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Assessment of Comparative *in-vitro* antioxidant potential of *Asparagus racemosus*, *Glycyrrhiza glabra*, *Piper betle* and *Withania somnifera* extracts

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Abstract
The present study aimed to assess and compare the *in vitro* antioxidant potential of ethanolic extracts of *Asparagus racemosus*, *Glycyrrhiza glabra*, *Piper betle*, and *Withania somnifera* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Extracts were tested at concentrations ranging from 10 to 500 µg/ml, with ascorbic acid serving as the reference standard. All extracts exhibited concentration-dependent radical scavenging activity, with significant differences ($p<0.01$) observed between species. *P. betle* consistently demonstrated the highest activity across all concentrations, achieving 90.64±0.31% inhibition at 500 µg/ml, followed by *A. racemosus* (89.17±0.30%) and *G. glabra* (85.57±0.34%). *W. somnifera* showed the lowest activity (79.15±0.33% at 500 µg/ml). The results suggest that the superior antioxidant activity of *P. betle* and *A. racemosus* may be linked to their higher phenolic and flavonoid content. These findings highlight the potential application of these medicinal plants as natural antioxidant agents in nutraceutical and pharmaceutical formulations, warranting further investigation into their bioactive compounds and *in vivo* efficacy.

Keywords: *Asparagus racemosus*, *Glycyrrhiza glabra*, *Piper betle*, *Withania somnifera*, DPPH assay, antioxidant activity, herbal extracts, phytochemicals, radical scavenging, nutraceuticals

Introduction
Oxidative stress arises when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. This imbalance is implicated in the pathogenesis of several chronic and degenerative diseases, including cardiovascular disorders, cancer, diabetes, and neurodegenerative conditions (Lobo *et al.*, 2010) [12]. The search for effective antioxidants, particularly from natural sources, has intensified due to growing concerns about the safety and long-term effects of synthetic antioxidants.

Medicinal plants are valuable reservoirs of bioactive compounds such as phenolics, flavonoids, alkaloids, and tannins, many of which exhibit potent antioxidant activities (Pisoschi and Pop, 2015) [15]. In the traditional Ayurvedic system, *Asparagus racemosus* (Shatavari), *Glycyrrhiza glabra* (Licorice), *Piper betle* (Betel leaf), and *Withania somnifera* (Ashwagandha) are renowned for their therapeutic properties, including rejuvenating, anti-inflammatory, immunomodulatory and antioxidant effects (Sharma *et al.*, 2018; Singh *et al.*, 2011) [19, 18].

Asparagus racemosus Linn. (Family *Asparagaceae*) is widely recognized in traditional Indian medicinal systems, including Ayurveda, Unani, and Siddha. Within Ayurvedic practice, it is notably employed as a galactagogue (Gupta and Shaw, 2011) [8], indicating its potential application in managing infertility. Classified as a *Rasayana* herb, *A. racemosus* is believed to support homeostasis, decelerate the ageing process, and provide protection against various ailments. Experimental studies have suggested possible therapeutic roles for this plant in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. Additionally, research has demonstrated its potential in alleviating stress-induced anxiety and depression (Bopana and Saxena, 2007; Majumdar *et al.*, 2021) [1, 13].

Phytochemical investigations have identified numerous compounds in *A. racemosus*, including tetranorlipoic acid, dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, 9,12-

octadecadienoic acid, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, octadecanoic acid, and 2-furancarboxaldehyde, 5-(hydroxymethyl) as well as octadecane, 2-methyl, phthalic acid, tetracosane and octadecane. According to Guo *et al.* (2022) [9], the phytochemical composition of *A. racemosus* can vary with environmental factors and geographic origin.

Among its bioactive constituents, polyphenols are particularly noteworthy due to their diverse health-promoting effects. These compounds possess antioxidant, anti-inflammatory, anti-ulcer, antistress, and wound-healing properties.

