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Anemone rivularis Buch.-Ham. ex DC. (Ranunculaceae) in India: A review on its taxonomy, ethnomedicinal and chemical profiling

Pravesh Rajput, Raza Waris, Priyanka Agnihotri*
Plant Diversity Systematics and Herbarium Division, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow, Uttar Pradesh, India

Abstract
Anemone rivularis Buch.-Ham. ex DC. belongs to family Ranunculaceae, a perennial herb mainly distributed in temperate and subtropical climates in both Northern and Southern hemispheres. In India, it abundantly found in the Indian Himalayan region. This species is chemically, genetically and geographically variable and have variable vernacular names and a wide range of medicinal uses, including rheumatoid arthritis, dysentery, stomach-ache, malaria, stress and anxiety, snakebite, burns, cuts and wounds. *A. rivularis* traditionally used to treat inflammation and cancer. The purpose of this study is to evaluate the existing literature on *A. rivularis* decoction from different aspects: taxonomical, pharmacological, chemical constituents and conservation status. It will provide reference for further research in different species of *Anemone*.

Keywords: *Anemone rivularis*, conservation, distribution, medicinal

Introduction
The genus *Anemone* L. is situated in the tribe Anemoneae belongs to family Ranunculaceae, mainly distributed in temperate and subtropical region of both Northern and Southern hemispheres. The genus encompasses *ca* 200 species, 20 subspecies, and 27 varieties divided into 15 subgenera, 23 sections, 4 subsections and 23 series which are mostly distributed across Europe, North America and Asia [1]. The Greek word "anemos," which means "wind," is the source of the name *Anemone* (Rajput *et al*. 2020). In various countries, more than 50 species of the *Anemone* are used as herbal medicine and having wide range of pharmacological uses. It holds a significant place in several traditional medical systems due to its potent medicinal properties. In India, the genus is known by 26 taxa, including 24 species, one subspecies and one variety from Himalayan Region (both Eastern and Western), some states of Northeast India and Western Ghat [2].
Anemone rivularis, a perennial herb native to the Indian subcontinent, Central China and Vietnam, as well as Sumatra. Common name of this species is "River anemone" or "River windflower" and known by several names in India as Chutrak, ageli, angeli, carbini, carbini mamiri, maruiri, mirchilee, and rattan [3]. Species characterised with rhizomes, basal leaf blades from entire to ternate, tepals which are sparsely pubescent (sometimes glabrous), achenes without projections, glabrous or subglabrous. *Anemone rivularis* produces as many as 20 triterpenoid saponins with anti-inflammatory, antitumor making it a crucial resource of saponin compounds with biological properties in traditional medicine [5-8]. Over the last few decades, ethnobotanical field surveys and pharmacological studies have been conducted to know more about the medicinal properties of *A. rivularis viz*. antioxidant, antibacterial [9], antitumor [10], anti- inflammatory [11] properties of the species. This species shows a broad spectrum of medical applications including rheumatoid arthritis, cancer, diabetes, dysentery, malaria, snakebite and wound healing. The majority of the current research in *A. rivularis* shows numerical chromosomal variation, with 2n=14, 16, 24, and 48 [12-17].

Methodology
The comprehensive information about the plant has been collected from a variety of peer-reviewed research articles, variety of internet sources, including Springer Link, Google Scholar, Google, Scopus, Research Gate, Pub Med, Med Line and Science Direct. The review article is divided into four key sections: distribution, secondary metabolites chemistry and pharmacological activities.

Natural Distribution and Habitat
Anemone rivularis occurs in a wide range of habitats in India such as Indian Himalayan Region (both Eastern Himalaya and Western Himalaya), Western Ghats and North eastern states of India. It covers total nine states of the country *viz*. Jammu & Kashmir, Arunachal Pradesh, Meghalaya, Himachal Pradesh, Nagaland, Uttarakhand, Tamil Nadu, Sikkim and West Bengal (Fig. 1). This species is predominantly found in Himachal Pradesh and Uttarakhand. It grows well in the moist and wet ecological zones of meadows, forest clearings, stream sides, and hedges at altitudes ranging from 2000 to 3900 metres [18]. It's a versatile plant that can tolerate temperatures as low as 20°C. Flowering and fruiting of this species occurs May to October.

