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A comprehensive review of the anticancer potential and other pharmaceutical effects of *Hemidesmus indicus* R. Br.

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Abstract

Ayurveda which is a traditional system of medicine used in various methods to promote health and well-being. Its primary goals are improving health, preventing disease and treating existing conditions. In developing countries, there is a growing demand for herbal medicines not only because they are affordable but also due to their cultural acceptance, compatibility with the human body and minimal side effects. *Hemidesmus indicus* R. Br., also known as Indian Sarsaparilla, is a versatile medicinal herb that has long played a significant role in an Indian traditional medicine. Among its many therapeutic uses includes the treatment of diseases like syphilis, diabetes and skin ailments. This plant has a wide range of phytoconstituents, including glycosides, flavonoids, tannins and sterols. Experimental studies conducted on various models have demonstrated a wide range of pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, anticancer, hepatoprotective and immunomodulatory effects, etc. This review is to promote the understanding of the potential of *H. indicus* in complementary and alternative medicine and to provide relevant information to the field of science and the pharmaceutical industry. Overall, *H. indicus* shows significant promise as a therapeutic agent across a range of conditions. Continued research, including clinical trials, is essential to fully understand its mechanisms of action and to validate its efficacy and safety in humans. The traditional uses of *H. indicus*, supported by experimental findings, highlight its potential as a valuable natural remedy in modern medicine.

1. Introduction

India has a very significant legacy of traditional medicine, which is represented by Ayurveda, Siddha, Unani, Sowa rigpa and other classical and folk medical systems (King *et al.*, 1998). The World Health Organization (WHO) found that a substantial portion of the world's population uses traditional medicines as self-administered remedy (Yuan *et al.*, 2016). Homemade therapies for treating various illnesses sometimes rely on the integration of crucial trace elements derived from medicinal plant extracts (Farnsworth *et al.*, 1985). *H. indicus*, an indigenous Ayurvedic herb, has been used since ancient times in traditional medical practices and also as a cooling beverage in various states of India, primarily in the southern regions. Traditional medicine has employed the roots and woody part of the plant to treat a number of illnesses including rheumatism, venereal illnesses, fever and stomach discomfort. It has long been used to treat urinary infections, skin conditions and genital warts. It also functions as an alternate tonic, demulcent and diaphoretic (Desai *et al.*, 2017; Pathan *et al.*, 2018). Several therapeutic herbs that can be used to treat peptic ulcers and lessen their effects in both western and Ayurvedic medicine (Singh *et al.*, 2022).

According to the Environmental Information System (EIS) 2014, *H. indicus* is a medicinal plant that is red listed, implying an urgent need

for the collection and protection of this species (Yazhni *et al.*, 2021). *H. indicus* has narrow, twining or prostrate perennial shrub with thickened nodes on the cylindrical stems and fragrant roots. The leaves are simple, opposite or whorled, short petiole and vary in shape from broadly ovate to oblong-elliptic, linear or linear lanceolate, 3-10 cm long and 0.33-8 cm wide, with an acute, rounded, or truncate base and rounded or emarginated and apiculate apex. They are also leathery and dark green, with petioles that are 0.1 to 0.6 cm long. Small, lobes thick, ovate-oblong flowers have an exterior that is greenish yellow and an inside that is purple (Murty, 2008). There are two varieties of the plant: a black variety known as Krishna Sariva and a white variety called Sariva. In Ayurvedic formulary, *H. indicus* is recognized as the white variety (Pansare *et al.*, 2018).