Withania somnifera (Ashwagandha) has been recently studied for its response to temperature stress, which affects phytochemical accumulation and antioxidant dynamics. Its immunomodulatory potential and rich withanolide content further amplify its relevance in oxidative-stress contexts. Studies also reveal that *A. racemosus* exhibits strong adaptogenic, neuroprotective, and antioxidant properties linked to shatavarin IV, which may mitigate oxidative damage via modulation of acetylcholinesterase activity

Although research on *G. glabra* and *P. betle* remains limited in these sources, both are traditionally recognized for their substantial antioxidant and phytochemical profiles

While the antioxidant properties of these plants have been individually reported, a comparative evaluation under uniform experimental conditions is limited. Such an assessment can provide insight into their relative potency, guide the formulation of polyherbal preparations, and support the scientific validation of traditional claims.

Therefore, the present study aims to assess and compare the *in-vitro* antioxidant potential of methanolic extracts of *A. racemosus*, *G. glabra*, *P. betle*, and *W. somnifera* using established free radical scavenging assays. The findings may contribute to identifying promising candidates for the development of natural antioxidant formulations.

Materials and Methods

Collection of Plant Materials

Fresh roots of *Asparagus racemosus* were collected from the herbal garden of Melathottam, Veterinary College and Research Institute (VCRI), Orathanadu, Tamil Nadu, India. The roots were placed in sterile polyethylene containers and transported to the Department of Livestock Products Technology, VCRI, Orathanadu.

Root portions of *Glycyrrhiza glabra*, leaves of *Piper betle*, and root portions of *Withania somnifera* were procured from reputed herbal shops located in Orathanadu and Thanjavur (Tamil Nadu, India).

Preparation of Herbal Powders

The collected plant materials were first cleaned under running tap water to remove adhering dirt, sand, and other extraneous contaminants. This was followed by washing with distilled water to ensure sterility.

The *Asparagus racemosus* roots were cut into small pieces (2-3 cm) using a sterile stainless steel knife. All plant materials were shade dried at ambient temperature (28±2 °C) for 4-5 days to prevent degradation of thermolabile compounds. The partially dried materials were then oven-dried at 40 °C for 5-6 hours in a hot air oven until constant weight was achieved, ensuring complete removal of residual moisture.

The dried samples were ground to a fine powder using a laboratory mixer grinder and sieved through a stainless steel mesh sieve to obtain a uniform particle size. The powders were packed in sterile, airtight, food-grade plastic containers and stored at room temperature in a moisture-free environment until further use.