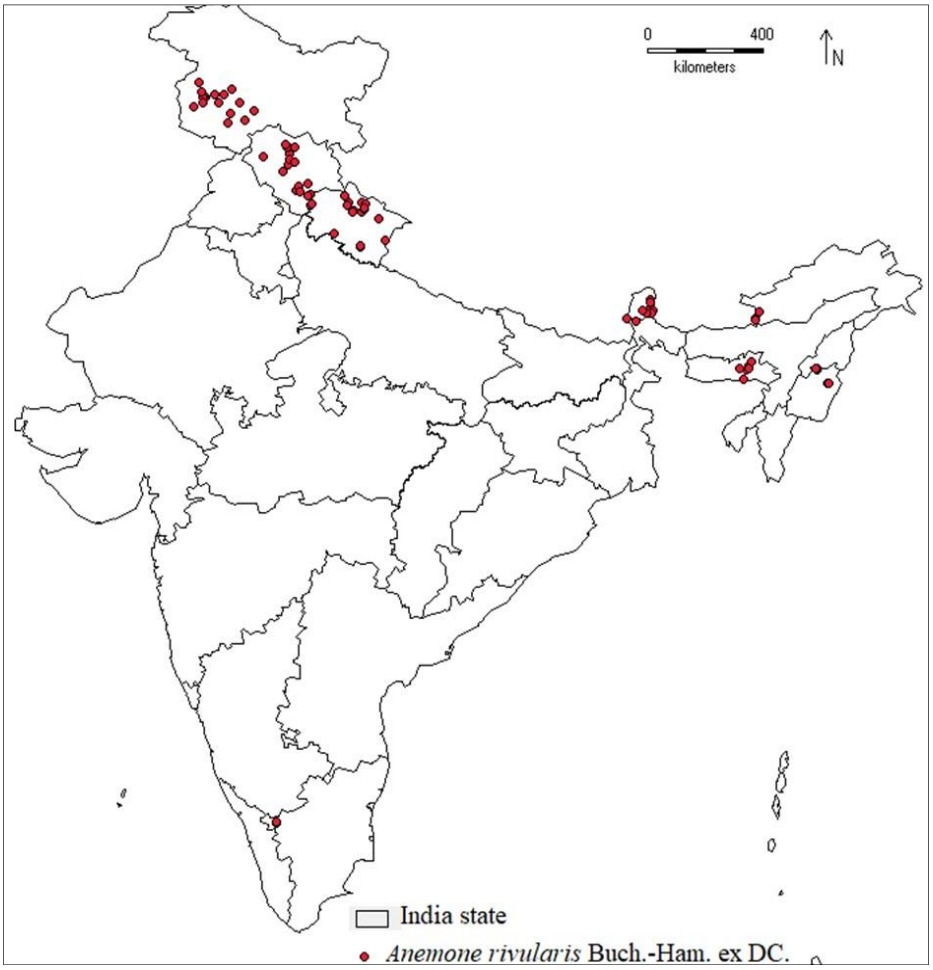

Fig 1: Distribution of *A. rivularis* Buch.-Ham. ex DC. in India.

Taxonomy of *Anemone rivularis* Buch.-Ham. ex DC.

A. rivularis is an annual or perennial herbaceous plant growing to a height of 1m with woody rootstock. Radical leaves of the plant are 10 – 35 cm in length, deeply palmately with long leaf stem, deeply 3–5 lobed, again each lobe 3–4 sub-lobed, serrate at margins, hairy on both abaxial & adaxial surface; cauline leaves palmately divided into many linear lobes; leaflets broadly elliptic, cuneate at base, apex acute, often deeply 3 – lobed, serrate at margins, 4 –7 × 3–5 cm, pubescent with stiff hairs; petioles 10–30 cm long. Scapes stout, 7–35 cm long; involucral leaves similar to leaves, deeply incised, serrate; segments linear, ca 2 mm broad; cymes compound, many-flowered; branches spreading, elongate, 2–bracteolate. White flower, 3 – 4.5 cm in diameter, purple bluish outside. Sepals 5–8, 4 – 10 mm long, elliptic, obovate, obtuse, Silky outside. Filaments slender; anther ovoid. Carpels many; style curved at tip. Achenes broadly elliptic, ca 7 × 5 mm, compressed with ca 1 mm long glabrous (Fig. 2).