The terms 'Magra', 'Salsa' and 'Anantamool' are used in Hindi and 'Anantamool' and 'Upalsari' are used in Marathi. Palasugandhi is the name given to it in Telugu, while Indian Sarsaparilla is now it is known in English. Tamil people refer to it as 'Nannari'. These diverse names are a reflection of the great linguistic diversity and cultural importance attached to this particular plant species (Pansare *et al.*, 2018). *H. indicus* is a component in approximately 46 Ayurvedic formulations. Some of the notable Ayurvedic preparations containing *H. indicus* include Dasamoolarishta, Dhanwamtharishta, Balamritham, Saribadyasavam, Anuthaila and so many other (Manjulatha *et al.*, 2014). To get the most bioactive components from the roots, the entire plant system is often removed (Shekhawat and Manokari, 2016). Dried root and bark parts are valued for their tonic, diaphoretic, diuretic and blood purifying properties. In some well-known Ayurvedic formulations, they are used to treat intestinal problems, elephantiasis, fever, hemiplegia, nausea, syphilis and

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vomiting. It is also used as a syrup, infusion or decoction or as a powder. The Paharia community of southern Bihar, root paste is applied on the front head to lower fever. To treat chronic cough and diarrhea in children, a hot infusion of the root bark with milk and honey is administered (Murty, 2008). In order to avoid taking contemporary drugs on their own, the general public is increasingly resorting to herbal remedies (Idris *et al.*, 2021). Overall, people can consume herbs and spices to improve their health and reduce their risk of chronic illness (Rani *et al.*, 2023). This paper explores ethnopharmacology of *H. indicus* along with the investigations done to study.

2. Chemical configurations

Using several *in vitro* and *ex vivo* models, the study evaluated the antioxidant capabilities of the methanolic extract produced from the root bark of *H. indicus*. The same extract was also subjected to preliminary phytochemical screening in order to determine the various chemical groups present and its phytochemical constitute was identified by creating a TLC fingerprint profile. The principal chemical groups found in the methanolic extract, according to the findings, were flavonoids, phenolic and tannins (Ravishankara *et al.*, 2002). To characterize certain trace components in the plant, a separate research project was initiated. Examining numerous trace elements present in varying quantities, which are essential in the creation of

traditional remedies, was made possible by the use of ICP-MS, which is renowned for its effectiveness and speed. This investigation highlights the significant contribution of traditional plants with both trace and major elements in combatting a range of human ailments. The ICP-MS method was used to identify a total of twenty elements in these medicinal plants, including macro and microelements. These substances contained the following elements: Li, Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cs, Ba and Tl. Findings from the study show that *H. indicus* contains trace elements in acceptable amounts, making it appropriate for treating skin conditions. When prescribing herbal medications made from these plant components, such findings can be used as a foundation for setting dose requirements. The following is a list of the trace elements in decreasing order of concentration: Fe, Al, Sr, Mn, Ba, Zn, Rb, Ga, Cu, Cr, Ni, V, As, Co, Se, Li, Cs, Ag, Be and Tl are arranged in order of importance (Balakrishna *et al.*, 2022). The therapeutic efficiency of these treatments is enhanced by the natural abundance of particular trace elements in medicinal plants (Balakrishna *et al.*, 2022). It is crucial to remember that toxicity can result from high trace element concentrations in a plant (Patnaik *et al.*, 2019). This emphasizes how crucial it is to study how medicinal plants affect human health because when people consume herbal medicines, their bodies absorb vital trace components from these plants (Jyothsna *et al.*, 2021; Nagarajan *et al.*, 2001; Gupta *et al.*, 1992). Table 1 briefs about the presence of phytochemicals by conducting various laboratory tests.

Table 1: Different tests for identifying trace compounds (Kumar *et al.*, 2007; Ravishankara *et al.*, 2002)

S.No.	Tests	Compounds	Result	Main content
1	Phoshomolybdc acid reagent	Phenols	Present	Hemidesmin 1, Hemidesmin 2, 2-hydroxy-4-methoxy benzoic acid
2	Braemer's test	Tanins	Present	-
3	Liebermann-Burchardt test	Steroids and terpenoids	Traces of terpenoids	-
4	Dragendorff's reagent with methanolic extract on TLC plate	Alkaloids	Negative result	-
5	Borntrager's test	Anthraquinones	Negative	-
6	Shinoda test	Flavonoids	Present	Rutin