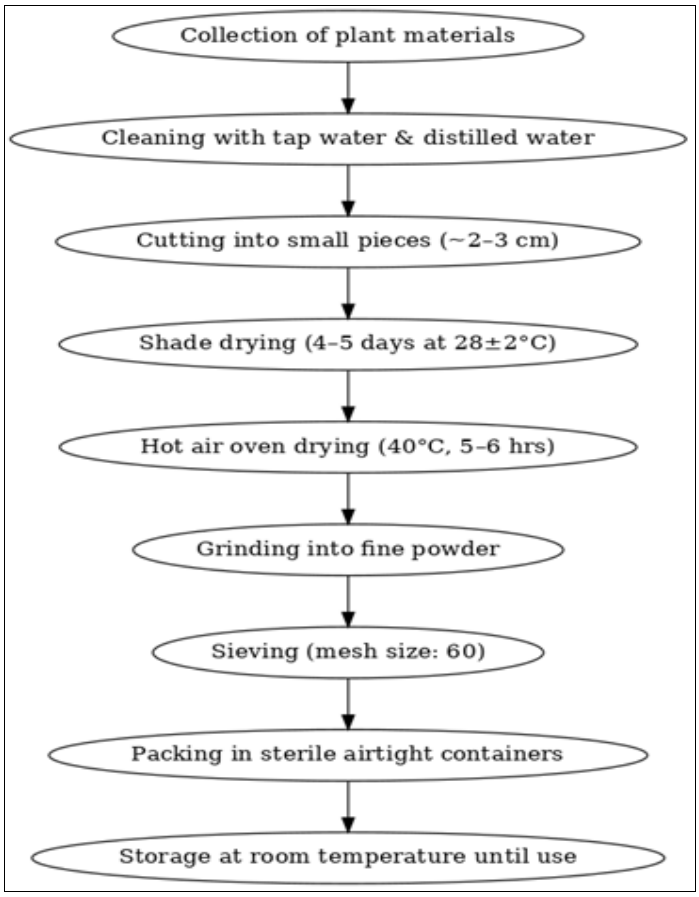

Fig 2: Flow chart for the preparation of herbal powder extracts

Preparation of Ethanolic Extracts

Ethanolic extracts of each herbal powder (*A. racemosus*, *G. glabra*, *P. betle*, and *W. somnifera*) were prepared by Soxhlet extraction using analytical grade ethanol (99% v/v).

For each extraction, 100g of herbal powder was placed in the Soxhlet extractor and continuously extracted with ethanol for 48 hours. The temperature was maintained below 50 °C to avoid thermal degradation of active constituents. The extracts were filtered through Whatman No. 1 filter paper to remove solid residues.

The filtrates were concentrated under reduced pressure using a rotary evaporator at 50 °C for 30 minutes to obtain a semisolid mass. The concentrated extracts were further dried to constant weight, weighed to determine extraction yield, and stored in airtight containers at 4 °C until further experimental use.

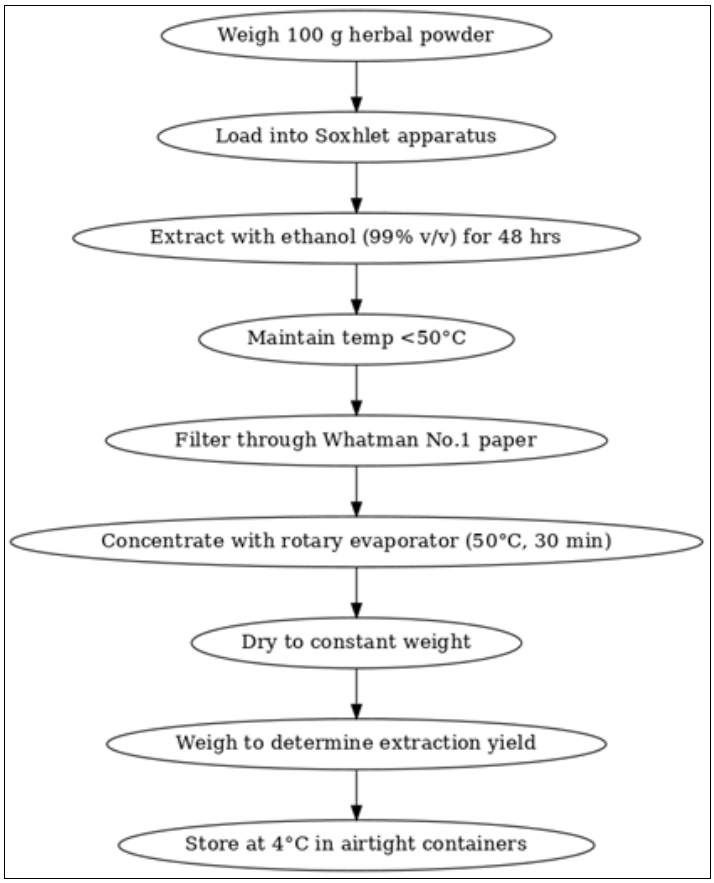

Fig 2: Flow chart for the preparation of ethanolic herbal extracts.

Storage and Use

The prepared ethanolic extracts were protected from light and humidity during storage to minimize oxidative degradation. These extracts were subsequently used for in-vitro antioxidant assays.

