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REVIEW ARTICLE

A Scoping Review on Ethnomedicinal and Ethnopharmacological Aspects of *Rhizoclonium riparium* (Roth) Harvey and Their Relationship with the Phytochemical Contents

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ABSTRAK

Rhizoclonium riparium (Roth) Harvey merupakan sejenis alga hijau air tawar yang telah digunakan secara tradisional dalam pelbagai budaya kerana dipercayai mempunyai khasiat perubatannya. Walaupun alga ini mempunyai pelbagai potensi manfaat terapeutik, pengetahuan saintifik mengenainya masih terhad. Kajian ini meneliti aspek etnobotani dan etnofarmakologi R. riparium dengan tujuan menjelaskan kegunaan tradisionalnya serta mengenal pasti kandungan fitokimia yang bertanggungjawab terhadap potensi terapeutiknya. Pencarian literatur secara kritikal telah dijalankan menggunakan pelbagai pangkalan data saintifik elektronik seperti SCOPUS, PubMed dan EBSCOhost, serta sumber tambahan termasuk Google Scholar dan ScienceDirect bagi artikel berkaitan yang diterbitkan dari tahun 2006 hingga 2025. Carian awal mengenal pasti sejumlah 1,409 artikel yang relevan. Melalui proses pemilihan yang teliti, hanya 12 artikel dipilih kerana memenuhi semua kriteria kemasukan yang ditetapkan. Kesemua kajian tersebut melaporkan kesan positif sederhana hingga ketara terhadap sifat etnoperubatan R. riparium. Ujian kimia in vitro menunjukkan aktiviti antimikrob, antikulat, anti-radang, antioksidan dan antimutagenik yang signifikan. Analisis fitokimia pula mendedahkan kehadiran pelbagai metabolit sekunder, termasuk sebatian fenolik, flavonoid, alkaloid, terpenoid dan polisakarida yang diketahui mempunyai aktiviti farmakologi. Memandangkan alga ini banyak terdapat di rantau Asia-Pasifik dan mengandungi fitokimia berpotensi tinggi seperti flavonoid, polisakarida, fenol dan karotenoid, ia berpotensi untuk dimanfaatkan dalam pembangunan ubat-ubatan baharu pada masa hadapan. Ulasan skop ini menyoroti kesan etnobotani dan etnofarmakologi ekstrak R. riparium serta hubung kaitnya dengan kandungan fitokimia yang terkandung di dalamnya.

Kata kunci: *Etnoperubatan; etnofarmakologi; fitokimia; Rhizoclonium riparium*

ABSTRACT

Rhizoclonium riparium (Roth) Harvey, a freshwater green alga, has been traditionally utilised across various cultures for its purported medicinal properties. Though it has many potential medicinal properties, there is not enough knowledge about this alga. This study delves into the ethnobotanical and ethnopharmacological aspects of *R. riparium*, aiming to elucidate its traditional uses and unravel the underlying phytochemical constituents responsible for its therapeutic potential. A critical literature search from various scientific electronic databases, such as SCOPUS, PubMed, EBSCOhost and additional sources, such as Google Scholar and ScienceDirect was conducted for related articles published from the

year 2006 to 2025. The initial search found total 1409 relevant articles. Through a meticulous selection process, only 12 articles were chosen as they successfully met all the inclusion criteria. All of the studies reported moderate to noticeable positive effects of *R. riparium* against ethnomedicinal properties. *In vitro* chemical assay revealed significant antimicrobial, antifungal, anti-inflammatory, antioxidant and antimutagenic properties. The phytochemical analysis unveiled a plethora of secondary metabolites, including phenolics, flavonoids, alkaloids, terpenoids and polysaccharides, which are known for their pharmacological activities. As this alga is commonly available in Asia-Pacific region and consist of effective phytochemicals like flavonoid, polysaccharide, phenol, carotenoid, it could be used in the future to develop new drugs. This scoping review highlights the ethnobotanical and ethnopharmacological effects and their relation with phytochemicals of *R. riparium* extracts.
Keywords: Ethnomedicinal; ethnopharmacological; phytochemicals; *Rhizoclonium riparium*

INTRODUCTION

Algae are a promising source of a wide variety of bioactive compounds that hold great potential for the development of new pharmaceutical agents because of their interesting biological properties, such as antihypertensive, antioxidant, antitumor, anticoagulant, neuroprotective, anti-inflammatory, antifungal, antibacterial, antidiabetic etc. (Kandale et al. 2011). Phytochemicals that are found in algae are categorised as primary or secondary constituents, based on their role and biological properties (Campos-Vega & Oomad 2013; Rabizadeh et al. 2022). Microscopic (microalgae) and macrophytic (macroalgae) are the two major types of algae. Microalgae, often known as phytoplankton, are microscopic algae found in aquatic environments. The macroalgae are called seaweeds (Pröschold & Leliaert 2007). Up to 50% of drugs approved by the Food and Drug Administration (FDA) in the US derived from marine organisms and 30% of them are obtained from algae. For example, an anti-angiocardiopathy drug, propylene glycol alginate sodium sulphate is produced from marine algae (Xue et al. 2018).

