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Review

Zanthoxylum rhetsa (Roxb.) DC: A systematic review on traditional uses, phytochemistry, and pharmacology

[*Zanthoxylum rhetsa* (Roxb.) DC: Una revisión sistemática sobre usos tradicionales, fitoquímica y farmacología]

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Abstract

Context: *Zanthoxylum rhetsa* (Roxb.) DC is a plant rich in essential oils, terpenes, coumarins, and alkaloids, among others, and has traditionally been used by tribal healers for decades. But as yet, there is no systematic and critical review on this plant to document the phytochemistry and pharmacological properties of its isolated compounds.

Aims: To systematically review reports about the ethnobotany, phytochemistry, pharmacological effects and clinical trials of the plant. It also attempts to provide critical assessment of the existing knowledge and research gaps.

Methods: Science Direct, PubMed, Wiley Online Library, and Google Scholar were used to collect the relevant research performed on *Z. rhetsa*, and the discussed dataset finally included 48 articles.

Results: The plant has been used traditionally to treat fever, pain, dysentery, cholera, eczema, and rheumatism, among others. *Z. rhetsa* was found to be abundant in terpenes, coumarins, lignans, alkaloids with broad pharmacological effects, including anti-photo aging, antimicrobial, anti-inflammatory, cytotoxic, antidiarrheal, anthelmintic activities. Studies also revealed its potential use as a food supplement rich in ALA and a functional ingredient in food as a spice. Most pharmacological studies were grounded on crude extracts of the plant, and there is a lack of evidence-based bioactivity-guided research to identify the bioactive compounds. Several research priorities have also been identified, which need to be addressed in the future.

Conclusions: Overall, the review attempts to critically assess the existing knowledge and research gaps that can contribute toward improving the prospect of *Z. rhetsa* as a source of lead molecules in drug discovery.

Keywords: essential oils; Indian prickly ash; terpenes.

Resumen

Contexto: *Zanthoxylum rhetsa* (Roxb.) DC es una planta rica en aceites esenciales, terpenos, cumarinas y alcaloides, entre otros y ha sido utilizada tradicionalmente por curanderos tribales durante décadas. Pero hasta el momento, no existe una revisión sistemática y crítica de esta planta para documentar las propiedades fitoquímicas y farmacológicas de sus compuestos aislados.

Objetivos: Revisar sistemáticamente los informes actualizados sobre la etnobotánica, fitoquímica, efectos farmacológicos y ensayos clínicos de la planta. También intenta proporcionar una evaluación crítica de los conocimientos existentes y las lagunas de investigación.

Métodos: Science Direct, PubMed, Wiley Online Library y Google Scholar se utilizaron para recopilar la investigación relevante realizada sobre *Z. rhetsa*, y el conjunto de datos discutido finalmente incluyó 48 artículos.

Resultados: La planta se ha utilizado tradicionalmente para tratar fiebre, dolor, disentería, cólera, eczema y reumatismo, entre otros. Se encontró que *Z. rhetsa* es abundante en terpenos, cumarinas, lignanos, alcaloides con amplios efectos farmacológicos que incluyen actividades antienvejecimiento, antimicrobianas, antiinflamatorias, citotóxicas, antidiarreicas y antihelmínticas. Los estudios también revelaron su uso potencial como complemento alimenticio rico en ALA y como ingrediente funcional en los alimentos como especia. La mayoría de los estudios farmacológicos se basaron en extractos crudos de la planta y hay una falta de investigación guiada por bioactividad basada en evidencia para identificar los compuestos bioactivos. También han sido identificadas varias prioridades de investigación que deben abordarse en el futuro.

Conclusiones: En general, la revisión intenta evaluar críticamente el conocimiento existente y las brechas de investigación que pueden contribuir a mejorar la perspectiva de *Z. rhetsa* como fuente de moléculas líderes en el descubrimiento de fármacos.

Palabras Clave: aceites esenciales; ceniza espinosa de la India; terpenos.

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INTRODUCTION

The alliance between humans and nature dates back to the very dawn of civilization. From foodstuffs to medicines, humans have invariably relied on nature for their existence and survival. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. According to the estimation of the World Health Organization (WHO), approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (Fabricant and Farnsworth, 2001). Natural products can be attributed to the discovery of potent anti-infective agents (e.g., antibacterial, antifungal, antimalarial, antiviral), cardiovascular, antineoplastic agents (Newman et al., 2000). Therefore, it is imperative to carry out scientific studies on plants to confirm the claims of community folks and traditional healers on their medicinal effects.

Zanthoxylum rhetsa DC. belongs to the genus *Zanthoxylum* of the *Rutaceae* family. It is prevalent in subtropical areas of the world and distributed widely in Bangladesh, India, Sri Lanka, Indonesia, Malaysia, Vietnam and China (Hartley, 1966; Thu et al., 2010). In Bangladesh, it is extensively distributed in Sylhet, Chittagong Hill Tracts, Cox's Bazar, Tangail and Gazipur and utilized by the traditional healers to treat different pathologies of local and tribal people (Yusuf et al., 1994). The plant is also popular among numerous indigenous tribes of the Indian subcontinent and enjoys a number of uses in ethnobotanical practice. But as yet, there is no systematic and detailed review of the secondary metabolites and biological activities of *Z. rhetsa*. Therefore, critical assessment of the existing phytochemical and pharmacological data is imperative to provide the perspectives and directions for future research and potential applications.

The review aims to provide an updated and comprehensive overview of the botany, traditional uses, chemical constituents, and pharmacological activities of *Z. rhetsa*. Furthermore, the assembled data were evaluated critically to offer the strategies and perspectives for further research on *Z. rhetsa*.

MATERIAL AND METHODS

The present study was designed following the guideline provided in the PRISMA statement (Moher et al., 2009). The study design includes the identification of research questions, selection of relevant literature, filtering of these studies based on inclusion and exclusion criteria, and collection and organization of the data (Fig. 1). Relevant literature was retrieved by scrutinizing key scientific databases (Science Direct, PubMed, Wiley Online Library) and search engines (Google Scholar). Books and dissertations were also used to obtain relevant information. The scientific name of the plant was validated using The Plant List. Regarding the search methodology, the following keywords were searched: "*Zanthoxylum rhetsa*", "Bazna", "Indian Prickly Ash". These papers were screened for the respective content in this review, e.g., "traditional uses", "chemical composition", "antimicrobial", "antioxidant", "volatile constituent", "anti-photoaging" and so on. All studies on traditional uses, phytochemistry, pharmacological properties, and bio application were obtained mainly from primary sources. All the identified articles in terms of title, abstract, and content were double-checked according to the inclusion and exclusion criteria (Table 1).

RESULTS

Based on the inclusion and exclusion criteria, 48 studies were finally chosen and critically analyzed to give an overview of the ethnobotany, traditional uses, reported compounds, pharmacology, and bioactivity of the extracts, as well as isolated compounds of *Z. rhetsa*.

Table 1. Inclusion and exclusion criteria for papers.

Inclusion criteria	Exclusion criteria
Journal articles, conference papers, and book chapters written between 1960 and 2020	Papers written in languages other than English
Papers published with title, abstract and full text	Papers with no available full text
Papers about traditional uses, phytochemistry and pharmacological properties of *Z. rhetsa*	Newspapers, posters, and letters to the editors

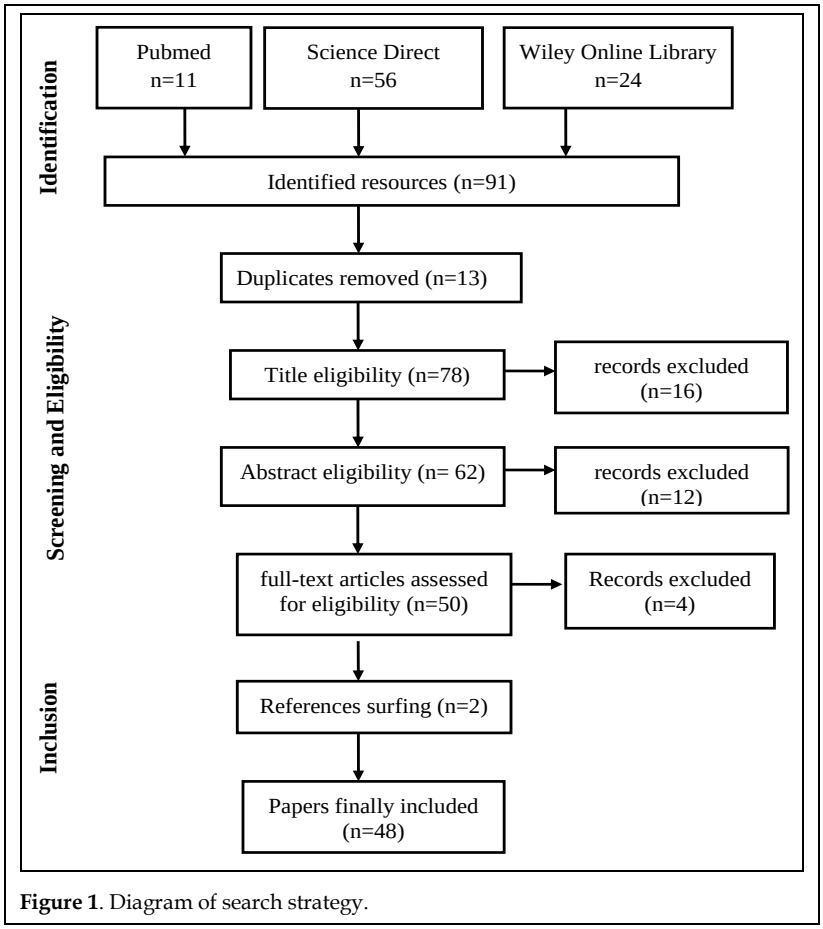

Figure 1. Diagram of search strategy.