Results

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of ethanolic extracts of *Asparagus racemosus*, *Glycyrrhiza glabra*, *Piper betle* and *Withania somnifera* at concentrations ranging from 10 to 500 µg/ml is presented in table 1. Ascorbic acid served as the reference antioxidant.

Table 1: Mean±SD values of DPPH radical scavenging activity of ethanolic extract of *A. racemosus*, *G. glabra*, *P. betle* and *W. somnifera* powder.

Sample concentration (µg/ml)	DPPH radical scavenging activity of different herbs extract					F value
	A. racemosus	*G. glabra*	*P. betle*	*W. somnifera*	Ascorbic acid	
10 µg/ml	67.58±0.37^{c}	55.60±0.26^{b}	77.73±0.34^{d}	47.02±0.00^{a}	92.58±0.08^{e}	5034.58**
50 µg/ml	71.79±0.18^{b}	72.15±0.46^{b}	82.37±0.20^{c}	66.24±0.43^{a}	92.58±0.08^{d}	1139.30**
100 µg/ml	75.92±0.12^{b}	78.85±0.14^{c}	84.39±0.26^{d}	69.29±0.20^{a}	92.58±0.08^{e}	2611.65**
250 µg/ml	82.66±0.55^{c}	80.71±0.17^{b}	87.45±0.37^{d}	77.41±0.36^{a}	92.58±0.08^{e}	293.87**
500 µg/ml	89.17±0.30^{c}	85.57±0.34^{b}	90.64±0.31^{d}	79.15±0.33^{a}	92.58±0.08^{e}	332.97**

Means bearing same superscripts row-wise do not differ significantly. (n=6).

At 10 µg/ml, scavenging activity ranged from 47.02±0.00% (*W. somnifera*) to 77.73±0.34% (*P. betle*), while ascorbic acid exhibited the highest activity (92.58±0.08%; F = 5034.58, $p<0.01$). Increasing the concentration to 50 µg/ml enhanced activity across all extracts, with *P. betle* (82.37±0.20%) outperforming *G. glabra* (72.15±0.46%) and *A. racemosus* (71.79±0.18%) (F = 1139.30, $p<0.01$).

At 100 µg/ml, activity values further increased, with *P. betle* (84.39±0.26%) and *G. glabra* (78.85±0.14%) showing significantly greater scavenging capacity than *A. racemosus* (75.92±0.12%) and *W. somnifera* (69.29±0.20%) (F = 2611.65, $p<0.01$).

At 250 µg/ml, *P. betle* remained the most potent extract (87.45±0.37%), followed by *A. racemosus* (82.66±0.55%) and

G. glabra (80.71±0.17%), with *W. somnifera* showing the lowest activity (77.41±0.36%) ($F = 293.87$, $p < 0.01$).

At the highest tested concentration (500 µg/ml), all extracts demonstrated strong scavenging activity, with *P. betle* (90.64±0.31%) and *A. racemosus* (89.17±0.30%) approaching the activity of ascorbic acid (92.58±0.08%) ($F = 332.97$, $p < 0.01$).

Discussion

The present study demonstrated that ethanolic extracts of *Piper betle*, *Asparagus racemosus*, *Glycyrrhiza glabra*, and *Withania somnifera* possess significant concentration-dependent DPPH radical scavenging activity, with *P. betle* consistently exhibiting the highest antioxidant potential across all tested concentrations. The scavenging capacity of all extracts approached that of the reference standard ascorbic acid at higher concentrations (500 µg/ml), indicating their strong electron- or hydrogen-donating abilities to neutralize free radicals.

