4	6	10	60
	50	10	3	6	6	10	60
	75	10	4	7	7	10	70
	100	10	5	5	8	10	80
	125	10	5	8	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Methanol	25	10	5	6	9	10	90
	50	10	5	5	9	10	90
	75	10	6	6	9	10	90
	100	10	6	7	10	10	100
	125	10	6	8	10	10	100
	3.5% Saline	10	0	0	0	0	0

	Vincristine sulphate	10	10 10 10 10	100
Ethanol	25	10	5 7 7 10	70
	50	10	5 5 8 10	80
	75	10	6 8 9 10	90
	100	10	7 7 9 10	90
	125	10	7 9 10 10	100
	3.5% Saline	10	0 0 0 0	0
	Vincristine sulphate	10	10 10 10 10	100
n-hexane	25	10	0 0 0 0	0
	50	10	0 0 0 0	0
	75	10	0 0 0 0	0
	100	10	0 0 0 2	20
	125	10	0 0 0 4	40
	3.5% Saline	10	0 0 0 0	0
	Vincristine sulphate	10	10 10 10 10	100

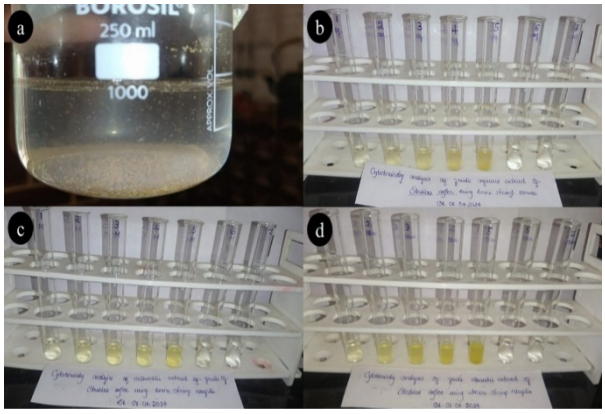

Fig. 4. Toxicity analysis of *S. asper* fruit extracts (**a**) Brine shrimp larvae, of the different solvents; (**b**) aqueous, (**c**) methanolic and (**d**) ethanolic.

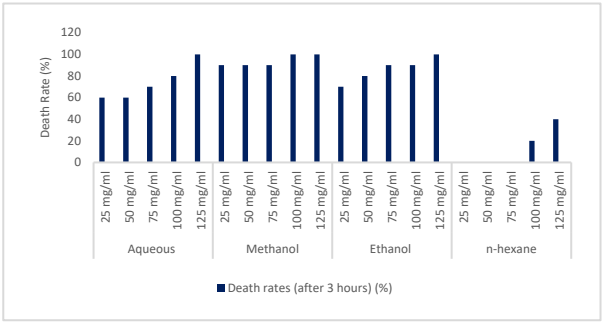

Fig. 5. Cytotoxicity of different fruits extracts against *Artemia salina*.

To further elucidate the bioactive compounds responsible for the observed cytotoxicity, TLC analysis was performed on the three most active extracts (aqueous, methanol, ethanol and n-hexane). The results revealed that the aqueous extract exhibited the highest number of spots (3), followed by methanol and ethanol extracts (Table 3). In n-hexane extract, no spots are observed.

Table 3. TLC analysis of *S. asper* fruit extracts.

Solvents	Extracts	Rf value of spots		
		S_1	S_2	S_3
Chloroform: Methanol (2:3)	Aqueous	0.58	0.70	0.88
	Methanol	0.81	0.60	-
	Ethanol	-	-	-
	n-hexane	-	-	-

Other researchers also documented its secondary metabolites and pharmacological potentials. In 2006, Rastogi and his team reported that the various chemical classes of compounds have been isolated from the roots of *S. asper* like cardiac glycosides, β-sitosterol, lupnol and vijaloside (Rastogi et al., 2006). In 2022, Kumar and his co-workers reported a lot more glycosides being cardiac the root bark of *S. asper* (Kumar et al., 2022). In 2001, Wongkham and his team reported that the ethanol extract of *S. asper* leaves possessed a selective bactericidal activity towards *Streptococcus*, especially to *S. mutans* (Wongkham et al., 2001). In 2004, Phutdhawong and his co-workers reported that the volatile oil from leaves of *S. asper* showed significant anticancer activity (Phutdhawong et al., 2004). In 2011, Tara studied the parenteral administration of extracts of aerial parts of *S. asper* indicated good analgesic activity (Tara, 2011). In 2018, Rawat and his co-workers reported that the methanolic extract of *S. asper* is rich source of glycosides, fatty acids, and phytosterol. The chloroform fraction is highly active on hepatoma cancer cell line whereas methanolic and, and hexane fractions have highly cytotoxic potency against leukaemia cancer cell line. Also, all the extracts of *S. asper* found potential anticancer activity against lung cancer cell line (Rawat et al., 2018). In 2022, Sivamaruthi and his team reviewed that the ethanolic extract of *S. asper* exhibited antimicrobial activity against *C. albicans* in human buccal epithelial cells (Sivamaruthi et al., 2022). The above findings revealed that there is a need of more exploration to experimental works on *S. asper*. Future research directions for *S. asper* include investigating its pharmacological potentials, such as anticancer, anti-inflammatory, and antimicrobial activities. Further studies can also focus on the isolation and characterization of bioactive compounds responsible for its medicinal properties. Additionally, research can explore the plant's potential in sustainable agriculture, such as its use as a natural pesticide or fertilizer. Investigating the plant's ecological role and its impact on biodiversity can also provide valuable insights. There are several research gaps that need to be addressed to fully understand the potential of *S. asper*. One major gap is the lack of comprehensive phytochemical analysis of the plant's different parts, including its roots, stems, and leaves. Another gap is the limited understanding of the plant's traditional uses and cultural significance, particularly among indigenous communities. Furthermore, there is a need for more in-depth studies on the plant's pharmacological and toxicological properties to

ensure its safe use. Addressing these gaps can provide a more complete understanding of the potential of this wild edible fruiting plant and promote its sustainable use.

CONCLUSIONS

The current study concludes that tribal areas of Odisha consume the fruits of *S. asper*, which are wild edible fruits with diverse secondary metabolites and potential cytotoxicity. TLC analysis also showed the presence of different active constituents. Therefore, fruits can be good nutritionally. Additional research is required to explore the nutritional and medicinal aspects of fruits. Also, there is a need for value addition at the regional level for sustainable utilization and conservation of these wild edible fruits in Odisha state.

CONFLICTS Of INTEREST

The authors declare no conflict of interest.

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