Vernacular names

Z. rhetsa (Roxb.) DC (syn. *Fagara rhetsa* Roxb., *Zanthoxylum budrunga* (Roxb.) DC.), commonly known as "Indian Prickly Ash," "Cape Yellowwood," or "Indian Ivy-rue" in English, "Mullilavu," "Mullilam," "Kothumurikku," in India (Joy et al., 1986; Jirovetz et al., 1998; Poornima and Krishnakumar, 2018), "Bazna," "Bazinali," "Kantahorina" in Bangladesh (Alam and Hassan, 2006), "Hantu duri," and "Chenkring" in Malaysia (Wong, 2002), "Makhwaen" in Thailand (Bubpawan et al., 2015), "Rukhboke Timur," and "Gaai Simal Rukh" in Nepal (Rajbhandari and Amatya, 2019).

Taxonomic classification

Kingdom: *Plantae*
Phylum: *Tracheophyta*
Class: *Magnoliopsida*
Order: *Sapindales*
Family: *Rutaceae*
Genus: *Zanthoxylum*
Species: *Zanthoxylum rhetsa* (Roxb.) DC.

Morphology

Zanthoxylum rhetsa DC. (syn. *Fagara rhetsa* Roxb., *Zanthoxylum budrunga* (Roxb.) DC., Fam. *Rutaceae*) is a medium-sized deciduous tree (upto 35 m) with a spreading crown covered with defensive prickles. The plant is widely distributed in the tropical and subtropical regions, including India, Bangladesh, Sri Lanka, Indonesia, Vietnam, China and Malaysia (Hartley, 1966; Thu et al., 2010), and lesser concentrations of species are found in North America and Australia (Brophy et al., 2000). The main stem is generally broad with conical spines and branches, usually armed with tiny ascending conical prickles (Fig. 2). The leaves are compound, imparipinnate, alternate, spirally arranged, and clustered at twig ends. Petioles are glabrous, having 5-8 pairs of oblong or elliptic-oblong leaflets. The plant contains small, greenish-yellow polygamous flowers, which are borne in panicles, at branch-ends or from uppermost leaf axils. Fruits are small, almost round, blackish when ripe, contain black seeds. The whole plant of *Zanthoxylum rhetsa* was collected from Gazipur, Bangladesh, and a voucher specimen (DACB Accession no. 42528) was identified and deposited at the National Herbarium, Bangladesh.

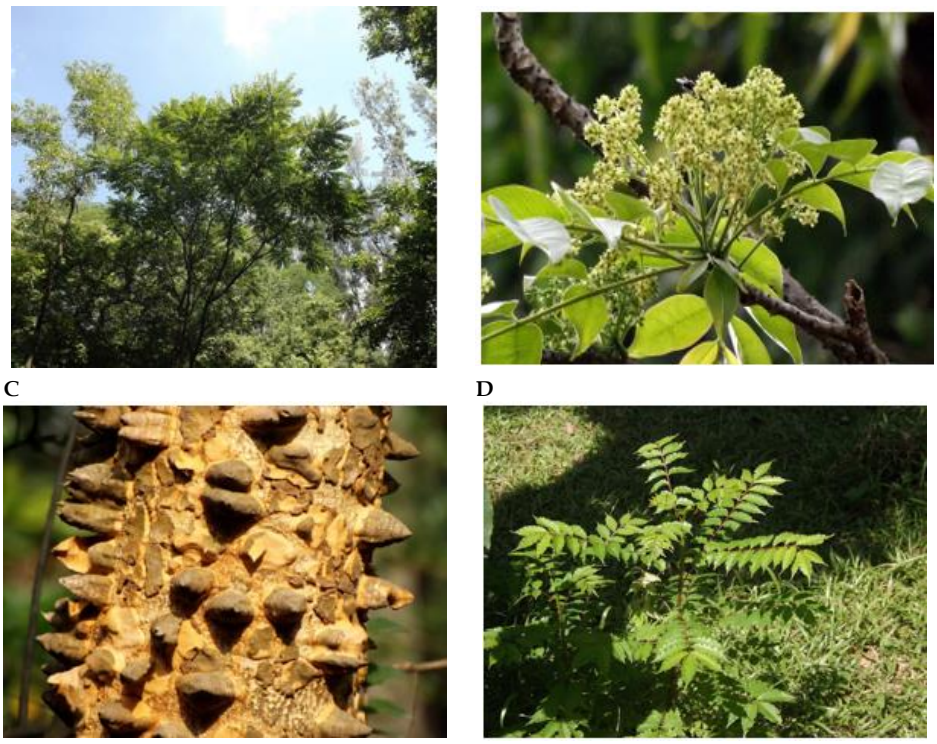

Figure 2. Different parts of *Z. rhetsa*: Mature plant **(A)**; flowers **(B)**; bark **(C)**; leaves **(D)**.
Plant photos reproduced from (Ali, 2018) with permission.

Traditional uses

The plant has long been valued for its medicinal uses, and almost every part of the plant has its own function. It has been traditionally used in folk medicine to reduce toothache as the paste prepared from the plant produces a numbing sensation (Wijaya et al., 2019).

The fruits are used to treat stomachache and diarrhea (Alphonso and Saraf, 2012), and the juice of the bark is considered beneficial in vomiting, cough, dysentery and headache (Kirtikar and Basu, 1993). In India, a paste made from the prickly thorns of *Z. rhetsa* is used by the Kannikar tribes (Tamil Nadu) to treat breast pain and to increase lactation in breast-feeding mothers (Santhanam et al., 2016). Moreover, a tincture of the seeds is usually utilized to treat cholera, and crushed seeds are applied to strengthen bones (Shafi et al., 2006). The extract prepared from the leaves is used as deworming agent (Pai et al., 2009; Yadav and Tangpu, 2009) by Naga tribes of North-Eat India. In Bangladesh, the essential oil collected from the seeds are utilized by local people as an astringent, antiseptic, disinfectant, and to treat dry eczema, dandruff and rheumatism (Yusuf et al., 1994).

In addition, the dried fruits are a popular traditional spice, and the fruits and leaves are used for cooking both sweet and savory preparations (Jirovetz et al., 1998; Rana and Blazquez, 2010). The tender shoots of *Z. rhetsa* are also consumed as food by the Adi tribe (Payum et al., 2013).

Phytochemistry

A diverse array of secondary metabolites has been isolated from the plant *Z. rhetsa* by conducting a number of studies on the constituents of the plant. More than 150 compounds have been isolated and identified, such as alkaloids, alkamides, lignans, coumarins, terpenes, flavonoids and phenolic compounds (Table 2).

Alkaloids

Alkaloids are a large and complex group of cyclic compounds and are important secondary metabolites isolated from *Z. rhetsa*. To date, 29 alkaloids have been isolated (**1-29**) and primarily classified into benzophenanthridine, quinazoline, isoquinoline and quinolone-terpene alkaloids (Fig. 3). The most common alkaloids found in *Z. rhetsa* are benzophenanthridine viz. dihydronitidine (**22**), chelerythrine (**24**), nitidine (**25**) (Santhanam et al., 2016), arnottianamide (**27**), fagaridine (**28**), oxynitidine (**29**) (Zohora et al., 2018). Ahsan et al., 2014 isolated four quinolone-terpene alkaloids chelerybulgarine (**12**), 2′-episimulanoquinoline (**14**), 2,11-didemethoxyvepridimerine B (**15**) and rhetsidimerine (**16**) from the methanolic extraction of *Z. rhetsa* root bark. The quinazoline alka-

loids isolated from *Z. rhetsa* comprise rhetsine (**1**) and rhetsinine (**2**) (Chatterjee et al., 1959). Columbamine (**18**), reticuline (**19**), allocryptopine (**20**) and usambanoline (**21**) are isoquinoline alkaloids collected from the methanolic extract of air-dried, powdered bark material (Santhanam et al., 2016) (Fig. 3).