The macroalgae *Rhizoclonium riparium* (RR) is a green filamentous alga that typically found in any fresh water environment. It is a member of the Cladophoraceae family, belonging to the class Chlorophyceae and placed within the division Chlorophyta, order Cladoporales (Zhao et al. 2016). It is mainly found in Asia-Pacific region, Britain, India, China and Australia. In the northern and northeastern regions of Thailand it is locally referred as “Kai”. During dry season it grows faster (November-March). The most interesting feature of this algae is its richness of cell wall polysaccharides (Mungmai et al. 2014).

Although RR possesses diverse potential pharmacological activities, there remains a lack of comprehensive understanding of existing findings. This knowledge gap underscores the need for a scoping review to consolidate available evidence and identify areas for future research. The purpose of this scoping review is to compile the medicinal properties and phytochemicals derived from RR that can provide novel insights to the future research endeavours for further study in this field. Nowadays, there has been a rising interest in natural medicines due to multiple drug resistance, high cost and adverse effects of synthetic drugs. Seaweeds are proven to be the effective and cheaper alternative source of synthetic drugs as they proved to successfully aid various ailments. The extracts of the RR exerts several biological properties. As this algae is commonly available in Asia-Pacific region and consists of effective phytochemicals, it could be used in the future to develop new drugs.

MATERIALS AND METHODS

Search Strategy

The study aimed to find relevant research on the biological properties of RR. Different online electronic databases, such as Scopus, PubMed, EBSCOhost and additional online database, such as Google scholar and ScienceDirect, were retrieved to identify the studies until August 2025. All articles searched in English and carefully scrutinised according to the other steps of the review. In each database, the following two sets of search terms, (i) *Rhizoclonium riparium* OR *Rhizoclonium Kützing* OR *Conferva riparia* OR *Cladophora riparia* OR *Conferva implexa* OR *Conferva riparia* OR *Lola implexa* OR *Rhizoclonium implexum* OR *Rhizoclonium hieroglyphicum* OR *Rhizoclonium kerneri* OR *Rhizoclonium panniosum* OR *Rhizoclonium salinum* OR *Rhizoclonium kochianum* OR *Rhizoclonium kochianum var. kerneri* OR *Rhizoclonium riparium var. implexum* OR *Tiresias riparia* AND (ii) oxida* OR inflammat* OR microb* OR biolog* OR therapeut* OR pharmacolog* OR medicin* were used.

Selection Criteria

- Inclusion criteria

The following inclusion criteria were applied: (i) the articles should discuss the biological activity, (ii) only original papers were considered, (iii) studies undertaken using RR and (iv) manuscripts written in English language.

- Exclusion criteria

The following criteria were used: (i) duplicate studies, and (ii) books, book chapters, editorial letters, review articles, news, case studies and conference proceedings.

Articles Screening

The article screening process consisted of three sequential steps. In the first step, we filtered out the articles published as a case study, review, book chapter, book, conference proceeding, editorial letters by reading the study title. In the second step, articles that are not related to the biological properties of RR were excluded by reading the abstracts. Finally, the remaining articles did not meet all the inclusion criteria were omitted. Duplicate articles from the databases were removed after final screening. The remaining articles were evaluated for eligibility based on the inclusion criteria and chosen for final qualitative synthesis. Two independent reviewers (H.T. and L.A.) conducted the screening process, and any discrepancies were resolved through discussion and consensus.

Data Extraction Process

Data extraction was drawn and standardised using data collection sheet. The data was extracted according to the characteristics of studies including (i) study name, (ii) study design, (iii) results, (iv) outcomes and (v) reference. The data were also recorded based on phytoconstituents of the studies such as (i) study name, (ii) plant sources, (iii) plant species, (iv) type of extracts, (v) phytochemicals, (vi) ethnobotany (pharmacological effectss) and (vii) reference of each study.

RESULTS AND DISCUSSION

Search Results

A total of 1,409 records were identified through database and additional source searching, including 29 from PubMed, 156 from Scopus, 10 from EBSCOhost and 1,214 from additional sources. After removing 78 duplicate records, 1,331 articles remained for screening. A total of 1,152 records were excluded as they were reviews, case reports, books, conference proceedings, editorials or protocols from the selected database. Thus, 179 records remained for the primary screening. During the primary screening, 167 records were excluded for not meeting the inclusion criteria. After the secondary screening, 12 articles were selected and included in the final qualitative analysis. The PRISMA-ScR flowchart illustrated the selection process was depicted in Figure 1.