3. Phytochemical distribution in parts of the plant

The root extract of *H. indicus* contains a substance called lupeol acetate, which has been shown to significantly reduce the lethality, bleeding, defibrinogenation and edema brought on by several toxic effects (Meenatchisundaram, 2008). *H. indicus* root includes a unique chemical called lupeol octacosanoate. A HPTLC method has been devised to quantify markers in dry root powder of *H. indicus*. This

procedure can be used for quality control on *H. indicus*. Lupeol is potentially valuable in pharmaceutical or industrial applications. (Darekar *et al.*, 2008; Pathak *et al.*, 2017). Various classes of phytochemicals are present in different parts of plants, each contributing to their unique medicinal and therapeutic properties. Table 2 shows the evident papers studied for the presence of phytochemicals in various parts of the plant. Figure 1 shows the phytochemical presence in parts of the plant.

Table 2: Phytochemical constituents in parts of the plant *H. indicus*

Class of phytochemical /compound	Phytochemical/ compound	Plant part	References
Flavonoids	Rutin	Flower and leaf	Pathak <i>et al.</i> , 2017; Nandy <i>et al.</i> , 2020; Subramanian and Nair, 1968
Glycosides	Hindicusine, hemidesmosides a-c	Stem, root	Sigler <i>et al.</i> , 2000; Das and Devraj, 2006; Zhao <i>et al.</i> , 2013

Terpenoids	Ledol, nerolidol, borneol, lupeol, lupeolacetate, lupeol, dehydrolupanyl-3-acetate	Stem, root	Mishra <i>et al.</i> , 2018
Aromatic aldehydes	2-hydroxy-4-methoxybenzaldehyde, 3-hydroxy-4-methoxybenzaldehyde (isovanillin)	Root	Mishra <i>et al.</i> , 2018
Phytosterols	β -amyirin, β -amyirin acetate	Roots	Mishra <i>et al.</i> , 2018
Lignins	Hemidesmin, hemidesmin-1 and 2(coumarinolignoids)	Roots and leaf	Pansare <i>et al.</i> , 2018

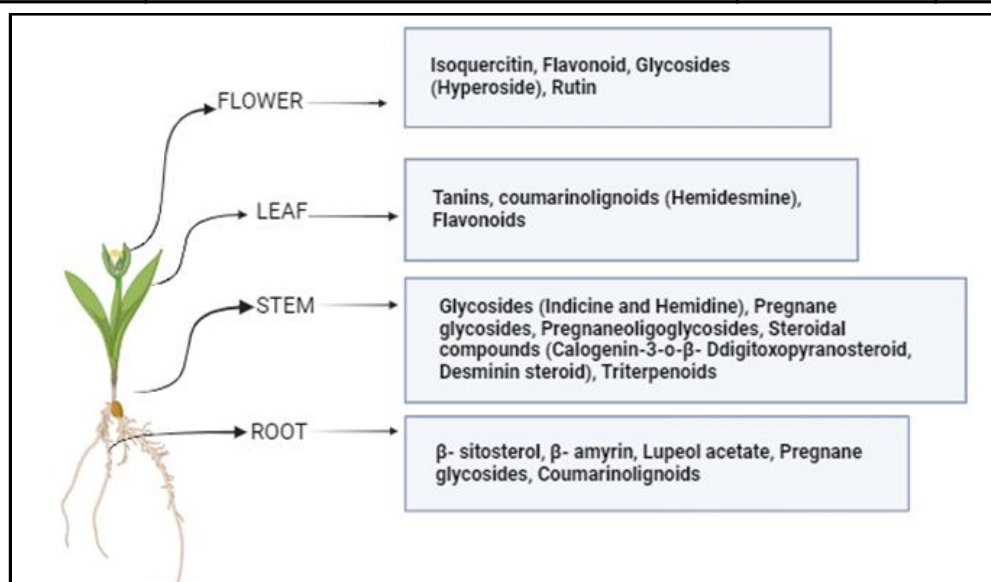


Figure 1: Phytochemical constituents.