Table 2. Chemical group, part of plant studied, and chemical constituents isolated from *Z. rhetsa.*

No.	Chemical group	Part of plant	References
ALKALOIDS			
1	Rhetsine	Stem bark	(Chatterjee et al., 1959)
2	Rhetsinine	Stem bark	(Chatterjee et al., 1959)
3	Rhetine	Stem bark	(Chatterjee et al., 1959)
4	Hydroxy evodiamine	Bark	(Gopinath et al., 1960)
5	Skimmianine	Heartwood	(Gupta and Seshadri, 1957)
6	Quinazoline-6-carboxylic acid	Spines on the bark	(Krohn et al., 2011)
7	1-Methoxy-7,8-dehydrorutaecarpine	Spines on the bark	(Krohn et al., 2011)
8	6-Acetonyldihydro-chelerythrine	Spines on the bark	(Krohn et al., 2011)
9	Dihydrochelerythrine	Stem bark and roots	(Tantapakul et al., 2012)
10	8-Acetonyldihydronitidine	Stem bark and roots	(Tantapakul et al., 2012)
11	Dictamine	Stem bark and roots	(Tantapakul et al., 2012)
12	Chelerybulgarine	Root bark	(Ahsan et al., 2014)
13	Simulanoquinoline	Root bark	(Ahsan et al., 2014)
14	2′-Episimulanoquinoline	Root bark	(Ahsan et al., 2014)
15	2,11-Didemethoxyvepridimerine B	Root bark	(Ahsan et al., 2014)
16	Rhetsidimerine	Root bark	(Ahsan et al., 2014)
17	8-Methoxy-N-methylflindersine	Stem bark	(Ahsan et al., 2000)
18	Columbamine	Bark	(Santhanam et al., 2016)
19	Reticuline	Bark	(Santhanam et al., 2016)
20	Allocryptopine	Bark	(Santhanam et al., 2016)
21	Usambanoline	Bark	(Santhanam et al., 2016)
22	Dihydronitidine	Bark	(Santhanam et al., 2016)
23	N-Methyllaurotetanine	Bark	(Santhanam et al., 2016)
24	Chelerythrine	Bark	(Santhanam et al., 2016)
25	Nitidine	Bark	(Santhanam et al., 2016)
26	Zanthodioline	Root bark	(Zohora et al., 2018)
27	Arnottianamide	Root bark	(Zohora et al., 2018)
28	Fagaridine	Root bark	(Zohora et al., 2018)
29	Oxynitidine	Root bark	(Zohora et al., 2018)
TERPENES			
30	α-Pinene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
31	β-Pinene	Fruits	(Joy et al., 1986)
32	Camphene	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998)
33	Sabinene	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998)
34	Myrcene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
35	α-Phellandrene	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998)

Table 2. Chemical group, part of plant studied, and chemical constituents isolated from *Z. rhetsa* (continued…)

No.	Chemical group	Part of plant	References
36	β-Phellandrene	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998)
37	α-Terpinene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
38	γ -Terpinene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
39	Limonene	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998; Shafi et al., 2006)
40	Carene	Fruits	(Brophy et al., 2000)
41	*p*-Cymene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
42	Terpinolene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
43	α-Terpineol	Fruits	(Joy et al., 1986)
44	Linalool	Seeds	(Jirovetz et al., 1998)
45	Trans-β-ocimene	Seeds	(Jirovetz et al., 1998)
46	Linalyl acetate	Seeds	(Jirovetz et al., 1998)
47	Terpinen-1-ol	Seeds	(Shafi et al., 2006)
48	Terpinen-4-ol	Seeds/seed coat	(Shafi et al., 2000; Shafi et al., 2006; Rana and Blazquez, 2010)
49	α-Thujene	Seeds/seed coat	(Shafi et al., 2006; Rana and Blazquez, 2010)
50	1,8-Cineole	seeds	(Shafi et al., 2006)
51	*p*-2,4(8) Menthadiene	seeds	(Shafi et al., 2006)
52	Cryptone	Fruits	(Bubpawan et al., 2015)
53	Sabinol	Fruits	(Bubpawan et al., 2015)
54	Carveol	Fruits	(Bubpawan et al., 2015)
55	Cuminol	Fruits	(Bubpawan et al., 2015)
56	1-Carvone	Fruits	(Bubpawan et al., 2015)
57	Phellandral	Fruits	(Bubpawan et al., 2015)
58	Carvacrol	Fruits	(Bubpawan et al., 2015)
59	Geranyl acetate	Fruits	(Bubpawan et al., 2015)
60	Safranal	Leaf	(Shafi et al., 2000)
61	Terpeneolene	Seed coat	(Rana and Blazquez, 2010)
62	*cis*-Sabinene hydrate	Seed coat	(Rana and Blazquez, 2010)
63	*trans*-Sabinene hydrate	Seed coat	(Rana and Blazquez, 2010)
64	*cis*-p-Menth-2-en-1-ol	Seed coat	(Rana and Blazquez, 2010)
65	*trans*-p-Menth-2-en-1-ol	Seed coat	(Rana and Blazquez, 2010)
66	α-Terpinyl acetate	Seed coat	(Rana and Blazquez, 2010)
67	Neryl acetate	Seed coat	(Rana and Blazquez, 2010)
68	3,5-Dimethoxy-4 geranyloxycinnamyl alcohol	Stem bark	(Ahsan et al., 2000)
69	*p*-Menthan-1α,2β,4β triol	Whole plant	(Paknikar and Kamat, 1993)
70	γ-Cadinene	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998)
71	β-Elemene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
72	β-Caryophyllene	Fruits/seeds/leaf	(Joy et al., 1986; Jirovetz et al., 1998; Brophy et al., 2000)
73	α-Farnesene	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998)

Table 2. Chemical group, part of plant studied, and chemical constituents isolated from *Z. rhetsa* (continued…)

No.	Chemical group	Part of plant	References
74	Farnesol	Fruits/seeds	(Joy et al., 1986; Jirovetz et al., 1998)
75	δ-Cadinene	Seeds	(Jirovetz et al., 1998; Shafi et al., 2006)
76	δ-Cadinol	Seeds	(Jirovetz et al., 1998)
77	β-Cubebene	Seeds/leaf	(Jirovetz et al., 1998; Brophy et al., 2000)
78	Germacrene D	Seeds/leaf	(Jirovetz et al., 1998; Brophy et al., 2000)
79	β-bisabolene	Seeds	(Jirovetz et al., 1998)
80	Spathulenol	Seeds/leaf	(Jirovetz et al., 1998; Brophy et al., 2000; Shafi et al., 2000)
81	α-Selinene (s)	Seeds	(Jirovetz et al., 1998; Shafi et al., 2006)
82	β-Selinene	Seeds	(Shafi et al., 2006)
83	Germacrene B	Seeds	(Jirovetz et al., 1998)
84	α-Humulene	Seeds/leaf	(Jirovetz et al., 1998; Brophy et al., 2000)
85	α-Cubebene	Leaf	(Brophy et al., 2000)
86	δ-Elemene	Leaf	(Brophy et al., 2000)
87	Bicycloelemene	Leaf	(Brophy et al., 2000)
88	α-Copaene	Leaf/seed	(Brophy et al., 2000; Shafi et al., 2006)
89	β-Copaene	Leaf/seed	(Shafi et al., 2000; 2006)
90	β-Bourbonene	Leaf	(Brophy et al., 2000)
91	α-Gurjunene	Leaf	(Brophy et al., 2000)
92	Aromadendrene	Leaf	(Brophy et al., 2000)
93	Alloaromadendrene	Leaf	(Brophy et al., 2000)
94	Viridiflorene	Leaf	(Brophy et al., 2000)
95	α-Muurolene	Leaf	(Brophy et al., 2000)
96	Caryophyllene oxide	Leaf	(Brophy et al., 2000)
97	Epi-globulol	Leaf	(Brophy et al., 2000)
98	Ledol	Leaf	(Brophy et al., 2000)
99	Cubeban-11-ol	Leaf	(Brophy et al., 2000)
100	Cubenol	Leaf	(Brophy et al., 2000)
101	Epi-cubenol	Leaf	(Brophy et al., 2000)
102	Globulol	Leaf	(Brophy et al., 2000)
103	Viridiflorol	Leaf	(Brophy et al., 2000)
104	Cadinol T	Leaf	(Brophy et al., 2000)
105	Muurolol T	Leaf	(Brophy et al., 2000)
106	α-Muurolol	Leaf	(Brophy et al., 2000)
107	α-Cadinol	Leaf	(Brophy et al., 2000)
108	(*E*)-Nerolidol	Seed coat	(Rana and Blazquez, 2010)
109	Lupeol	Root bark	(Chatterjee et al., 1959; Ahsan et al., 2014)
FLAVONOID			
110	Hesperetin	Bark	(Santhanam et al., 2016)

Table 2. Chemical group, part of plant studied, and chemical constituents isolated from *Z. rhetsa* (continued…)