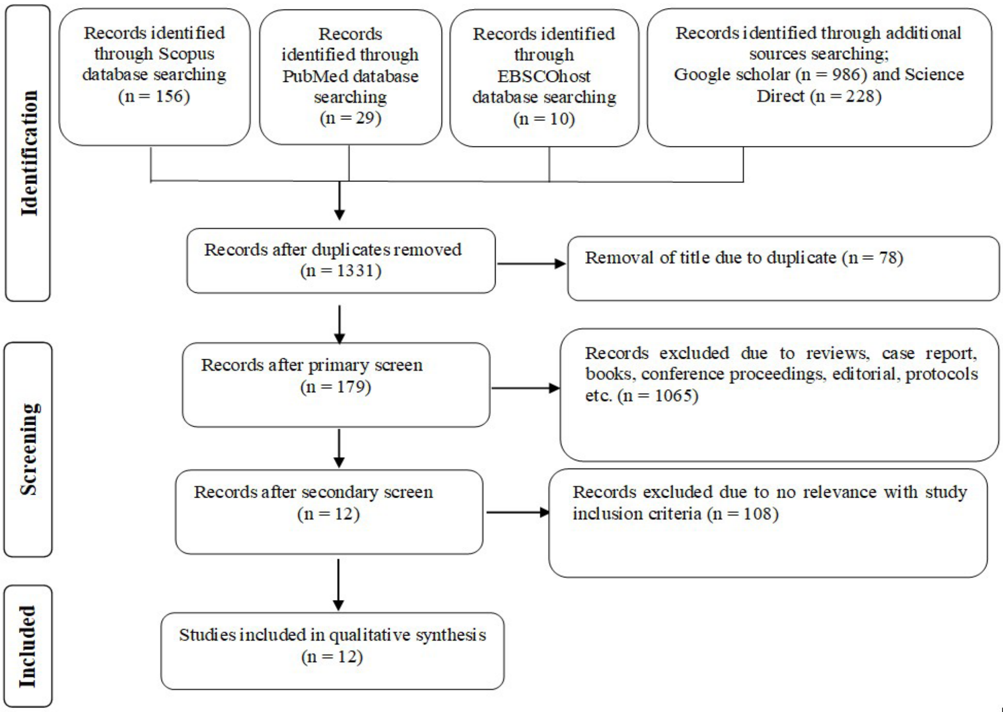

FIGURE 1: The flowchart made according to PRISMA-ScR guideline shows the selection process of the articles in the review

Study Design Characteristics

All studies were published between the years 2006 to 2025 were characterised in Table 1 and consisted of eight chemical assay studies, three cell culture study and one combined chemical assay with *in-vitro* and *in-vivo* study. Based on the types of activities, four studies focused on antioxidative activities (Mungmai et al. 2014; Seal et al. 2014; Osuna-Ruíz et al. 2016; Osuna-Ruíz et al. 2019). One of them was conducted on both antioxidant and antimutagenic (Osuna-Ruíz et al. 2019), another two studies focused on antioxidant and antimicrobial (Mungmai et al. 2014), and antioxidant, antimutagenic and antiproliferative properties (Osuna-Ruíz et al. 2016). Three studies focused on antibacterial activities (Mungmai et al. 2014; Perez Gutierrez and Garcia Baez 2011; Rani et al. 2018). The remaining five studies focused on antiproliferative, algicidal, moisturising, immunomodulating and anti-inflammatory properties (Hsu et al. 2006; Leelapornpisid et al. 2014; Paul & Kundu 2013; Ripol et al. 2018; Roychoudhury et al. 2018). One study evaluated moisturising properties using both human healthy volunteers (n = 30) and pig skin models (Leelapornpisid et al. 2014). In the *in-vitro* cell culture studies murine derived macrophage cell line J774A.1 and HeLa cell line was used (Paul & Kundu 2013). Several chemical assays such as ferric reducing antioxidant potential, 1-diphenyl-2-picrylhydrazyl, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), thiobarbituric acid reactive substances, total flavonoid content, total carotenoid content, total phenolic content were carried out (Mungmai et al. 2014; Osuna-Ruíz et al. 2016; Osuna-Ruíz et al. 2019; Seal et al. 2014). Biochemical tests such as ascorbate peroxidase, catalase activity, superoxide dismutase were also conducted