4. Experimental studies on pharmacological effects using extracts on various models

Different studies investigated on pharmacological effect of sarsaparilla are mention in Table 3.

Table 3: Different studies on *H. indicus* extracts (*in vivo*, *ex vivo*, *in vitro*)

Types of activity	Model used	Methods of extraction			References
Antisnake venom effects	Albino rat, mouse			Methanolic	Cvitkovic, 1998; Kaur <i>et al.</i> , 2011
Anti hyperlipidemic activity	Rat		Ethanollic		Baroi <i>et al.</i> , 2023
Renoprotective activity	Rat		Ethanollic		Kotnis <i>et al.</i> , 2004
Antiulcerogenic effect	Rat		Ethanollic		Vishali <i>et al.</i> , 2011; Austin <i>et al.</i> , 2003
Anti-inflammatory/analgesic antipyretic	Mouse, rat		Ethanollic	Methanolic	Kharat and Mokat, 2020; Das and Singh, 2013; Lakshman <i>et al.</i> , 2006
Antioxidant/Antithrombotic (<i>in vivo</i>)	Rabbit			Methanolic	Mary <i>et al.</i> , 2003; Mishra <i>et al.</i> , 2018
Rat		Ethanollic			
Antidiarrhoeal properties	Rat	Aqueous			Gayathri and Kannabiran, 2009
Antidiabetic effects	Rat	Aqueous			Gayathri and Kannabiran, 2008
Genotoxic and antigenotoxic activity	Mouse	Aqueous	Ethanollic		Ananthi <i>et al.</i> , 2010; Turrini <i>et al.</i> , 2018

Diuresis	Rat	Aqueous		Gadge and Jalalpure, 2011
Antiasthmatic activity	Rat, mouse		Ethanollic	Bhujbal <i>et al.</i> , 2009; Singh <i>et al.</i> , 2014
Cytotoxicity	HepG2	Aqueous	Ethanollic	Thabrew <i>et al.</i> , 2005; Samarakoon <i>et al.</i> , 2010
MCF7			Methanollic	Statti <i>et al.</i> , 2015; Das and Singh, 2013
Antimicrobial activity	<i>Enterobacteria</i> , <i>Helicocter pylori</i> , <i>S. Typhimurium</i> , Rat colon	Bacteria	Aqueous	Das and Singh, 2013; Nandy <i>et al.</i> , 2020
Acne bacteria		Ethanollic		
Antioxidant/Antithrombotic effects (<i>in vitro</i> and <i>ex vivo</i>)	Platelet			Methanollic
Wound healing effect	Wister rats	Aqueous		Methanollic
				Methanollic
				Mary <i>et al.</i> , 2003; Ravishankara <i>et al.</i> , 2002; Zahin <i>et al.</i> , 2009
				Ganesan <i>et al.</i> , 2012