No.	Chemical group	Part of plant	References
ALKAMIDE			
111	Zanthorhetsamide	Stem bark and roots	(Tantapakul et al., 2012)
112	N-(4-Methoxyphenethyl) benzamide	Stem bark and roots	(Tantapakul et al., 2012)
113	Alatamide	Stem bark and roots	(Tantapakul et al., 2012)
LIGNAN			
114	Asarinin	Stem bark and roots	(Tantapakul et al., 2012)
115	Horsfieldin	Stem bark and roots	(Tantapakul et al., 2012)
116	Sesamin	Stem bark	(Ahsan et al., 2000)
117	Kobusin	Bark	(Santhanam et al., 2016)
118	Yangambin	Bark	(Santhanam et al., 2016)
119	Eudesmin	Bark	(Santhanam et al., 2016)
120	*epi*-Eudesmin	Bark	(Santhanam et al., 2016)
121	8-Hydroxy-4'-methoxypinoresinol	Bark	(Santhanam et al., 2016)
122	Magnolin	Bark	(Santhanam et al., 2016)
123	Sinapyl alcohol	Bark	(Santhanam et al., 2016)
124	Pluviatilol	Root bark	(Zohora et al., 2018)
COUMARIN			
125	Suberosin	Heartwood	(Gupta and Seshadri, 1957)
126	5,7,8-Trimethoxycoumarin	Stem bark and roots	(Tantapakul et al., 2012)
127	Xanthyletin	Stem bark	(Ahsan et al., 2000)
128	Scoparone	Bark	(Santhanam et al., 2016)
FATTY ACIDS			
129	Nonanoic acid	Seeds	(Jirovetz et al., 1998)
130	Dodecanoic acid	Bark (spine)	(Lalitharani and Mohan, 2010)
131	Tetradecanoic acid	Bark (spine), seeds	(Lalitharani and Mohan, 2010; Naik, 2015)
132	n-Hexadecanoic acid	Bark (spine)	(Lalitharani and Mohan, 2010)
133	9,12-Octadecadienoic acid	Bark (spine)	(Lalitharani and Mohan, 2010)
134	Oleic acid	Bark (spine)	(Lalitharani and Mohan, 2010)
135	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	Bark (spine)	(Lalitharani and Mohan, 2010)
136	α-Linolenic acid	Fruits	(Bubpawan et al., 2015)
137	Linoleic acid	Fruits	(Bubpawan et al., 2015)
138	Palmitic acid	Fruits	(Bubpawan et al., 2015)
139	Stearic acid	Seeds	(Naik, 2015)
140	Arachidic acid	Seeds	(Naik, 2015)
141	Palmitoleic acid	Seeds	(Naik, 2015)
142	Cis-10-pentadecenoic acid	Seeds	(Naik, 2015)

Table 2. Chemical group, part of plant studied, and chemical constituents isolated from *Z. rhetsa* (continued…)

No.	Chemical group	Part of plant	References
PHENOLIC COMPOUNDS AND ALIPHATIC HYDROCARBONS			
143	Quinic acid	Bark	(Santhanam et al., 2016)
144	4-Vinyl syringol	Bark	(Santhanam et al., 2016)
145	Octanal	Seeds	(Jirovetz et al., 1998)
146	Nonanal	Seeds	(Jirovetz et al., 1998)
147	Decanal	Seeds	(Shafi et al., 2000)
148	Dodecanal	Seed coat	(Rana and Blazquez, 2010)
149	Tetradecanal	Seed coat	(Rana and Blazquez, 2010)
150	2-Tridecanone	Leaf	(Brophy et al., 2000)
151	2-Nonanone	Seed coat	(Rana and Blazquez, 2010)
152	2-Undecanone	Seed coat	(Rana and Blazquez, 2010)
153	Undecane	Seeds	(Jirovetz et al., 1998)
154	1-Hexen-3-ol	Seeds	(Jirovetz et al., 1998)
155	Decyl acetate	Seed coat	(Rana and Blazquez, 2010)
156	Pentyl cyclopropane	Fruits	(Bubpawan et al., 2015)

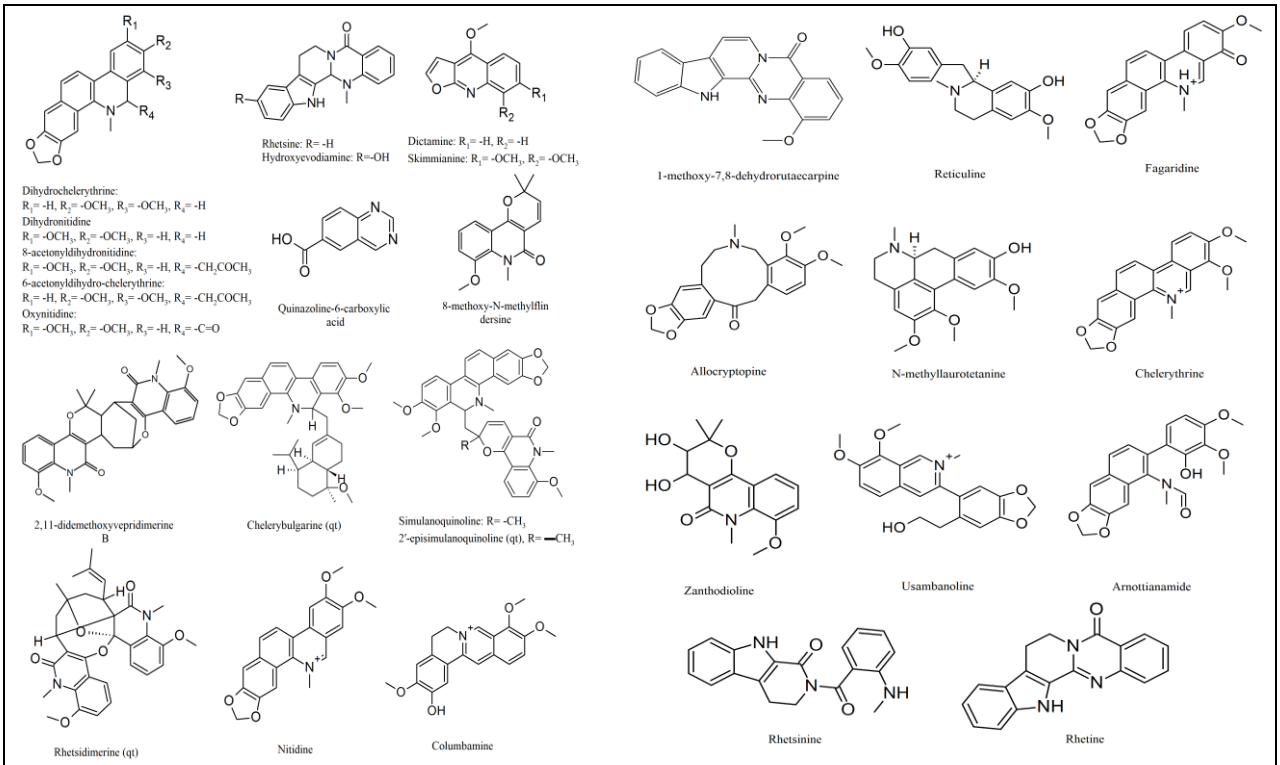

Figure 3. Chemical structures of alkaloids isolated from *Z. rhetsa*.

Terpenes

Terpenes and oxygenated monoterpenes are the most significant secondary metabolites of the plant. Till date, 79 terpenes have been isolated (**30-109)** from different parts of *Z. rhetsa* and classified as monoterpenes (**30-69**), sesquiterpenes (**70-108**) and triterpene (**109**) (Fig. 4). Lupeol (**109**) is the only triterpene that has been isolated from the methanol extract of the *Z. rhetsa* root bark (Chatterjee et al., 1959; Ahsan et al., 2014). Monoterpenes and sesquiterpenes are the major terpenes in *Z. rhetsa* and most common are the

bicyclic monoterpenes α- pinene (**30**), β- pinene **(31**), sabinene, and limonene (**39)** found in the oil collected from the air-dried fruits and seeds (Joy et al., 1986; Shafi et al., 2000; Bubpawan et al., 2015); bicyclic sesquiterpene β-caryophyllene (**72**), α-copaene (**88**) and β-copaene (8**9**) isolated from the leaf oil (Brophy et al., 2000; Shafi et al., 2000). A number of monoterpene alcohols, namely α-terpineol (**43**), linalool (**44**), terpinen-1-ol (**47**), terpinen-4-ol (**48**), *p*-menthan-1α,2β,4β triol (**69**) were isolated from *Z. rhetsa* seeds and fruit oil (Joy et al. 1986; Jirovetz et al., 1998; Rana and Blazquez, 2010) among which terpinene-4-ol (**48**) is a major constituent of the collected essential oil. Brophy et al. (2000) also isolated some sesquiterpene alcohols from the leaf oil of *Z. rhetsa* viz. spathulenol (**80**), epiglobulol (**97**), cubeban-11-ol (**99**), globulol (**102**), cadinol T (**104**), muurolol T (**105**), α-cadinol (**107**). It is evident from the literature study that monoterpenes such as sabinene (**33**), α-pinene (**30**), cymene (**41**), limonene (**39**), terpinene-4-ol (**48**) are predominant in *Z. rhetsa* fruits and seeds, whereas the leaf oil is rich in sesquiterpenes viz. β-caryophyllene (**72**), β-copaene (**89**), spathulenol (**80**) and germacrene D (**78**) (Brophy et al., 2000; Shafi et al., 2000).