TABLE 1: Characteristics of studies included in the review

Study No	Study Design	Result	Outcome	References
1	Chemical Assay study. RR fractions: ACE, FH, FM, FM1 and FM2 extracts. Parameters: Phytochemical (TChl, TCC, TPC and TFC), antioxidant (ABTS, DPPH, FRAP) and antimutagenic (Ames test) studies	FH: ▲TPC, TFC ($p < 0.05$); FM1& ACE: ▼▼TPC, TFC; ACE, FM1, FM2: ↓TCC, TChl ($p < 0.05$); FM1: ▲▲DPPH; FM: ▲▲ABTS, FRAP; FM, FM1: ▲ Antimutagenic activity.	RR fraction enhanced the antioxidant and antimutagenic activity.	Osuna-Ruíz et al. 2019
2	Chemical Assay Study RH aqueous (RW) and ethanolic (RE) extracts. Parameters: Antimicrobial (Agar well diffusion method) and antioxidant (DPPH, ABTS and TBARS) activities	RH, RW: no effective antibacterial activity. ▲Antioxidant activity in RW than RE.	RH can be utilized as pharmaceutical nutritional, and cosmetic products.	Mungmai et al. 2014
3	In-Vitro Cell Culture: 293T and HeLa cell line Algal (RR) methanolic extract (AME) Parameters: Cytotoxicity assay (MTT assay) and morphological studies	AME showed cytotoxicity. HeLa cells showed morphological changes and 293T cell had normal morphology.	RR can derive new lead anticancer molecules.	Paul & Kundu 2013)
4	Chemical Assay Study SNP of RR silver nitrate extracts. Parameters: Biochemical analysis (chlorophyll, carotenoid, protein content, SOD, APX, CAT) and algicidal activity.	SNP showed algicidal activity. ↑ protein followed by ↓ drastic chlorophyll, carotenoid content, ↑ APX, CAT, SOD activity.	SNP of RR may have potential algicidal activity.	Roychoudhury et al. 2018
5	Chemical Assay Study Various organic extracts of RH. Parameters: Antibacterial activity (Disc diffusion method)	The chloroform and methanol extracts showed maximum inhibitory effect.	RH can used as antibacterial products.	Rani et al. 2018
6	Chemical Assay Study Ethanol, methanol, and acetone extracts of RH. Parameters: Antibacterial activity (Agar disc diffusion technique) and GC-MS	RH ethanolic extract showed potent antibacterial effect. GC-MS analysis identified 30 bioactive compounds.	RH can be a potential antibacterial agent.	Morsi et al. 2023
7	Cell culture study: J774A.1 cell line Potassium hydrate extracts of RR. Parameters: ELISA: IL-1	↓ IL-1	RR has immunomodulating properties.	Hsu et al. 2006
8	In-vitro Study Aqueous extract of RR Parameters: Anti-inflammatory activities (COX-2) and fatty acid profile (SFA, MUFA, PUFA)	RR exhibited anti-inflammatory activity with high COX-2 activity inhibition. RR was richer in ω3 PUFA.	RR can be a potential source of bioactive compounds.	Ripol et al. 2018

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Study No	Study Design	Result	Outcome	References
9	Chemical assay study, in-vitro, in-vivo pig and human studies. RH aqueous extracts (RW) Parameters: In-vivo pig and human evaluation (Skin irritation and moisturizing test).	↑ moisture content with no irritation. More than 80 % of the volunteers were satisfied.	RH might be used in skin care cosmetics as natural skin moisturizer.	Leelapornpisid et al. 2014
10	Chemical assay study. Various organic RH extracts Parameters: Antioxidant activity (DPPH, TFC, TPC, reducing power)	In RH chloroform extract ▲▲TPC, TFC, reducing power In RH benzene extract ▲▲DPPH	RH can be used as natural antioxidant.	Seal et al. 2014
11	Chemical assay study. Hexane extract of RH Parameter: Antimicrobial activity: Agar disk diffusion.	Hexane extract showed significant antimicrobial activity.	RH can be used as antimicrobial agent.	Perez Gutierrez & Garcia Baez 2011
12	Chemical assay and in vitro (Murine B-cell lymphoma cell line) studies. Methanol, hexane and acetone extract of RR Parameters: Antioxidant (DPPH, ABTS, TPCC, TFC, TCC), antimutagenic (Ames test) and Antiproliferative (MTT assay) activities.	▲▲mutagenesis, antiproliferative inhibition observed. ▲Antioxidant capacity.	RR seaweed can be potential candidate of novel chemoprotective agent.	Osuna-Ruíz et al. 2016