4.1 Anticarcinogenic

In a research, extract of sarsaparilla applied to mouse skin before being exposed to cumene hydroxide effectively prevented the activation of DNA synthesis and ornithine decarboxylase activity, which are vital biochemical markers for determining a substance's potential to promote tumours. As a result, this extract prevented tumour development in mouse skin, making it a strong chemopreventive agent (Sultana *et al.*, 2003). The effects of a decoction produced from *H. indicus*, *Smilax glabra* and *Nigella sativa* on diethyl nitrosamine (DEN), induced hepatocarcinogenesis in a separate investigation. By examining the quantity, size, staining intensity and density of glutathione S-transferase placental (GST-P) give positive foci in rat livers, researchers determined the propensity substance to cause cancer. As a consequence of the decoction's considerable inhibition of DEN induced GST-P expression in rat livers, the early phases of DEN initiated hepatocarcinogenesis were successfully halted, according to the study's findings. The authors speculated that, although the precise mechanism of action was yet unknown, it may entail cytotoxic effects, antioxidant capabilities, immunomodulation, or the detoxification of carcinogens (Iddamaldeniya *et al.*, 2003). Das and Singh, (2013), examined the chloroform fraction to see, if it could protect against cell death caused by *Salmonella typhimurium* bacteria in human intestinal cell cultures. This fraction contained phytosterols and fatty acids that were obtained from the crude methanollic extract of *H. indicus* roots. Int 407 cells (human intestinal epithelial cell line) infected with *Salmonella typhimurium* showed a tenfold decrease in cytotoxicity when exposed to 100 g/ml of the chloroform fraction as compared to cells infected with the wild type bacterium. Additionally, the chloroform fraction treatment greatly reduced the ability of *S. typhimurium* to attach and invade Int 407 cells by 40-fold and 10-15 fold, respectively (Iddamaldeniya *et al.*, 2006). Additionally, Int 407 cells infected with *S. typhimurium* that had been exposed to chloroform fraction showed virtually normal morphology, including intact mitochondrial cristae (Iddamaldeniya *et al.*, 2003; Iddamaldeniya *et al.*, 2006).

4.2 Antiosteoclastic activity

People with osteoporosis have reduced bone mass and are more likely to sustain a bone fracture after modest stress. Senior citizens are more likely to have the disorder. Osteoclasts are specialised cells that have the rare capacity to degrade bone tissue, which is essential for both the reconstructive mechanisms during bone healing (Vegad *et al.*, 2019).

Asparagus racemosus, *Embllica officinalis*, *H. indicus* and *Rubia cordifolia*, four traditional Ayurvedic herbs, were chosen because of their ethnobotanical uses and probable usefulness in treating disorders associated with bone loss. The study prepared decoctions from dried plant material using conventional methods, which were eventually standardized using high performance liquid chromatography (HPLC) and marker elements particular to each plant species. The total polyphenol and flavonoid content of these decoctions, as well as their ability to scavenge free radicals, were also assessed. Researchers conducted cytotoxicity testing on the mouse monocytic RAW 264.7 cell line to determine safe dosages for further investigation. They were compared to the medicine alendronate for its ability to suppress osteoclast genesis and promote osteoclast death while protecting bone forming mesenchyme stem cells by performing cytotoxicity screening on mouse monocytic RAW 264.7 cells. Researchers carried out a cytotoxicity screening to gauge the potency of plant decoctions and establish their IC₅₀ values. It is significant to note that depending on the particular cell type employed, the reaction to these substances might change. They chose the mouse monocytic cell line RAW 264.7 for their screening. This was selected because to the biochemical similarities between these cells and osteoclast (OC) precursors. This cell line was used to investigate the potential cytotoxicity of various plant decoctions and how they would impact OC precursor like cells. As *H. indicus* displayed the anti osteoclastic activity of bisphosphonates, a high efficacy at low doses and a lack of toxic effects, it emerged as the most viable choice (Granchi *et al.*, 2013; Russel 2011; Di *et al.*, 2014).

4.3 Nephrotoxicity inducing activity and antioxidant effect

Cisplatin is a very efficient chemotherapy agent used to treat a variety of malignancies, although its usage in treatment may be

constrained due to dose dependent nephrotoxicity, which prevents it from reaching its full potential (Cvitkovic, 1998).

In rats with cisplatin induced renal damage, the ethanolic extract from the roots of *H. indicus* was investigated for its kidney protective activities. The extract indicated a dose dependent decrease in high blood urea and serum creatinine levels when given as part of a curative regimen at dosages of 250 and 500 mg/kg. The antioxidant molecules GSH and GST enzymes were also elevated and the lipid peroxidation brought on by cisplatin was inhibited. Overall, our results show that the ethanolic extract of the roots of *H. indicus* has strong nephroprotective efficacy (Kaur *et al.*, 2011).