The superior activity of *P. betle* in this study aligns with previous reports attributing its antioxidant efficacy to high levels of phenolic compounds such as chavibetol, eugenol, and hydroxychavicol (Pradhan *et al.*, 2013; Choudhary and Kale, 2002) [16, 3]. These phytochemicals are known for their ability to donate electrons, scavenge free radicals, and inhibit lipid peroxidation (Shen *et al.*, 2010) [20]. Similarly, *A. racemosus*, which ranked second in antioxidant potency, has been reported to contain abundant saponins (shatavarins), flavonoids, and polyphenolic constituents contributing to its free radical scavenging capacity (Bopana and Saxena, 2007; Goyal *et al.*, 2010) [1, 6]. The high activity observed for *G. glabra* may be attributed to its glycyrrhizin, liquiritigenin, and glabridin content, which have demonstrated potent *in vitro* antioxidant effects through radical scavenging and ferric ion reduction mechanisms (Fiore *et al.*, 2005; Visavadiya and Narasimhacharya, 2006) [4, 22].

W. somnifera exhibited the lowest scavenging activity among the tested species, which is consistent with earlier findings that although rich in withanolides and alkaloids, its phenolic content is comparatively lower than that of polyphenol-dense herbs (Mishra *et al.*, 2000; Kulkarni and Dhir, 2008). However, *W. somnifera* is still recognized for its *in vivo* antioxidant potential through upregulation of endogenous enzymatic antioxidants such as superoxide dismutase and catalase (Singh *et al.*, 2001) [21], which may not be fully reflected in the DPPH assay.

The concentration-dependent increase in scavenging activity observed in this study is in agreement with earlier reports indicating that polyphenolic-rich plant extracts exhibit greater antioxidant efficiency at higher concentrations due to increased availability of active compounds to interact with the stable DPPH radical (Brand-Williams *et al.*, 1995; Prior *et al.*, 2005) [2, 17]. The statistical significance ($p < 0.01$) between plant species across concentrations underscores the influence of phytochemical diversity and abundance on radical neutralization capacity.

It is noteworthy that both *P. betle* and *A. racemosus* achieved scavenging levels close to ascorbic acid at the highest concentration tested, highlighting their potential as natural alternatives to synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which have raised safety concerns (Gülçin, 2012) [7]. Given the increasing consumer preference for plant-based bioactives in functional foods and nutraceuticals, these findings provide a scientific basis for incorporating these herbs into antioxidant formulations.

However, the DPPH assay reflects only one aspect of antioxidant activity-amely, the ability to scavenge stable free radicals in a non-biological system. Antioxidant behavior *in vivo* is influenced by factors such as absorption, metabolism, and interaction with endogenous antioxidant systems (Halliwell and Gutteridge, 2015) [10]. Therefore, future studies should include complementary assays such as ABTS, FRAP, and ORAC, alongside *in vivo* models, to provide a more comprehensive antioxidant profile. Additionally, phytochemical profiling through HPLC or LC-MS could help identify the specific compounds responsible for the observed activity and assess their synergistic interactions.

In conclusion, the present findings indicate that *P. betle* and *A. racemosus* are promising sources of natural antioxidants, with potential applications in the nutraceutical and pharmaceutical industries. Their high radical scavenging efficiency, coupled with traditional therapeutic use, supports further investigation into their bioactive compounds and clinical efficacy in mitigating oxidative stress-related disorders.

Conclusion

The present study demonstrates that ethanolic extracts of *Asparagus racemosus*, *Glycyrrhiza glabra*, *Piper betle*, and *Withania somnifera* exhibit significant in-vitro DPPH radical scavenging activity in a concentration-dependent manner. Among the tested extracts, *P. betle* consistently displayed the highest antioxidant potential, followed closely by *A. racemosus* and *G. glabra*, whereas *W. somnifera* showed comparatively lower activity. The superior performance of *P. betle* and *A. racemosus* is likely attributable to their high phenolic and flavonoid contents, which enhance their ability to neutralize free radicals.

These findings highlight the potential of these medicinal plants particularly *P. betle* and *A. racemosus* as natural antioxidant sources for use in nutraceutical, pharmaceutical, and functional food applications. Further studies involving *in vivo* models, bioactive compound isolation, and mechanism-based evaluations are warranted to validate their efficacy and explore synergistic effects with conventional antioxidants.

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Author's Contribution

Not available

Conflict of Interest

Not available

Financial Support

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