↑: Increased; ↓: Decreased; ▲: Higher; ▼: Lower; ▲▲: Highest; ▼▼: Lowest; ACE: Acetone crude extract; APX: Ascorbate peroxidase; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); CAT: Catalase activity; COX-2: Cyclooxygenase 2; DPPH: 1-diphenyl-2-picrylhydrazyl; EDAX: Energy dispersive X-ray spectroscopy; FH: Fraction of hexane; FM: Fraction of methanol; FM1: Sub-fraction of FM in ethyl acetate; FM2: Sub-fraction of FM in water; FRAP: Ferric reducing antioxidant potential; GC-MS: Gas chromatography-mass spectrometry; IL-1: Interleukin-1; MUFA: Monounsaturated fatty acids; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PUFA: Polyunsaturated fatty acids; RASP: Alkali-soluble polysaccharides of *R. riparium*; RR: *Rhizoclonium riparium*; RH: *Rhizoclonium hieroglyphicum*; FT-IR spectroscopy: Fourier-transform infrared spectroscopy; RT-PCR: Reverse transcription polymerase chain reaction; SEM: Scanning electron microscope; SFA: Saturated fatty acid; SNP: Silver nanoparticle; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substances; TCC: Total carotenoid content; TChl: Total chlorophyl content; TFC: Total flavonoid content; TLC: Thin layer chromatography; TPC: Total phenolic content; TPCC: Total phenolic compound content; UV-Vis: Ultraviolet-visible spectroscopy; XRD: X-ray powder diffraction

(Roychoudhury et al. 2018). Antimutagenic assay done by Ames test, antimicrobial assay done by agar well diffusion method, antiproliferative activity done by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide′ (MTT) assay, cyclooxygenase-2 (COX-2) inhibition method was used to investigate anti-inflammatory activities (Perez Gutierrez & Garcia Baez 2011; Paul & Kundu 2013; Mungmai et al. 2014; Rani et al. 2018; Ripol et al. 2018). Studies revealed that specific fractions of RR and *Rhizoclonium hieroglyphicum* (RH) extracts exhibited strong antioxidant activities, with methanol and hexane fractions showing enhanced total phenolic and flavonoid content, correlating with superior antioxidant and antimutagenic effects (Mungmai et al. 2014). RH extracts, particularly those from chloroform, methanol, ethanol and hexane, demonstrated significant antibacterial activity, while aqueous extracts showed limited effects

(Perez Gutierrez & Garcia Baez 2011; Mungmai et al. 2014; Rani et al. 2018). Methanolic extracts of RR showed antiproliferative effects on cancer cell lines such as HeLa with minimal toxicity to normal cells, suggesting potential anticancer applications (Paul & Kundu 2013; Osuna-Ruíz et al. 2016). Anti-inflammatory activity was evident through inhibition of COX-2 and reduction of pro-inflammatory cytokines by RR extracts, which are rich in polyunsaturated fatty acids implicated in inflammation regulation (McCauley et al. 2015; Jin et al. 2006; Ripol et al. 2018). In vivo and human studies confirmed moisturising benefits of RH aqueous extracts with high volunteer satisfaction and no irritation, supporting their potential in natural skincare products (Leelapornpisid et al. 2014). Additionally, silver nanoparticle extracts of RR exhibited algicidal effects by disrupting chlorophyll and carotenoid content while enhancing antioxidative enzyme activities, indicating possible applications in environmental or agricultural management to control algal growth (Roychoudhury et al. 2018).

Phytochemical Characteristics of *Rhizoclonium riparium*

The phytochemicals from the selected articles in this review were presented in Table 2 and Figure 2. All the studies had used the primary species RR or RH (Rani et al. 2018). The type of RR extracts used in the studies varies from each other. Fractionated solvents were used in six studies. Though most of extracts were organic, however, aqueous extracts were reported in four different studies (Leelapornpisid et al. 2014; Mungmai et al. 2014; Osuna-Ruíz et al. 2019 Ripol et al. 2018). Phytoconstituents were identified in nine of the studies (Hsu et al. 2006; Morsi et al. 2023; Mungmai et al. 2014; Perez Gutierrez & Garcia Baez 2011; Osuna-Ruíz et al. 2016; Ripol et al. 2018; Roychoudhury et al. 2018; Osuna-Ruíz et al. 2019; Seal et al. 2014).

