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BIOACTIVE POTENTIAL OF FRESHWATER ALGAE AS A SOURCE OF ANTIOXIDANT AND ANTIBACTERIAL COMPOUNDS: A REVIEW WITH REFERENCE TO THE UNEXPLORED FRESHWATER SYSTEMS OF WASHIM DISTRICT

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Abstract: Freshwater algae constitute a renewable and largely untapped source of bioactive secondary metabolites with recognised antioxidant and antibacterial properties. This review synthesises published literature on the phytochemical composition and antioxidant/antibacterial potential of freshwater algal genera commonly reported from Washim district, Maharashtra — a semi-arid region drained by rivers including the Adan, Arunavati, Painganga, Katepurna, and Morna, and impounded by reservoirs such as the Ekburji, Sonala, and Adan dams — with particular reference to Spirogyra, Chlorella, Scenedesmus, Oscillatoria, Chara, and Nitella. Qualitative phytochemical screening of these genera has consistently detected alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids using simple colour and precipitation tests, while basic spectrophotometric assays (DPPH radical scavenging, total phenolic and flavonoid content) and agar well/disc diffusion antibacterial assays have demonstrated activity comparable to standard antioxidants and antibiotics against common Gram-positive and Gram-negative pathogens. Despite this promise, the algal flora of Washim district has so far been surveyed only for taxonomic composition and water quality, and no study has evaluated its antioxidant or antibacterial potential. This review outlines a simple, laboratory-feasible screening approach — built around standard extraction, qualitative phytochemical tests, and basic spectrophotometric and agar-diffusion assays rather than chromatographic or molecular techniques — and identifies systematic bioprospecting of Washim's freshwater algae as an accessible research opportunity suited to laboratories with limited instrumentation.

Index Terms **-** Freshwater algae; antioxidant activity; antibacterial potential; phytochemical screening; DPPH assay; agar well diffusion; Washim district; Maharashtra

I. INTRODUCTION

Freshwater algae are a taxonomically diverse group of photosynthetic organisms that serve as primary producers and water-quality bioindicators in rivers, lakes, and reservoirs [1]. Beyond this ecological role, they synthesise a wide range of bioactive secondary metabolites — phenolics, flavonoids, carotenoids, alkaloids, and terpenoids — many of which show antioxidant and antimicrobial activity [2].

Interest in these compounds has grown alongside two converging problems: the rising burden of oxidative-stress-related disease, which has intensified the search for safe natural antioxidants [3], and the global

antimicrobial resistance (AMR) crisis, which the World Health Organization has identified as one of the leading public health threats and which has renewed interest in natural sources of antibacterial agents [4].

India's freshwater ecosystems support a rich and largely underexplored algal flora. In Maharashtra's Vidarbha region, Washim district — located at approximately 20.11°N, 77.13°E and drained by the Painganga, Adan, Arunavati, Katepurna, and Morna rivers, and impounded by reservoirs including the Ekburji, Sonala, and Adan dams [5,6] — supports diverse freshwater habitats. A small number of studies have documented the phytoplankton and macrophytic algal flora of these water bodies [7–10], but none has evaluated their antioxidant or antibacterial potential.

This review aims to (i) summarise the documented algal diversity of Washim district; (ii) outline a simple, laboratory-feasible methodology for antioxidant and antibacterial screening suited to a resource-limited setting; (iii) summarise the phytochemical composition reported for the relevant algal genera; (iv) review their reported antioxidant and antibacterial potential; and (v) identify realistic research gaps and directions for bioprospecting the freshwater algae of Washim district.

II. Study Area and Documented Algal Diversity of Washim District

2.1 Hydrological Profile

Washim district occupies approximately 3,145 sq. km in the western Vidarbha region of Maharashtra, within the Godavari river basin. The climate is semi-arid, with average annual rainfall of about 850 mm concentrated in the southwest monsoon (June–September), producing marked seasonal variation in water availability [5]. The principal river, Painganga, receives the Adan, Arunavati, Kaas, and Bembala as tributaries, while the Katepurna and Morna flow northward to join the Purna river system. Several reservoirs and lakes provide additional standing-water habitat for algal growth (Table 1).

Table 1: Major freshwater water bodies of Washim district [5,6,9]

Water Body	Type	Notes
Painganga, Adan, Arunavati, Katepurna, Morna	Rivers	Principal rivers of the district, part of the Godavari river system
Ekburji Dam	Reservoir	Built 1964, on the Chandrabhaga river
Sonala Dam	Reservoir	Built 1981, on the Adan river
Adan Dam	Reservoir	Built 1977, near Karanja (Lad)
Upper Morna Reservoir	Reservoir	At Medshi village
Shaha, Rishi, Borala, Yedshi Lakes	Lakes	Documented for algal/hydrophyte diversity

2.2 Documented Algal Diversity

Solanke et al. [7] documented representatives of Bacillariophyceae, Chlorophyceae, and Cyanophyceae in the Upper Morna Reservoir; Halwe [8] recorded 26 species of Chlorophyceae from Shaha Lake, Karanja (Lad); Dhore et al. [9] reported Chara species from Adan Dam and adjoining lakes; and Shende [10] documented hydrophyte diversity, including Characeae, from Borala and Mirzapur lakes. These local surveys, together with broader regional reviews of freshwater algal diversity in Maharashtra [11], indicate that Chlorophyceae, Bacillariophyceae, Cyanophyceae, and Charophyceae are the dominant algal classes across the region (Table 2), several genera of which have been independently reported elsewhere to show antioxidant or antibacterial activity.