Flavonoids and alkamides

Till date, a flavanone named hesperetin (**110)** has been identified from the ethyl acetate fraction of *Z. rhetsa* bark (Santhanam et al., 2016). Alkamides are natural products formed by connecting straight-chain, mostly unsaturated, aliphatic acids with various amines by an amide linkage (Greger, 2016). Zanthorhetsamide (**111**), N-(4-methoxyphenethyl)-benzamide (**112**), and alatamide (**113**) are alkamides isolated from the acetone fraction of the stem bark and root of the plant (Tantapakul et al., 2012) (Fig. 5).

Lignans and coumarins

Lignans are a group of compounds that are dimers of phenylpropanoids units linked by the central carbons of their side-chains (MacRae and Towers, 1984). Till now, 11 lignans have been isolated (**114-124**) from *Z. rhetsa* stem bark and roots (Fig. 6). Suberosin (**125**), 5,7,8-trimethoxycoumarin (**126**), xanthyletin (**127**), and scoparone (**128**) are the coumarins isolated from different parts of the plant (Fig. 6).

Fatty acids and phenolic compounds

A huge collection of fatty acids is synthesized by plants, and in the case of *Z. rhetsa,* the most abundant are n-hexadecenoic acid (**132**) and oleic acid (**134**) collected from the ethanolic extract of the bark spine of *Z. rhetsa* (Lalitharani and Mohan, 2010). Essential oil collected from the air-dried fruits also contains a high quantity of α-linolenic acid (**136**) and linoleic acid (**137**) (Bubpawan et al., 2015). Colorless and fragrant liquid aldehydes viz. octanal (**145**), nonanal (**146**) (Jirovetz et al., 1998), decanal (**147**) (Shafi et al., 2000), dodecanal (**148),** tetradecanal (**149**) (Rana and Blazquez, 2010) were also collected from the freshly dried seed coats (Fig. 7).

Biological activities

Anti-photo aging activity

Plant extracts and phytochemical constituents, which have the ability to inhibit elastase and collagenase are in high demand nowadays because of their potential use in delaying the skin's aging process (Thring et al., 2009). Santhanam et al. (2018) tested different solvent extracts obtained from the stem bark of *Zanthoxylum rhetsa* and hesperidin, a major constituent isolated from the ethyl acetate extract, for a number of anti-photo aging assays. All fractions exhibited moderate to strong inhibition of elastase, and the strongest inhibition was seen in ethyl acetate fraction with an IC_{50} = 65 ± 2.29 µg/mL, compared to the IC_{50} = 58 ± 1.10 µg/mL of standard epigallocatechin gallate (EGCG). Santhanam et al. (2018) also determined the SPF value of the most promising ethyl acetate fraction and the bioactive isolated constituent hesperidin to be 13.36 and 13.39, respectively, both of which were higher than the 10.34 SPF value of EGCG. Both the bark extract and pure hesperidin demonstrated significant inhibition of UVB-induced cytotoxicity in human dermal fibroblast (HDF) cells in a dose-dependent manner. In addition to that, the ethyl acetate fraction and hesperidin both were successful in preventing expressions of a number of pro-inflammatory mediators such as NF-κB and matrix metalloproteinases (MMP 1, MMP 3 and MMP 9) in the HDF cells. This suggests that bioactive compounds isolated from *Z. rhetsa* may have a potential therapeutic effect as active constituents in sunscreen and anti-photo aging preparations (Table 3).

Ethyl acetate fractions of *Zanthoxylum rhetsa* bark were also utilized as natural sunscreen formulations by Santhanam et al. (2017). The formulations were rated to have a category of two stars in the Boot Star Rating system, which meant that they had a moderate level of UVA protection (Wang et al., 2008) and also exhibited high ranges of critical wavelength (365.3 and 360.4 nm, respectively), which according to FDA guidelines nearly qualifies it as a 'broad spectrum sunscreen' (Wang and Lim, 2011).

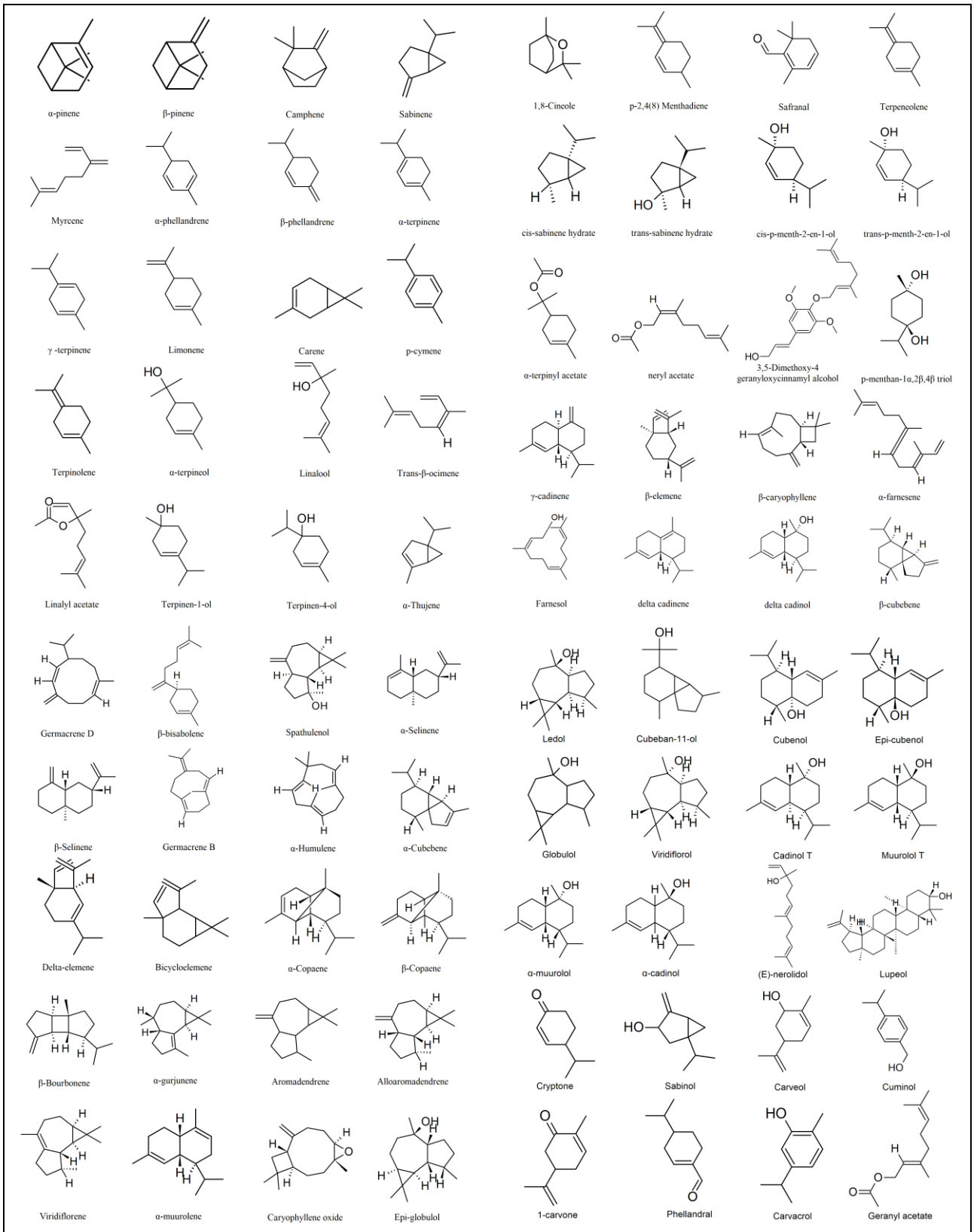

Figure 4. Chemical structures of terpenes present in *Z. rhetsa*.