Anti-oxidative Activities

The antioxidative effect of *Spirulina platensis* was considerably higher in the ethanolic extracts. Previous studies have reported that *S. platensis* contains non-polyphenolic substances such as vitamin E, carotenoids and chlorophyll, which have the potential to exhibit higher antioxidative effects (Jaime et al. 2005; Metekia & Ulusoy 2023; Shukla et al. 2009). However, the aqueous extract of RH showed higher levels of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activities compared to the ethanolic extracts. Similar effects had been reported by previous studies, noting that the aqueous extracts of some algae contained higher amounts of polar polyphenolic compounds, which can exhibit stronger antioxidant activity (Cikoš et al. 2021; Karimi et al. 2018; Kuda & Ikemori 2009). Five different algae extracts were analysed from four different solvents (chloroform, acetone, benzene and methanol) to determine the effect of antioxidant properties (Seal et al. 2014). Phenolic compounds are key plant antioxidants due to their redox properties, enabling free radical neutralisation. Flavonoids and flavonols, widely present in plants, also exhibit strong antioxidant activity through scavenging and chelation processes. These natural compounds significantly contribute to the antioxidant potential of plants (Florence et al. 2011). The acetone extract had the highest phenolic and flavonoid content, while the chloroform extract had the highest flavonol content. The highest reducing power and radical scavenging activity were found in the methanol extract, likely due to the higher amount of phenolic compounds such as flavonols and flavonoids (Wong et al. 2013). Previous studies have reported that these phenolic compounds are the major plant constituents responsible for strong antioxidant activity (Jimoh et al. 2011; Stanojevi et al. 2009). Generally, the antioxidant activity of natural extracts was associated with phytochemicals, mainly polyphenols and flavonoids (Farasat et al. 2014; Tenorio-Rodriguez et al. 2017). Antioxidant capacity sometimes depended on the nature of the sample, assay conditions and the solvent used to prepare the extracts (Awika et al. 2003). In one study, selected seaweed species extracted with

TABLE 2: Phytochemistry and ethnobotany of RR among the studies included in the review

Study No	Plant Species	Plant Sources	Type of Extracts	Phytochemicals	Ethnobotany (Pharmacological effects)	Ref.
1	*Rhizoclonium riparium*	NA	Acetone, methanol, hexane, aqueous and ethyl acetate extracts	Chlorophyll, carotenoid, flavonoid, phenol.	Antioxidative, antimutagenic	Osuna-Ruíz et al. (2019)
2	*Rhizoclonium hieroglyphicum*	Northern Thailand	Aqueous and ethanolic extracts	Vitamin E, gallic acid, rhamnose, xylose, arabinose, galactose, sulfated polysaccharides.	Antioxidative, antimicrobial	Mungmai et al. (2014)
3	*Rhizoclonium riparium*	Sundarbans mangrove ecosystem, India	Methanolic extract	NA	Antiproliferative	Paul & Kundu (2013)
4	*Rhizoclonium riparium*	NA	Silver Nitrate extract	Chlorophyll, protein, carotenoid.	Algicidal	Roychoudhury et al. (2018)
5	*Rhizoclonium heiroglyphicum*	Tamil Nadu, India	Benzene, methanol, chloroform, petroleum ether and hexane extracts	NA	Antibacterial	Rani et al. (2018)
6	*Rhizoclonium hieroglyphicum*	Kafr El-Sheik Governorate, Egypt.	Ethanol, methanol and acetone extracts	Myristic, palmitic, -linolenic, oleic, stearic acids.	Antibacterial	Morsi et al. (2023)
7	*Rhizoclonium riparium*	Taiwan Fishery Research Institute	Potassium hydrate extracts	Polysaccharides, galactose, glucose, xylose, mannose.	Immunomodulation properties	Hsu et al. 2(006)
8	*Rhizoclonium riparium*	Fish pond aquaculture systems	Aqueous extract	PUFA, lipid, protein, carbohydrates, phenol carotenoids, phytosterols, alkaloids.	Anti-inflammatory	Ripol et al. (2018)
9	*Rhizoclonium hieroglyphicum*	Nan Province, Thailand	Aqueous extract	NA	Skin moisturizer	Leelapornpisid et al. (2014)

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Study No	Plant Species	Plant Sources	Type of Extracts	Phytochemicals	Ethnobotany (Pharmacological effects)	Ref.
10	*Rhizoclonium hieroglyphicum*	West Bengal, India	Methanol, chloroform, benzene and acetone extracts	Flavonoid, flavonol, phenol.	Antioxidative	Seal et al. (2014)
11	*Rhizoclonium hieroglyphicum*	South central Mexico	Hexane extract	Isopimarane diterpenoids.	Antibacterial	Perez Gutierrez & Garcia Baez (2011)
12	*Rhizoclonium riparium*	Mexican Pacific Ocean	Methanol, acetone and hexane extracts	Flavonoids, phenol, chlorophyll a and b, carotenoids.	Antioxidative, antimutagenic, antiproliferative	Osuna-Ruíz et al. (2016)

NA: Data is not available

acetone, hexane and methanol were evaluated. The brown and green seaweeds exhibited higher antioxidative activity compared to red seaweeds, which was attributed mainly to variations in flavonoid and chlorophyll content (Osuna-Ruíz et al. 2016). The antioxidant activity of crude extracts varied depending on the solvent type and its polarity (Marinova & Yanishlieva 1997). So, multiple studies consistently demonstrated strong antioxidant activity in extracts of *Rhizoclonium* species, particularly when using organic solvents such as methanol, acetone and ethanol.