Table 2: Freshwater algal genera reported from Washim district and the adjoining Vidarbha region [7–11]

Algal Class	Representative Genera Reported
Chlorophyceae	Spirogyra, Scenedesmus, Chlorella, Oedogonium, Volvox, Ulothrix
Bacillariophyceae	Navicula, Gyrosigma, Cymbella
Cyanophyceae	Nostoc, Oscillatoria, Spirulina, Anabaena
Charophyceae	Chara, Nitella

III. Methodology for Antioxidant and Antibacterial Screening

The methods outlined below rely on standard glassware, common laboratory reagents, and a basic UV–Vis spectrophotometer and microbiology incubator, and are designed to be achievable in a modestly equipped laboratory without recourse to chromatographic (HPLC/GC–MS) or molecular techniques.

3.1 Sample Collection and Extraction

Algal biomass is collected from the target water body and identified morphologically under a light microscope using standard taxonomic keys [12]; material is then shade-dried and powdered. Extraction is carried out by cold maceration (or Soxhlet extraction, where available) using solvents such as methanol, ethanol, chloroform, and acetone, applied singly or sequentially, consistent with the solvents most commonly used in published algal phytochemical and bioactivity studies [13,14].

3.2 Qualitative Phytochemical Screening

Crude extracts are screened using standard colour and precipitation tests [15], which require only basic reagents and no specialised instrumentation:

- Alkaloids — Mayer's and Dragendorff's tests
- Flavonoids — Shinoda test and alkaline reagent test
- Tannins and phenolics — ferric chloride test
- Saponins — froth formation test
- Terpenoids and steroids — Salkowski test and Liebermann–Burchard test
- Glycosides — Keller–Kiliani test

3.3 Antioxidant Activity Assessment

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is the most accessible and widely used antioxidant test, requiring only a UV–Vis spectrophotometer to measure decolorisation of the DPPH radical at 517 nm; activity is expressed as percentage inhibition or IC50 relative to a standard antioxidant such as ascorbic acid [2]. Total phenolic content (Folin–Ciocalteu method, expressed as mg gallic acid equivalents/g) and total flavonoid content (aluminium chloride method, expressed as mg quercetin equivalents/g) can be determined using the same instrument [2]. Other assays such as ABTS and FRAP are also reported in the literature but require additional reagents; DPPH combined with total phenolic and flavonoid content provides a reliable and reproducible baseline antioxidant profile without chromatographic instrumentation.

3.4 Antibacterial Activity Assessment

Antibacterial potential is evaluated using the agar well diffusion or disc diffusion method against reference bacterial strains, typically Staphylococcus aureus and Bacillus subtilis (Gram-positive) and Escherichia coli and Pseudomonas aeruginosa (Gram-negative). Zones of inhibition are measured after incubation, and, where resources permit, minimum inhibitory concentration (MIC) can be estimated by simple two-fold broth microdilution. Non-polar to mid-polar solvents such as chloroform and ethyl acetate generally recover more antibacterial metabolites than aqueous extracts, which are consistently the least active across published studies — a useful consideration when choosing extraction solvents with limited reagent stocks [14,16].

IV. Phytochemical Composition of Freshwater Algae

Qualitative screening studies on genera relevant to Washim district report a broadly similar phytochemical profile across taxa (Table 3) [13,14,16,17].

4.1 Alkaloids

Detected across most genera tested, including Spirogyra, Chlorella, Scenedesmus, and Oscillatoria [13,14].

4.2 Flavonoids

Consistently present, and considered a major contributor to radical-scavenging activity through electron donation and metal-ion chelation [18].

4.3 Phenolic Compounds

The most extensively documented class of algal antioxidants; total phenolic content varies considerably between genera and extraction solvent [16,18].

4.4 Tannins

Widely present, though reported as variable or absent in some Oscillatoria and Nitella extracts [16].

4.5 Terpenoids and Steroids

Broadly present across genera and readily detected by simple qualitative tests; associated in the literature with antimicrobial activity [14,16].

4.6 Saponins and Glycosides

Present in most genera but reported as variable across studies and extraction solvents [13,16].