In rats with ethanol induced kidney injury, the antioxidant properties of *H. indicus* ethanolic root extract (EHI) were examined. Rats fed ethanol for 60 days exhibited elevated levels of oxidative stress markers and kidney related markers. EHI may be able to guard against ethanol induced kidney toxicity since it greatly decreased kidney damage and oxidative stress when given over the final 30 days of the trial (Saravanan and Nalini, 2007).

4.4 Antivenom activity

First ever tests were done to see if *H. indicus* and *Pluchea indica* methanol root extracts might counteract the venom of the snake venom (*Vipera russellii*). Both extracts successfully counteracted the hemorrhagic, coagulant and fatal effects of the venom in albino rats and mice. With polyvalent snake venom antiserum, the extracts did not exhibit any precipitating interactions. The most potent neutralizing potential was shown by the root extract of *H. indicus*. According to this study, several Indian medicinal herbs have the potential to neutralize snake venom, which justifies additional research into the plants' active ingredients (Alam *et al.*, 1996).

Extracts from plants have been tested on Swiss albino mice at three different doses: 200 mg/kg, 400 mg/kg and 600 mg/kg for their capacity to counteract *Naja nigricollis* venom. *H. indicus* root extract at a concentration of 600 mg/kg resulted in 100% survival in experiments with albino mice and *N. nigricollis* (Cobra venom), demonstrating high antivenom potential. This shows that *H. indicus*, which has a strong antivenom effect, may be a useful therapy for snake venom (Saravanan and Nalini, 2007). The venom of *N. nigricollis* contains dangerous proteins, such as potent post synaptic neurotoxins, which, because to their tiny size, move throughout the body very fast. It also has phospholipases, which can stop presynaptic sites from allowing nerve muscle transmission (Fattepur and Gawade, 2007).

4.5 Antiarthritis effect

The components of the hydroalcoholic extract and its three fractions, the ethyl acetate fraction, the chloroform fraction and the residual fraction of the root of *H. indicus* were analysed using preliminary phytochemical analysis and thin layer chromatography. Complete Freund's adjuvant (CFA) was used to create arthritis related rat models. Paw edema, body weight, the arthritic index, the erythrocyte sedimentation rate, the serum rheumatoid factor, the serum C reactive protein, the serum nitrite level and the synovial joint histology were all measured. The positive control was taken to be methotrexate. The results of this study imply that *H. indicus* possesses antiarthritic properties and that these properties may be due to the presence of terpenoids in both the hydroalcoholic extract and the ethyl acetate fraction (Mehta *et al.*, 2012).

4.6 Cardio protective and inotropic effect

In a rat model of congestive heart failure, the study discovered that extracts of *H. indicus*, specifically aqueous and methanolic extracts, significantly decreased the severity of salt water induced micro albuminuria, elevated serum urea and creatinine levels, myocyte diameter and sodium retention, while also increasing serum calcium levels (Das and Devaraj, 2007). In Langendorff perfused rat hearts, the cardioprotective effects of *H. indicus* were investigated (Khandelwal *et al.*, 2010). *H. indicus* demonstrated increased recovery of left ventricular end diastolic pressure and a reduction in ventricular premature beats (VPB). *H. indicus* has been investigated in rat models of Ischemia/Reperfusion (I/R) damage and has demonstrated potential for shielding the heart by (i) avoiding or lessening the effects of contractile dysfunction during ischemia, (ii) lowering the frequency or intensity of arrhythmias caused by reperfusion, (iii) reducing the degree of irreparable tissue damage brought on by I/R injury (Sudarshan and Patel, 2009).