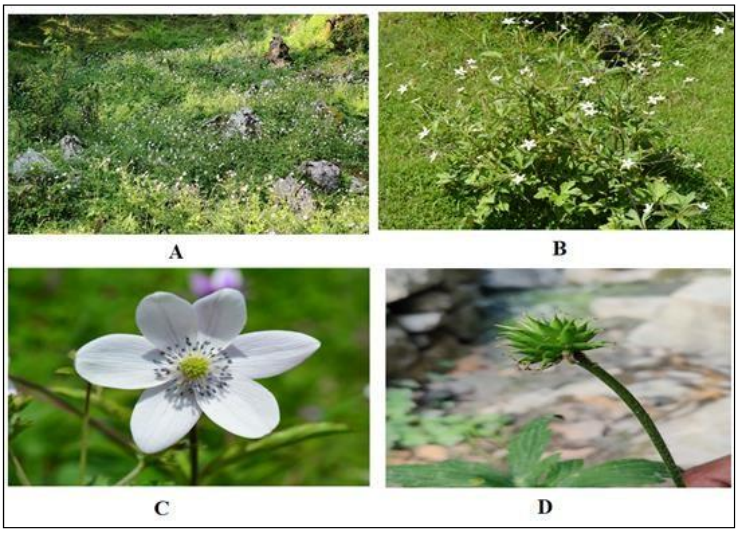

Fig 2: *Anemone rivularis* A. Habitat; B. Habit; C. Flower; D. Achene.

Chemical compounds in *Anemone rivularis*

Phytochemical study of the genus *Anemone* L. has been mainly focused on *Anemone rivularis*. Many different types of secondary metabolites (mostly terpenoids) have been isolated from *A. rivularis*. So far, lots of chemical constituents including triterpenoids, saponins, steroids, coumarins and flavonoids, phenolics, glycosides, carotenoids, lactones, fats and oils, carbohydrates and alkaloids. It also contains ranunculin, anemonin, and protoanemonin. The most bioactive constituents in this plant are triterpenoid saponins (Li *et al*. 2008) [20], the majority of which are oleanane-type saponins, according to comprehensive phytochemical and pharmacological studies (Sun *et al*. 2011). The rhizomes of *A. rivularis* were analyzed phytochemically found new oleanane-type triterpenoid saponins (Mizutani *et al*. 1984). Different types of secondary metabolites displayed in table 1.