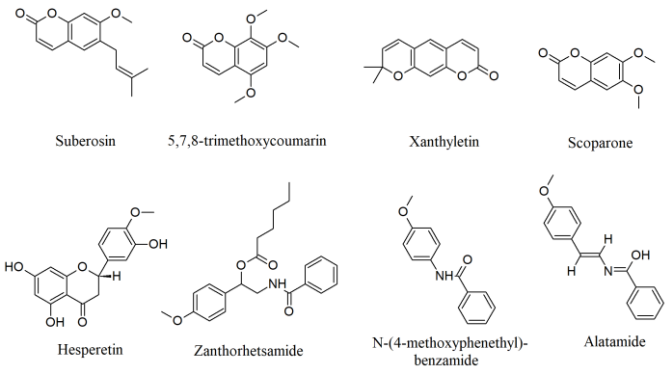

Figure 5. Chemical structures of flavonoid, coumarin and alkamides found in *Z. rhetsa.*

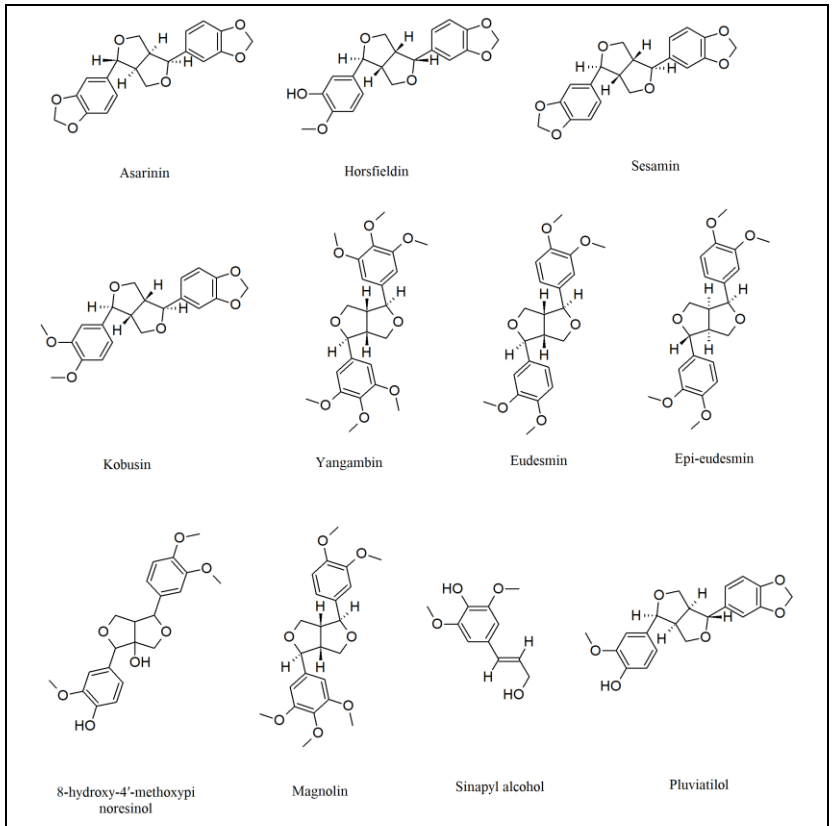

Figure 6. Chemical structures of lignans present in *Z. rhetsa.*

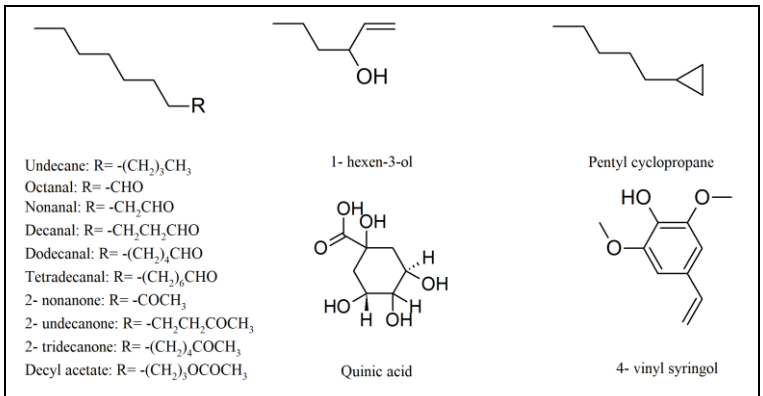

Figure 7. Chemical structures of phenolic compound and hydrocarbons present in *Z. rhetsa.*

Table 3. Summary of the pharmacological activities of *Z. rhetsa.*

Pharmacological activity	Tested substance	Model used	Tested dose	Results	Reference
Anti-photo aging activity	Multiple solvent extract of stem bark and an isolated bioconstituent hesperidin	Antielastase assay	0-400 µg/mL doses	All fractions exhibited moderate to strong inhibition of elastase. Maximum ethyl acetate fraction with an IC_{50} value of 65 ± 2.29 µg/mL	(Santhanam et al., 2018)
	Multiple solvent extract of stem bark and an isolated bioconstituent hesperidin	Inhibition of bacterial collagenase via disk diffusion method using gelatin	500-15.625 µg/mL serially diluted doses	Butanol and ethyl acetate fractions showed significant inhibition of collagenase with 45% and 50% inhibition respectively	(Santhanam et al., 2018)
	Ethyl acetate extract of stem bark and an isolated bioconstituent hesperidin	UVB induced cytotoxicity in Human Dermal Fibroblast (HDF) cells	500, 250 and 125 µg/mL	The ethyl acetate extract and pure hesperidin demonstrated significant inhibition of UVB induced cytotoxicity in the cells in a dose dependent manner	(Santhanam et al., 2018)
	Ethyl acetate extract of stem bark and an isolated bioconstituent hesperidin	Inhibition of expressions of several pro-inflammatory mediators such as NF- κB and matrix metalloproteinases (MMP 1, MMP 3 and MMP 9) in the HDF cells	250 and 125 µg/mL	The ethyl acetate fraction and hesperidin both were successful in preventing expressions of the pro-inflammatory mediators	(Santhanam et al., 2018)
	Ethyl acetate extract of stem bark and an isolated bioconstituent hesperidin	SPF value	-	Ethyl acetate fraction = 13.36 µg/mL Hesperidin = 13.39 µg/mL	(Santhanam et al., 2018)
	Ethyl acetate and butanol extract of stem bark	SPF value	-	Ethyl acetate fraction = 13.36 µg/mL Butanol fraction = 8.62 µg/mL	(Santhanam et al., 2013)
Antimicrobial activity	Five bioactive alkaloids obtained from the bark	Zone of inhibition determination by disc diffusion method	50 µg of test substance per test filter disc	Several alkaloids gave moderate to good activity against *Escherichia coli*, *Bacillus megaterium*, *Microbotryum violaceum* and *Chlorella fusca*	(Krohn et al., 2011)
	Alkaline, acidic and aqueous extracts obtained from the seeds	Zone of inhibition determination by disc diffusion method	50 µL per disc	All extracts showed good activity against *Staphylococcus aureus*, *Proteus vulgaris, Klebsiella pneumoniae* and *Escherichia coli*	(Reddy and Jose, 2011)
	Ten compounds isolated from the roots and stem bark	Minimum inhibition concentrations (MICs) determination using Mueller Hinton broth	-	Only dihydrochelerythrine showed strong activity against MRSA and mild activity against *E. coli*	(Tantapakul et al., 2012)
	Different fraction of essential oil extracted from the pericarp	Minimum inhibition concentrations (MICs) determination using agar diffusion method	50 µL of sample in 30% DMSO	The fractions gave MIC value ranging between 35-140 µg/mL against *S aureus, E. coli and K. pneumonia* species	(Naik et al., 2015)

Table 3. Summary of the pharmacological activities of *Z. rhetsa* (continued…)

Pharmacological activity	Tested substance	Model used	Tested dose	Results	Reference
Antioxidant activity	Ethyl acetate, butanol, methanol, chloroform and hexane extracts obtained from bark	Total phenolic content determination by Folin-Ciocalteu's method	1 mg/mL	Ethyl acetate fraction = 20.47±0.09 GAE/g Butanol fraction = 14.14±0.19 GAE/g	(Santhanam et al., 2013)
	Edible portion extract	Total phenolic content determination by Folin-Ciocalteu's method	100 µL of extract	6.01 mg GAE/g of fresh weight	(Tukun et al., 2014)
	Ethyl acetate, butanol, methanol, chloroform and hexane extracts obtained from bark	Total flavonoid content assay using $AlCl_3$	1 mg/mL	Ethyl acetate fraction = 1.59±0.12 mg QE/g Butanol fraction = 3.07±0.24 mg QE/g	(Santhanam et al., 2013)
	Ethyl acetate, butanol, methanol, chloroform and hexane extracts obtained from bark	DPPH free radical scavenging assay	20 – 500 µg /mL	Ethyl acetate and butanol fraction gave IC_{50} values of 140 and 168 µg/mL, respectively	(Santhanam et al., 2013)
	Edible portion extract	DPPH free radical scavenging assay	200, 400 and 800 µL of extract	7.54 µmol trolox equivalent per gram of fresh weight	(Tukun et al., 2014)
	Hexane and ether extract of seeds	DPPH free radical scavenging assay	125-2000 µg /mL	Hexane and ether fraction gave IC_{50} values of 266 and 265 µg/mL, respectively	(Naik, 2015)
	Essential oil obtained from the pericarp	DPPH free radical scavenging assay	3-750 µg/mL	IC50 value of 7.5 µg/mL	(Naik et al., 2015)
	Ethyl acetate, butanol, methanol, chloroform and hexane extracts obtained from bark	Nitric oxide (NO) free radical scavenging assay	20–500 µg /mL	Ethyl acetate and butanol fraction gave IC_{50} values of 50 and 69 µg/mL, respectively	(Santhanam et al., 2013)
Anti-inflammatory activity	Methanol extract of stem bark	Lipopolysaccharide (LPS) activated RAW 264.7 cells	5-20 µg/mL	The extract dose dependently inhibited the expression of COX-2, iNOS, TNF-α, IL-1β, NF-κB and AP1- all of which are involved in the expression of inflammation	(Thu et al., 2010)
Cytotoxic activity	Chloroform extract of bark and four active bio constituents	Human Dermal Fibroblasts (HDF) and B16-F10 melanoma cells	500-7.81 µg/mL dilutions of extract and active constituents each	Chloroform extract was nontoxic in both HDF and B6-B10 melanoma cells. The four active constituents kobusin, columbamine, lupeol and yangambin gave IC_{50} values of 112.2, 195.6, 377.8 and 442.4 µg/mL respectively	(Santhanam et al., 2016)
	Nine compounds isolated from methanol extract of root bark	Six human stomach-cancer cell lines, SCL, SCL-6, SCL37'6, SCL-9, Kato-3 and NUGC-4	250-15.63 µg/mL dilutions of active constituents each	No cell mortality at tested concentrations	(Ahsan et al., 2014)