5. Other potential of *H. indicus*

5.1 Phytoextraction of lead

Utilizing the spiked soil samples, the studies were carried out in a lab setting. Under various pH, starting metal concentration, competing metal ions and contact duration conditions, *H. indicus* absorption of lead was observed. When compared to the controls, plants treated with Zn and Cd did not exhibit any overt signs of phytotoxicity or growth inhibition. However, the Ni⁺² and Cr⁺⁶ plants produced a little chlorosis of the leaves. With the exception of Ni, where there was a decrease in lead absorption, *H. indicus* efficiently eliminated lead in the presence of these metal ions. This could be caused by the development of soluble inorganic complexes inside the plant and in the soil, which can greatly decrease the effectiveness of the plant's phytoextraction. According to the findings of the time duration investigations, *H. indicus* can absorb 99.4% of soil lead in three months. The experiments with different soil lead concentrations show that at a very high concentration of 1000 ppm of soil lead, around 65% of lead can be efficiently eliminated. Soil amendments were made using various chemical modifiers to shorten the harvestable period and improve the metal absorption capacity (at higher concentrations) (Sekhar *et al.*, 2005).

5.2 Insect repellent

The root essential oil of *H. indicus* is a new and effective repellent and insecticide against stored product pests, offering an eco-friendly way to reduce crop damage and economic losses (Narayanankutty *et al.*, 2021). The aqueous extract of *H. indicus* roots showed 100% larval mortality in *Culex quinquefasciatus* mosquitoes within two days at a 5% concentration, suggesting its potential as a natural mosquito control insecticide (Sujatha and Anusha, 2010). When applied at a 5 % concentration for 2 days, the 20 mg/ml chloroform extract had a 100 % larvicidal effectiveness against *C. quinquefasciatus* (Mallikarjun *et al.*, 2010).

5.3 Other herbal drug products

Herbal medicines that are rich in phytochemicals from a range of botanical sources, which influence and nourish healthy skin and hair (Nupur 2021). The benefits of herbal products outweigh those of synthetic ones, which are harmful to human health due to ingredients like paraphenylenediamine that why the term 'Herbal' signifies

security (Bhuvaneswari *et al.*, 2021). Much more herbal product manufacturers like Himalaya Drug Company (Antidandruff shampoo, Evicare syrup, Renalka), Charak (Skinelle cream), Patanjali (Ashvagandharist, Beauty cream), Baidyanath (Surakta syrup and tablets), Sri sritattva (Sariva syrup, Replenishing foot cream), *etc.*, many more with combined other herbal roots and plants are manufactured with scientifically proven benefits both internal and external use satisfying market demands (Nandy *et al.*, 2020).

6. Conclusion

The medicinal herb *H. indicus* has a long history of conventional usage and ayurvedic applications. Its supply is threatened by overuse by people. This review focused on the various phytochemical content of *H. indicus* demonstrating its appeal in traditional and alternative medicine. The growing desire for novel, safe and effective medicines highlight the promise of naturally produced phytochemicals, notably those derived from *H. indicus*, as sources of new medications. The roots of *H. indicus* are a primary source of numerous phytochemicals with anticancer properties, including tannins, sterols, glycosides, saponins, flavonoids and terpenoids. The stems, leaves and flowers also contain important compounds like glycosides, tannins and flavonoids, which have been extensively studied for their therapeutic potential. Recent research in controlled laboratory conditions with experimental models like rats have progressively supported the traditional benefits of *H. indicus*, particularly as an antidiabetic, anti-inflammatory, neuroprotective and anti-ophidian drug. In numerous cases, these pharmacological studies have verified traditional medical claims by employing rigorous scientific procedures and criteria. However, overharvesting poses a severe threat to this ethnomedicinal plant, stressing the necessity of conservation. By enabling *in vitro* regeneration and enhancing phytochemical synthesis, genetic engineering and plant tissue culture techniques can play a critical role in conserving *H. indicus*. However, further research is needed to explore many of these phytochemicals for biomedical applications, assess their toxicity and safety, and understand their molecular mechanisms and effective doses. The paper emphasizes the potential of *H. indicus* extracts in drug development despite challenging such as high attrition rates, sustainable supply and intellectual property issues. Advances in scientific and biotechnological research provide a strong foundation for the continued exploration and development of *H. indicus* based drugs.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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