Table 1: Bioactive compounds present in *A. rivularis*

S. No.	Classes	Bioactive Compounds	References
1.	Essential oils	Acetophenone, ethyl-2-methyl-hexane, 5,6-dimethyl-decane and 4,5-diethyl-octane, 3-cyclohexanone, 5-methyl-Undecane, Benzyl Alcohol, 2,4-dimethyl Undecane, 2,4-Dodecadienal, Benzaldehyde, α-terpineol, 4- methylbenzoic acid, 2-methyl hexadecane, palmitic acid, linoleic acid and oleic acid, *trans*-caryophyllene oxide, pentadecane, tridecane and undecane or α-cadibene and aromadendren	(9,19,20)
2.	Saponins	A, B, C and D form of huzhangosides, rivularin, oleanolic acid 3-O-α-arabinopyranosyl-(1~2)- α-rhamnopyranosyl-(1~4)-β-glucopyranosyl-(1~4)-β-glucuronopyranoside, rivularinin, sapindoside B, 3-*O*-β-D- xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L arabinopyranosyl oleanolic acid, sieboldianoside A, B, 3-*O*-α-L-arabinopyranosyl gypsogenin 28-*O*-α- L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, hederasaponin B, cauloside D, F, 3β-O-{β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl} oleanolic acid 28-O-β-D-glucopyranoside, pulsatilloside D, kalopanax saponin A, 3β-O-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl} betulinic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester	(5,8,10,21,22)
	Triterpenoid saponins	Olean-9 (11), 12-dien-3-O-palmitate, lupeol, betulin, betulic acid, oleanolic acid, ursolic acid and β-amyrin, 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1 →2)-[β-D-glucopyranosyl-(1→4)]-α-L arabinopyranosyl gypsogenin 28-*O*-β-D-glucopyranosyl ester, 3-*O*-β- D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabino-pyranosyl gypsogenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-*O*-β- D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)-β-D-gluco-pyranosyl-(1→4)]-α-L-arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D- glucopyranosyl-(1→4)]-α-L-arabinopyranosyl 21α-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-[α-L-rhamno-pyranosyl-(1→2)]-α-L-arabinopyranosyl 21α-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D- glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, gypsogenin, 3β-O-β-D-xylopyranosyl-(1→3)-α-L rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl gypsogenin 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3β-O-α-L-arabinopyranosyl betulinic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3β-O-α-L-arabinopyranosyl 21α-hydroxy-oleanolic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3β-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl 21α-hydroxy-oleanolic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester	(8,10, 23,24,25)
3.	Glycosides	Labdane-type diterpene glycoside, trihydroxyfuranoid lignanoid glycoside, b-D-glucopyranosyl (13S)-13-hydroxy-7-oxo-labda-8,14-diene-18-oate and (7S,70 R,8R,80 S)-70 -butoxy-7,90 -epoxy-4,40, 9-trihydroxy-3,30 -dimethoxylignane 9-O-b-D-glucopyranoside	(26)
4.	Aglycones	Gypsogenin, Betulinic acid, 21-hydroxy-oleanolic acid, Hederagenin and Oleanolic acid	(27)

Essential oils

Previous studies have established the presence of essential oils in the genus *Anemone*, which have been demonstrated to be highly efficient against a variety of pathogenic microorganisms. The most common types of phytoconstituent found in all essential oils, tested were diterpenoids and fatty acids. The predominant compounds of *A. rivularis* are essential oils, aliphatic compounds and benzene derivatives, which were obtained by hydro distillation method. Three monoterpenoids (3.6%), six benzene derivatives and ten aliphatic compounds (34.5%) were found in the oil (58.0%). Acetophenone, 3-ethyl-2-methyl-hexane, 5,6-dimethyl-decane, and 4,5-diethyl-octane were the main components in 55.9%,16.2%, 5.7% and 4.5% respectively, depicted in fig. 3 [19, 28-30].