Table 3. Summary of the pharmacological activities of *Z. rhetsa* (continued…)

Pharmacological activity	Tested substance	Model used	Tested dose	Results	Reference
Antinociceptive activity	Methanol extract of stem bark	Acetic acid induced writhing in albino mice	250 and 500 mg/kg body weight, oral	Significant reduction in acetic acid induced writhes. 47.8% reduction in case of 250 mg/kg dose and 58.3% reduction in case of 500 mg/kg dose	(Rahman et al., 2002)
Insecticidal activity	Acetone, methanol and aqueous extract of seed and leaves	Mean mortality percentage of red flour beetle *Tribolium castaneum*	5.0, 7.5, 10.0 and 12.5% diluted dose of extracts	Moderate mortality rate with a highest 38.0% mortality 72 hours after treatment for 12.5% diluted dose	(Mamun et al., 2009)
Antidiarrhoeal activity	Methanol extract of stem bark	Castor oil induced diarrhea in albino mice	250 and 500 mg/kg body weight, oral	Dose-dependent reduction in number of diarrheal episodes up to 5 hours. 100% reduction till 2 hours for 500 mg/kg dose	(Rahman et al., 2002)
	Seed oil extract	Inhibition of the gastrointestinal motility in mice using charcoal plug	50, 100, 200 and 300 mg/kg doses, oral	Moderate reduction in gastrointestinal transit. Maximum of 31.5% inhibition in transit at 300 mg/kg dose	(Naik, 2015)
	Essential oil extracted from the pericarp, different fractions obtained from the oil and an isolated compound terpinen-4-ol	Castor oil induced diarrhea in albino mice	100 and 200 µL/kg dose per body weight, oral	Dose-dependent reduction in number of diarrheal episodes after 2 hours. A maximum 83.3% reduction in diarrheal episodes in 200 µL/kg dose of essential oil	(Naik et al., 2015)
Anthelmintic activity	Methanol extract of the plant leaves	Adult albino rats infected by oral inoculation with a cestode parasite- *Hymenolepis diminuta*	100, 200, 400 and 800 mg/kg doses, oral	800 mg/kg dose extract showed significant anthelmintic effect on the larval stage of the parasites. Reduced EPG count to 0 and worm load by 86.6%	(Yadav and Tangpu, 2009)
Spasmolytic activity	Oil extracted from the pericarp of the mature fruits	Acetylcholine, histamine and nicotine induced inflammation in guinea pigs	50 g/mL of extract dissolved in 30% aqueous solution of DMSO, oral	Weak inhibition of contractions. IC_{50} 23.0 ± 1.2 and 39.0 ± 1.8 µg/mL against contractions induced by acetylcholine and histamine	(Naik et al., 2015)

Antimicrobial activity

Several alkaloids isolated from the bark of *Zanthoxylum rhetsa* were biologically active in the disk diffusion assay against Gram-negative bacterium *Escherichia coli*, Gram-positive bacterium *Bacillus megaterium*, fungi *Microbotryum violaceum,* and alga *Chlorella fusca* (Krohn et al., 2011). At doses of 50 µg of test substance per test filter disc, all the five bioactive alkaloids showed moderate to good activity against *Chlorella fusca* with the highest zone of inhibition (10 mm) given by arnottianamide, 6-acetonyldihydrochelerythrine, and skimmianine compared to the 10 mm zone of inhibition given by tetracycline.

Reddy and Jose (2011) determined the antibacterial activity of *Zanthoxylum rhetsa* seed essential oils steam distilled from alkaline, acidic, and aqueous media against four bacterial species- *Staphylococcus aureus*, *Proteus vulgaris, Klebsiella pneumoniae,* and *Escherichia coli*. The oils were steam distilled from *Z. rhetsa* seeds (300 g each) using 500 mL water for aqueous media, 500 mL of 10% Na_2CO_3 for the alkaline media, and 500 mL of 4% H_2SO_4 for the acidic media. The zone of inhibition measured in the agar disk diffusion method revealed that the acidic media caused the largest zone of inhibition in all 4 bacterial species with a maximum diameter of 47 mm in *Staphylococcus aureus* disk compared to 25 mm caused by standard gentamicin (200 µg/mL) (Table 3).

Tantapakul et al. (2012) isolated 10 compounds from the roots and stem barks of *Zanthoxylum rhetsa,* including a new compound zanthorhetsamide, and tested them all for their antibacterial property against Gram-positive bacteria *Staphylococcus aureus* and MRSA SK1, as well as Gram-negative bacteria *Salmonella Typhimurium* and *Escherichia coli.* The only dihydrochelerythrine showed strong activity against MRSA with a MIC value of 8 µg/mL compared to the 1 µg/mL MIC of standard vancomycin used in this test. The same compound also showed mild activity against *E. coli* (MIC = 16 µg/mL, standard gentamicin MIC = 0.25 µg/mL), while all other compounds showed weak to no activity against all four bacterial species.

Antioxidant activity

Santhanam et al. (2013) tested the ethyl acetate, butanol, methanol, chloroform, and hexane extracts obtained from the bark of *Zanthoxylum rhetsa* and for their antioxidant properties by a number of different established assays. In the total phenolic content determination test by the Folin-Ciocalteu's method, ethyl acetate and butanol fractions of the bark showed the highest phenolic content of 20.47 ± 0.09 and 14.14 ± 0.19 mg gallic acid equivalent (GAE)/g of the dry weight of extract, respectively. Even in the total flavonoid content assay using $AlCl_3$, the ethyl acetate and butanol fractions of the extract showed significant results with values of 1.59 ± 0.12 and 3.07 ± 0.24 mg quercetin equivalent (QE)/g of the dry bark extract, respectively. These findings were parallel to the results of antioxidant activity determination test using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay where ethyl acetate and butanol fractions demonstrated the highest free radical scavenging ability with IC_{50} values of 140 and 168 µg/mL, respectively, compared to the 5 µg/mL IC_{50} value of ascorbic acid. Finally, in the nitric oxide (NO) free radical scavenging assay, the ethyl acetate and butanol fractions were able to scavenge 50% of NO free radical with concentrations as low as 50 and 69 µg/mL, respectively, compared to the 23 µg/mL value of standard ascorbic acid.

Tukun et al. (2014) attempted to compare the antioxidant activity of the edible portions from fifteen different plants available in Bangladesh, which included *Zanthoxylum rhetsa.* Of all the species tested *Zanthoxylum rhetsa* showed the highest phenolic content in the Folin-Ciocalteu's test (6.01 mg GAE/g of fresh weight) and highest DPPH radical scavenging capability with a value of 7.54 µmol Trolox equivalent per gram of fresh weight content.

Oils extracted from the seeds of *Zanthoxylum rhetsa* using hexane and ether were tested for their antioxidant activity using the DPPH radical scavenging assay (Naik, 2015). The hexane and ether extracted oils had IC_{50} values of 266 and 265 µg/mL, respectively, compared to the 85 µg/mL of standard α-tocopherol.

The essential oil obtained from the pericarp of *Zanthoxylum rhetsa* gave an IC_{50} value of 7.5 µg/mL compared to the 5.1 µg/mL of ascorbic acid in the DPPH radical scavenging assay (Naik et al., 2015). Different fractions obtained from the oil also showed mild radical scavenging activity.

Anti-inflammatory activity

Thu et al. (2010) conducted a study to investigate the effect of *Zanthoxylum rhetsa* extracts on the expression of inflammatory mediators COX-2, iNOS, TNF-α, and IL-1β using lipopolysaccharide-stimulated RAW 264.7 cells. Methanol extract obtained from the dried stem bark of *Z. rhetsa* demonstrated significant anti-inflammatory properties by inhibiting the formation of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) via the nuclear factor kappa beta (NF-κB) pathway in the lipopolysaccharide (LPS) activated RAW 264.7 cells. At doses of 5-20 µg/mL,

methanol extract of *Z. rhetsa* inhibited the production of prostaglandin and nitrite and also inhibited the expression of iNOS protein, which acts as a driver of the inflammatory response after injury. The extracts also inhibited cyclooxygenase (COX)-2 protein, as well as its gene expression in the LPS, stimulated macrophage cells at 5-20 µg/mL. NF-κB, which is responsible for inducing the expression of a number of pro-inflammatory genes, was also inhibited by the *Z. rhetsa* extra at 5-20 µg/mL. The extract also inhibited the expression of inflammation precursors TNFα and IL-1β.