Antimicrobial Activities

A study revealed that antimicrobial ability depended on the strain, the solvent used for extraction, the method of extraction and culture conditions (Mungmai et al. 2014). A previous study reported that *S. platensis* at various concentrations exhibited antimicrobial activity against three gram-positive bacteria (*Bacillus subtilis*, *Bacillus pumilus* and *Staphylococcus aureus*) and three gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) (Bhowmik et al. 2009). In contrast, the aqueous extract of *S. platensis* showed no effective antimicrobial action against *P. aeruginosa* and *E. coli* (Pradhan et al. 2012).

The antibacterial activity varied based on the concentration of algal extracts and the solvent system used, including benzene, methanol, chloroform, petroleum ether and hexane. Among these, the chloroform extract exhibited maximum antibacterial activity (Rani et al. 2018), a result consistent with previous studies (Dhanapal et al. 2016; Yi et al. 2017). Crude methanolic, ethanolic and acetone extracts of RH exhibited significant antibacterial activity, with the ethanolic extract showing the most potent antimicrobial action. This was attributed to its content of long-chain unsaturated and saturated fatty acids (Morsi et al. 2023). The study emphasised that the crude ethanolic extract possessed powerful antibacterial activities due to the presence of abundant bioactive constituents, especially unsaturated fatty acids. One limitation here is the

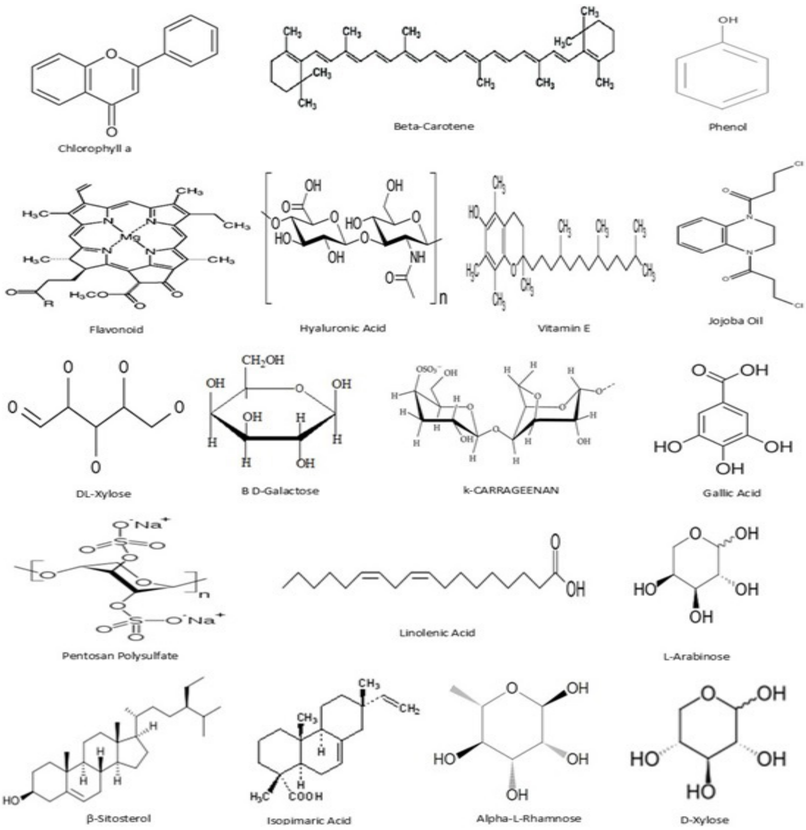

FIGURE 2: Phytochemical compounds obtained from *R. riparium. (Chemical structures were dragged from PubCham)*

variability in tested microbial strains and assay conditions, which makes it difficult to standardise efficacy claims. For example, some aqueous extracts lacked activity against *P. aeruginosa* and *E. coli*, highlighting strain-specific resistance.