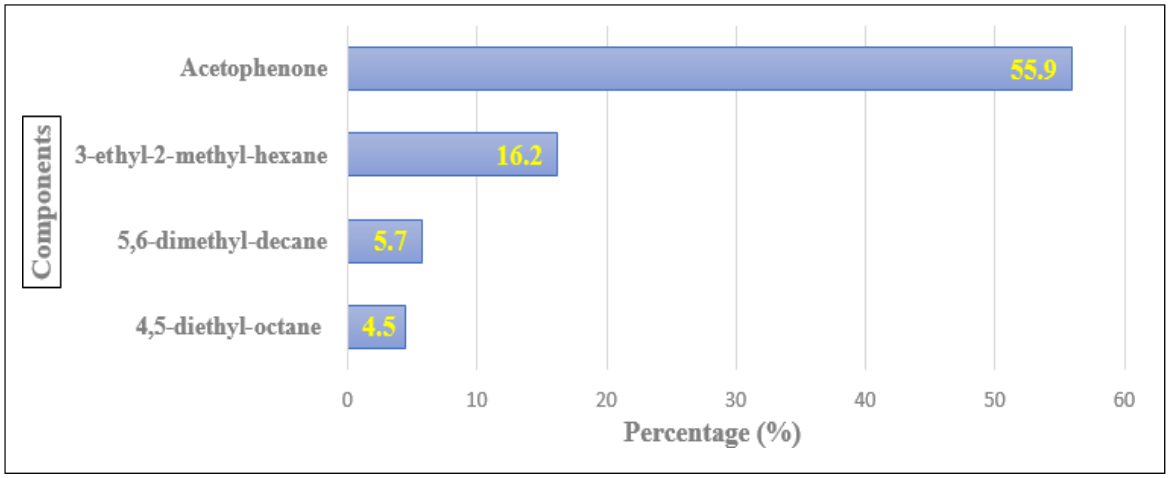

Fig 3: Main components of essential oils

Ethnomedicinal Importance

Anemone rivularis is the most studied species of the genus. Every part of the plant including the rhizomes, roots, leaf, flower and seed were used to treat various ailments [31] (Mabberley 2017) [32]. Figure - 4 showed different parts of plant utilised for therapeutic purposes. *A. rivularis* is used as blood-activating, purification of blood, detoxification, swell-dispersing, muscle contraction, pain-relieving and also used to treat arthritis, traumatic injury, stomach-ache, sore throat, toothache, hypothermia, chronic bronchitis, tonsillitis, hepatitis, gastric disease, jaundice, dysentery, diuresis detumescence, wound healing, pus drainage, peripheral nerve paralysis, snakebite, stubborn dermatitis [20, 32].

Topical application used for snake bites, skin diseases and wound healing. Oils are useful to treat rheumatism, headaches, joint pains and infection of ear. Fruits are used to treat gastritis and as an appetiser; seeds are used to reduce bleeding and to treat oedema. Root decoction is antiseptic, emetic and also be used to clean cuts and wounds [3].

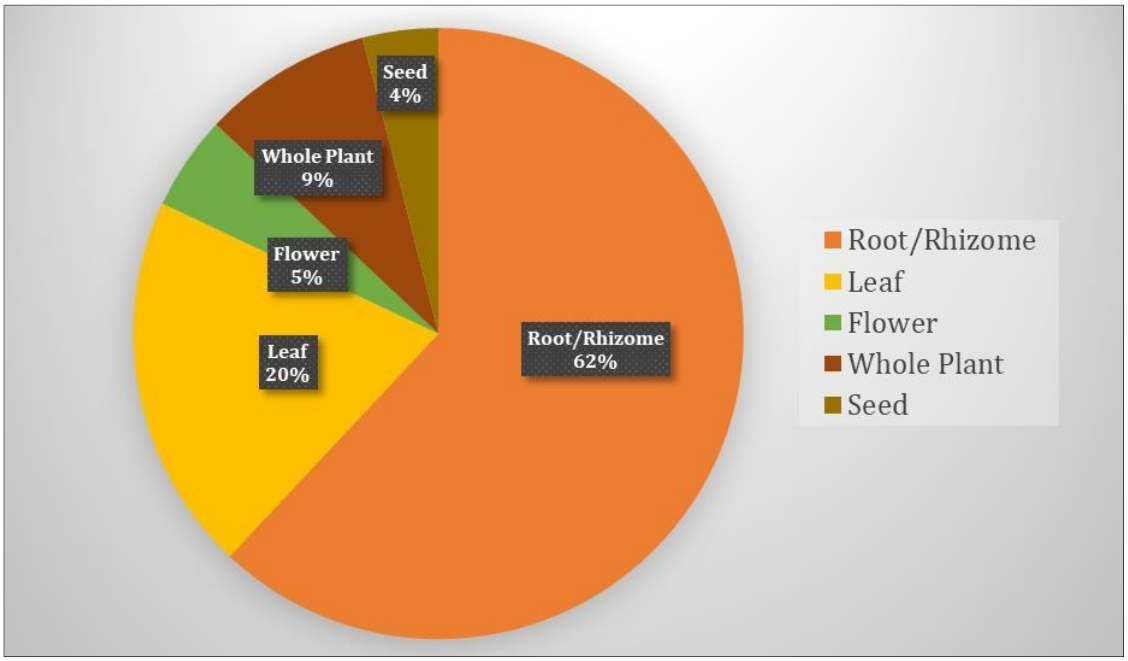

Fig 4: Pie chart showing plant parts used.