Table 3: Qualitative phytochemical screening of freshwater algal genera relevant to Washim district (+ = present; – = absent; +/– = variable across studies; ND = not determined in available literature) [13,14,16,17]

Phytochemical	Spirogyra	Chara	Chlorella	Scenedesmus	Oscillatoria	Nitella
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phenols	+	+	+	+	+/-	+
Tannins	+	+	+	+	+/-	-
Saponins	+	+	+/-	+	-	ND
Terpenoids	+	+	+	+	+	ND
Steroids	+	+	+	+	+	ND

V. Reported Antioxidant Potential of Freshwater Algae

Species-level studies confirm that the phytochemical richness of these genera translates into measurable radical-scavenging activity when tested by simple spectrophotometric assay. Spirogyra neglecta extracts showed a DPPH IC50 of approximately 7.46 µg/mL, comparable to the standard antioxidant Trolox (7.03 µg/mL) [19]. Chlorella vulgaris (strain BUACC25) showed strong DPPH activity, with an IC50 of approximately 2.82 µg/mL [17]. Scenedesmus obliquus extracts showed DPPH inhibition of up to 97.37%, alongside high total phenolic and flavonoid content [18]. Oscillatoria sp. extracts showed moderate DPPH scavenging, in the range of 22–45% [14]. Chara corallina showed a DPPH IC50 of 0.169 mg/mL, with beta-sitosterol identified as a key phytosterol [20]. Nitella showed the highest DPPH scavenging activity among five freshwater algal species compared in one study, together with a high total flavonoid content [21]. These findings are summarised in Table 4, alongside reported antibacterial activity.

VI. Reported Antibacterial Potential of Freshwater Algae

Freshwater algal extracts generally show stronger activity against Gram-positive bacteria, such as S. aureus and B. subtilis, than against Gram-negative bacteria, which are protected by a more complex outer membrane. Chlorella vulgaris (BUACC25) extracts active against S. aureus and related Gram-positive pathogens have also shown DPPH antioxidant activity in the same study, illustrating the dual bioactivity of some genera [17]. Similar Gram-positive activity, with MIC values generally in the sub-milligram to low-milligram-per-mL range, has been reported for Scenedesmus and Oscillatoria extracts [13,14]. Activity against Gram-negative bacteria such as E. coli has also been reported for some of these genera, though generally at higher extract concentrations than required against Gram-positive organisms [13,14]. Freshwater Cladophora and Vaucheria extracts from comparable habitats elsewhere have shown MIC values of 0.31–10 mg/mL against multiple bacterial species, with S. aureus among the most susceptible [16]. The activity of these extracts is generally attributed to disruption of the bacterial cell membrane by algal fatty acids and interference with bacterial protein or nucleic acid synthesis by alkaloids and phenolic compounds [14,16].

Table 4: Reported antioxidant and antibacterial activity of freshwater algal genera relevant to Washim district

Genus	Antioxidant Activity (DPPH)	Antibacterial Activity	Ref.
Spirogyra	IC50 ≈ 7.46 µg/mL, comparable to Trolox	Reported against Gram-positive and Gram-negative pathogens elsewhere	19
Chlorella	IC50 ≈ 2.82 µg/mL	Active against S. aureus and related Gram-positive pathogens	17
Scenedesmus	DPPH inhibition up to 97.37%	MIC in sub-mg/mL to low mg/mL range vs S. aureus; also active against E. coli	18, 13
Oscillatoria	Moderate scavenging (22–45%)	MIC ≈ 156 µg/mL against S. aureus	14
Chara	IC50 ≈ 0.169 mg/mL	Not well studied	20
Nitella	Highest among 5 species compared; high TFC	Not well studied	21

VII. Research Gaps and Future Directions

Despite the promising bioactivity reported for these genera elsewhere, several gaps remain that are directly addressable using the simple methods outlined in Section III:

- No published study has evaluated the antioxidant or antibacterial potential of algae collected specifically from Washim district's water bodies; existing local work is limited to taxonomic and water-quality surveys [7–10].
- Major water bodies, including Ekburji Dam, Sonala Dam, and Borala Lake, lack even a basic phytochemical or bioactivity screening, despite their documented algal diversity.
- Seasonal sampling across at least one full annual cycle would help capture how the district's pronounced monsoon-driven variation in water availability affects algal growth and, potentially, phytochemical yield.
- A straightforward first study would combine morphological identification, extraction in two to three solvents of differing polarity, qualitative phytochemical screening, DPPH-based antioxidant assessment, and agar well diffusion antibacterial testing against a small panel of reference bacterial strains — all achievable without chromatographic or molecular infrastructure.

VIII. Conclusion

Freshwater algal genera commonly reported from Washim district — including Spirogyra, Chlorella, Scenedesmus, Oscillatoria, Chara, and Nitella — have been shown elsewhere to contain a broad range of phytochemicals, including alkaloids, flavonoids, phenolics, tannins, and terpenoids, that confer measurable antioxidant activity and antibacterial efficacy against both Gram-positive and Gram-negative pathogens. These properties can be evaluated using simple, well-established techniques — qualitative colour tests, DPPH-based spectrophotometric assays, and agar well diffusion — that do not require chromatographic or molecular instrumentation. Yet the algal flora of Washim district itself remains entirely unevaluated for these properties. A systematic, laboratory-feasible bioprospecting study of the district's freshwater algae therefore represents an accessible and meaningful research opportunity.

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