Antiproliferative Activities

Antiproliferative activity was evaluated in two different studies, both focusing on the potential effects of methanolic extracts from two types of green algae, *Encephalitozoon intestinalis* and RR, on HeLa and 293T cell lines (Paul & Kundu 2013; Osuna-Ruíz et al. 2016). The results showed that the IC^{50} doses for the 293T cell line were significantly higher than for the HeLa cell line, suggesting that the algal methanol extracts were less toxic to non-cancerous cells. These results clearly indicated that the extracts exhibited potent cytotoxicity on HeLa cells, highlighted by a lower IC^{50} value and thus their antiproliferative properties (Paul& Kundu 2013). There were no prior records of their antiproliferative effects on cervical cancer cell lines. Similar results had been found in studies showing that green and brown seaweed extracts mainly exhibit cytotoxic and antiproliferative properties (Moo-Puc et al. 2009). Future studies should expand to include a wider range of cancer types and normal cells to confirm specificity and safety. However, the limited number of cell lines tested (mainly HeLa and 293T) restricted understanding of broader anticancer efficacy.

Other Ethnomedicinal Properties

Several studies had observed different ethnomedicinal properties, including antimutagenic, algicidal, anti-inflammatory activities and moisturising properties. The antimutagenic activities were assessed in acetone, methanol and hexane extracts. Among these, the acetone crude extract showed higher antimutagenic activity compared to extracts obtained with other solvents (Osuna-Ruíz et al. 2019). Additionally, crude extracts of seaweeds exhibited the highest antimutagenic activity when extracted with acetone and methanol (Osuna-Ruíz et al. 2016). Yet, variations in antimutagenic strength depending on the solvent used indicated the need for optimisation of extraction protocols. The anti-inflammatory properties of five green seaweed species were evaluated using the COX-2 inhibition assay as a screening method (Ripol et al. 2018). Several studies had demonstrated the anti-inflammatory activity of green seaweeds, confirming the presence of such properties in these species (McCauley et al. 2015; Jin et al. 2006). However, detailed mechanistic studies and in vivo validations are still sparse.

One study demonstrated that silver nanoparticles (SNPs), using the agar well diffusion method, exhibited effective algicidal activity against *Chlorococcum infusionum* (Roychoudhury et al. 2018). SNPs can easily penetrate algal cells through diffusion, releasing free radicals capable of breaking down the cell membrane, altering cell structure, creating porosity and ultimately resulting in cell death (Sondi & Salopek-Sondi 2004). Still, the ecological impact of nanoparticle use requires thorough investigation to avoid unintended consequences. A study by Leelapornpisid et al. (2014) demonstrated that the aqueous extract of RH has skin-moisturising properties and may be used as a natural moisturiser. Earlier investigations assessed the hydrating effects of this extract on both human and pig skin, comparing its performance to that of glycerin, hyaluronic acid, propylene glycol and carrageenan using a Corneometer (O'Goshi et al. 2000). Yet, human volunteer numbers were limited (n = 30), and longer-term safety and efficacy studies are warranted. More recent evidence indicates that the extract is an effective moisturiser, comparable to glycerin, hyaluronic acid, and propylene glycol, due to its occlusive properties that help reduce water loss from the skin and enhance the absorption of hydrophilic compounds (Draelos 2001).

Limitations and Potential Prospective

Despite the valuable insights gathered in this scoping review, which analysed 12 studies primarily centered on chemical assays, significant gaps remain in the current understanding of RR (Roth) Harvey. Notably, only a single study has explored its effects using animal and human models, and there is a complete absence of *in vivo* efficacy and safety evaluations through preclinical or clinical trials.

Given these limitations, future research should prioritise comprehensive biological evaluations, including robust *in vivo* studies and well-designed clinical trials, to establish the pharmacological effectiveness and safety profile of RR. Additionally, advanced phytochemical investigations, such as bioactivity-guided fractionation and metabolomics profiling, are essential to identify and characterise the active constituents responsible for its ethnomedicinal properties. Integrating ethnobotanical knowledge with modern pharmacological approaches could pave the way for the development of novel therapeutic agents derived from RR. Furthermore, exploring the ecological and sustainable harvesting aspects could support the conservation of this species while promoting its potential applications in pharmaceutical, nutraceutical, and cosmeceutical industries.

CONCLUSION

Exploring the vast world of algae reveals a rich reservoir of bioactive compounds with unique biological properties, offering promising avenues for the development of innovative pharmaceutical agents. This scoping review highlights that the extracts of RR (Roth) Harvey possess a range of noteworthy biological activities, largely attributed to the diverse phytochemical constituents present within the species. The documented antioxidative, antimicrobial, antiproliferative, and other ethnomedicinal properties underscore the significant therapeutic potential of RR. These findings suggest that RR represents an ideal candidate for future research, warranting detailed pharmacological studies, including *in-vivo* efficacy and safety evaluations. Continued exploration of this alga could contribute to the discovery of novel bioactive compounds and the development of next-generation therapeutic agents.

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