Pharmacological Activities

1. Anti-inflammatory activity

Anemonin, ranunculin and saponin are the potent anti-inflammatory compounds are found in genus *Anemone* [33, 34]. Crude saponins obtained from the rhizome of *A. rivularis*, exhibited suppressive effect on rat paw oedema that are induced by carrageenan, formaldehyde and dextran. It also having highest inhibitory effect on the formaldehyde-treated group of these three groups. Furthermore, raddeanoside R1 substantially decreased carrageenan-induced swelling, with a greater effect than crude saponins [5, 22].

2. Antioxidant activity

Essential oils of *A. rivularis* have been shown antioxidant activity, was tested *in-vitro* assays by comparing with synthetic antioxidant drugs like TBHQ and BHT. These synthetic drugs prevent the free DPPH radical & lipid peroxides with $FeSO_4$, H_2O_2, and CCl_4. Essential oils of *A. rivularis* converted the DPPH radical into its diminished form [29].

3. Antitumor activity

The ethanol extract of *A. rivularis* was used in *in-vitro* and cell-based pyruvate dehydrogenase kinases (PDHKs) activity assays after being fingerprinted by HPLC. In an *in vitro* kinase assay, ethanol extract of *A. rivularis* minimized PDHK activity and induced cancer cell apoptosis via the mitochondrial pathway in human DLD-1 colon cancer and murine LLC cells [11]. The cytotoxic effect of all saponin compounds was determined by using different cell line such as lung carcinoma A549 cells, hepatocellular carcinoma HepG2 cells, promyelocytic leukemia HL-60 cells, hepatic stellate cells (HSC-T6) and human cervical carcinoma HeLa cells [10, 27]. The mono desmosidic saponins were found to be cytotoxic to all cell lines, with IC_{50} value ranging from 7.25 to 52.65 M [10, 27].

4. Antibacterial activity

A triterpene ester, olean-9 [11], 12- dien-3-O-palmitate extracted from rhizome of *A. rivularis* by hydrolysed with KOH. This compound showed antimicrobial activity against the Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-positive bacteria (*E. coli, P. aeruginosa, S. typhi*) [25] (Zhao *et al*. 2012) [26]. The inhibition zones at 100 mg/disc and minimum inhibitory concentration (MIC) values for five bacterial strains were in the range of 7.0–14.0 mm and 50–110 mg/ml, respectively. The existence of 3-BHA, acetophenone, patchouli alcohol and benzyl alcohol in the essential oil is most likely responsible for its antibacterial activity. These compounds have been previously documented to disrupt cellular integrity by inhibiting the microbial cell's respiration mechanism [36]. The antibacterial property of essential oils can also be due to the antagonistic effects of almost all of the compounds contained in the oils. *B. subtilus* (1.88) was the most susceptible bacterium, while *P. aeruginosa* has been the most resistant [9] shown in fig. 5.

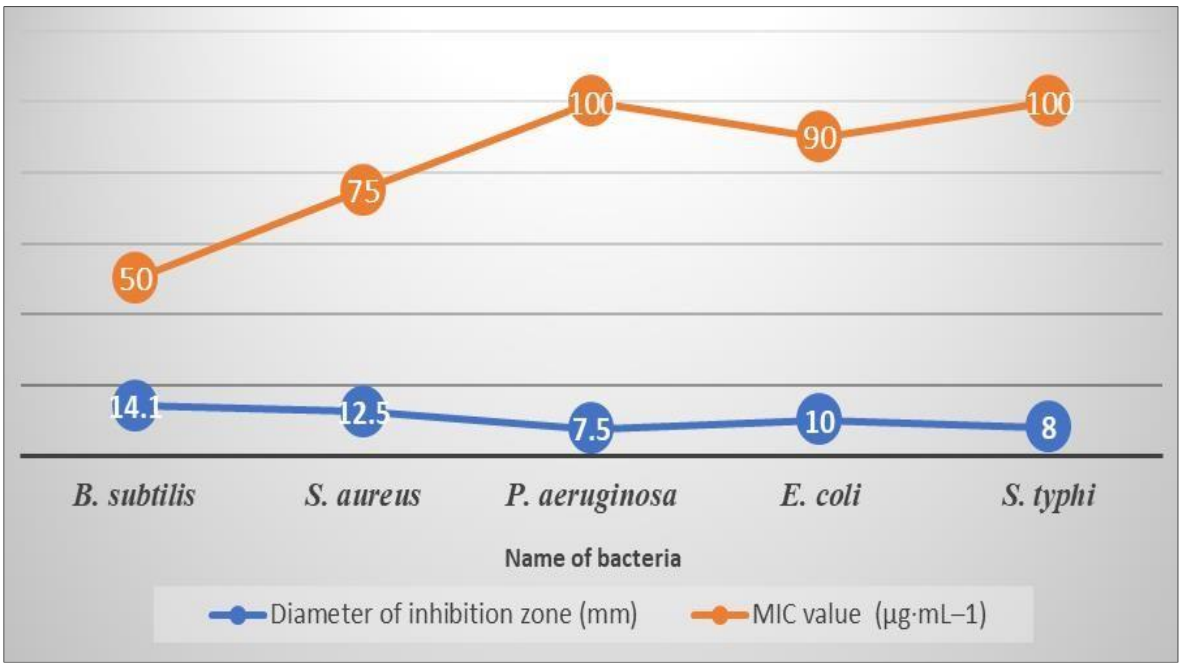

Fig 5: Antibacterial activity against different bacteria

5. Anti-viral Activity

The methanolic extract of *A. rivularis* demonstrated *in-vitro* cytotoxic activity in Vero cells/ HSV-1 (Herpes virus) and A/MDCK cells (influenza virus) with CC50 value 21 µg ml^{-1} and 40 µg ml^{-1} respectively. This extract showed highest antiviral activity against A/MDCK cells as compared to Vero cells/ HSV-1 [36].

Conservation status of *Anemone rivularis*

Anemone rivularis, a herb is abundantly distributed in Himachal Pradesh, Uttarakhand and certain high altitude areas as meadows, forest clearings, stream sides, and hedges of Kashmir, Sikkim, Meghalaya, Nagaland and Arunachal Pradesh in India. The IUCN criteria were used to determine the conservation status of specific medicinal and aromatic species growing in the region [18]. The current habitat, distribution and the populations of *Anemone rivularis* are severely affected by fragmentation, deforestation and selective logging, environmental destruction, livestock grazing, uncontrolled and unscientific species harvesting, unregulated tourism, and road building and other factors [37]. A major threat of *A. rivularis* because their underground components, such as rhizomes and roots, are used in medicine which are commercially important [38].

Conclusion

The current review summarized the taxonomical, phytochemical characterization, traditional medicine uses, pharmacological aspects and conservation status of the *Anemone rivularis* and it provides much more comprehensive understanding and potential of this species. Recent pharmacological findings on *A. rivularis*, validate traditional uses mentioned in ancient herbal medicine. Especially triterpenoids, saponins, steroids, coumarins and flavonoids, phenolic, lactones, fats and oils, carbohydrates, and alkaloids were reported to be responsible for wide range of biological activities such as antioxidant, antimicrobial, antibacterial, anti-inflammatory and anti-cancerous effects. According to recent studies, leaves are the most widely used material as poultice on sores and wounds to kill maggots, while leaves and roots of this plant are used to treat a variety of ailments, including kidney and liver disorders (diuretic, jaundice), skin infections (eczema, wounds, sores and snake bites), gastro- intestinal disorders (constipation, diarrhoea, ulcer), respiratory diseases (cough, bronchitis, asthma), rheumatoid problems and fever.

In conclusion, people are interested in phytochemical and pharmacological research on the genus *Anemone* L. Plants of this genus should be researched and standardize phytochemical formulations with a focus on resource conservation and clinical testing. For commercial use, modern cultivation techniques should be established. Tissue culture techniques must also be established in order to increase production and conserve germplasm of *A. rivularis*. In addition, a detailed pharmacological study and conservation on this genus and is needed to prevent severe genetic erosion of useful genotypes from the population.

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Conflict of Interest

The author declares that there is no conflict of interest.

Authors' Contribution

PA conceived the idea and its implementation of manuscript. PR contributed in data collection & its analysis, and manuscript writing. RW manuscript editing and also contributed to the data collection. Manuscript was critically revised by PA

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