Cytotoxic activity

Zanthoxylum rhetsa bark extract, along with four bioactive constituents obtained from the chloroform fraction of the bark extract, was investigated for their cytotoxic activity against human dermal fibroblasts (HDF) and B16-F10 melanoma cells using the MTT proliferation assay (Santhanam et al. 2016). While the extract itself seemed to be non-toxic to HDF and B16-F10 melanoma cells, the isolated compounds kobusin, columbamine, lupeol, and yangambin, obtained from the bioactive chloroform fraction, showed significant toxicity in B16-F10 melanoma cells. Kobusin caused the highest amount of cell rupture, giving an IC_{50} = 112.2 µg/mL followed by columbamine, lupeol and yangambin, which gave IC_{50s} = 195.6, 377.8 and 442.4 µg/mL compared to the IC_{50} value = 22.72 µg/mL of positive control quercetin used for this test. The result of this study indicated the potential of *Z. rhetsa* bark extract as a dermo protective agent in skincare products against skin cancer. Ahsan et al. (2014) isolated nine pure compounds from *Zanthoxylum rhetsa* root bark and tested them for their cytotoxic property on six human stomach cancer cell lines using the MTT assay. The compounds did not show any cell mortality between concentrations of 250 µg/mL to 15.63 µg/mL.

Antinociceptive activity

Methanolic extract of *Zanthoxylum rhetsa* stem bark significantly reduced acetic acid-induced writhing in Swiss albino mice (Rahman et al., 2002). Dose of 250 mg/kg and 500 mg/kg of the plant's stem bark methanolic extract inhibited writhing by 47.8% and 58.3%, respectively, compared to the 67% inhibition of acetylsalicylic acid (100 mg/kg) used as a control for this test.

Insecticidal activity

Mamun et al. (2009) tested the insecticidal activity of six indigenous botanicals of Bangladesh, which included the seed and leaf extracts of *Z. rhetsa* against red flour beetle *Tribolium castaneum* Herbst. Their study showed a direct toxic effect on the mean mortality rate of the insect. They tested different dilutions of acetone, methanol, and aqueous extracts of seeds and leaves, among which acetone extracts of the seed showed a maximum 38.0% mortality of insects 72 hours after treatment.

Antidiarrheal activity

The methanolic extract obtained from the stem bark of *Z. rhetsa* dose-dependently reduced castor oil-induced diarrheal episodes in mice (Rahman et al., 2002). At doses of 250 and 500 mg/kg body weight, the extracts significantly reduced the number of diarrheal episodes up to 5 hours of being treated with castor oil. 100% reduction in defecation was observed up to 2 hours in mice groups treated with 500 mg/kg extract. The effect lasted until 5 hours until when the reduction in defecation had decreased to 85%.

Naik tested the seed oil of *Z. rhetsa* for its ability to inhibit gastrointestinal motility in albino rats (Naik, 2015). At 300 mg/kg dose, the seed oil was able to inhibit intestinal transit by 31.5% compared to standard loperamide's 54.8% inhibition. The other doses exhibited moderate to weak inhibition.

Naik et al. (2015) also tested the different fractions of essential oil extracted from the pericarp of *Zanthoxylum rhetsa* for their antidiarrheal activity in albino mice using castor oil-induced diarrhea model, where they demonstrated dose-dependent inhibition of diarrhea for up to 2 hours. Essential oil dose of 100 µL/kg inhibited the diarrheal action by 67.3%, which increased up to 83.3% in case of 200 µL/kg doses. Different fractions of the oil and an isolated compound terpinen-4-ol, also showed moderate inhibition of diarrheal action at 100 µL/kg dose with values ranging from 33.3% to 66.7%. The standard used in this test was loperamide at 100 µL/kg doses, which gave a 100% reduction of diarrheal action.

Anthelmintic activity

The Naga tribesmen in India use *Zanthoxylum rhetsa* as a popular folk medicine for deworming. Leaf extract of *Z. rhetsa* was tested for its anticestodal activity against *Hymenolepis diminuta* (Cestoda), which was experimentally infected in albino rats. The therapeutic efficacy of the leaf extracts was determined at four different doses of 100, 200, 400 and 800 mg/kg doses on three different life stages of the cestode parasites- (i) the larval stage- where treatment was given 2-4 days after post-inoculation of cysticercoids, (ii) the immature stage- where treatment was given 8-10 days after post-inoculation of cysticercoids, and (iii) the adult stage- where treatment was given after 21-25 days after post-inoculation of cysticercoids. The leaf

extracts successfully reduced larval stage eggs per gram of feces (EPG) count in a dose-dependent manner ranging from 23.4 ± 2.4 at 100 mg/kg dose till up to 0 EPG at 800 mg/kg dose compared to the 8.1 ± 5.0 EPG of praziquantel (5 mg/kg). Worm load was also reduced by 86.6% at 800 mg/kg dose compared to the 80% reduction by 5 mg/kg dose of standard anthelmintic drug praziquantel. The same doses exhibited a moderate reduction in EPG count and worm load when they were given against the immature and adult stage worms. Against the immature and adult stages of *Hymenolepis diminuta* infections in rats, 800 mg/kg dose of extract reduced worm load by 60% compared to the 86.6% and 80% reduction in immature and stages, respectively, by 5 mg/kg praziquantel (Yadav and Tangpu, 2009).

Spasmolytic activity

The essential oil extracted from the pericarp of *Zanthoxylum rhetsa* gave weak non-specific muscle relaxing action when tested guinea pigs where muscle contractions were induced using acetylcholine (2.5×10^{-7} g/mL), histamine (3.0×10^{-7} g/mL) and nicotine (2.5×10^{-6} g/mL) (Naik et al., 2015). The IC_{50} values of the essential oils were 23.0 ± 1.2 and 39.0 ± 1.8 µg/mL against contractions induced by acetylcholine and histamine, respectively, while no activity was seen against nicotine-induced contractions. This study supported the fact that *Z. rhetsa* may contain constituents responsible for the non-specific spasmolytic activity. However, further work needs to be done for isolating these responsible, active constituents and tested for their muscle-relaxing action.

DISCUSSION

Though *Z. rhetsa* holds a strong place in the ethnomedicinal practice, preferably in Asian countries like India and Bangladesh, it is compulsory to perform more detailed and comprehensive ethnomedicinal studies of the plant in these countries. Proper documentation of the specific information regarding the mode and frequency of use, method of preparation, side-effects, and adverse effects is very much imperative, and as yet, there is no such study that summarizes and authenticates these facts.

We also found irregularity in some of the reported results; for instance, flavonoids have been reported as one of the major constituents of the plant (Pai et al., 2009; Alphonso and Saraf, 2012), but as yet only a flavanone named hesperetin and its 7-O-glycoside hesperidin (Santhanam et al., 2016; 2018) has been reported from *Z. rhetsa*. In addition, the scarcity of genuine comparative data from the literature makes the bioactivity-guided comparison of different plant parts more difficult. Furthermore, it has been observed that researchers tend to rely more on traditional methods like steam distillation and solvent extraction methods for the extraction of essential oil. It is proposed that modern extraction technologies such as microwave-assisted, and supercritical fluid extraction can be explored to increase the yield as well as minimizing the cost. As the process of mechanical drying and concentration for obtaining crude extract may cause destruction of some bioactive compounds, it is therefore proposed that the traditional methods of using *Z. rhetsa* in the form of a paste, extracted juice or decoction should be explored. Measures should be taken to authenticate and compare the traditional mode of use and confirm them clinically.

Different plant parts of *Zanthoxylum rhetsa* and the different bioactive constituents isolated from it have been tested for a number of different biological activities, perhaps most extensively for its antioxidant, antimicrobial, and sun-blocking properties. The stem bark of *Z. rhetsa* has proven to possess significant UVA and UVB absorption property, elastase and collagenase inhibitory action, and good SPF values. A sunscreen formulation was developed from the ethyl acetate fractions of the bark. However, we noticed that only the bark of the plant has been tested for anti-photo aging assay and even though the results were quite significant, not much follow-up research has been done regarding this activity. Most sunscreen formulations in the market are prepared from synthetic photoprotective agents with high SPF values, but oftentimes these chemical sunscreens can cause serious adverse effects (Morabito et al., 2011). These sunscreens are the most common cause of photoallergic contact dermatitis, and the synthetic photoprotective agents used in them sometimes diffuse into the systemic circulation from the skin resulting in lesser efficacy and possibly local and systemic toxicity (Sarveiya et al., 2004). Thus, the development of a natural sunscreen formulation has become a necessity, and *Z. rhetsa* can be a revolutionary natural source of photoprotective compounds, but further tests are required to improve its efficacy. The potential use of *Z. rhetsa* in sunscreen formulations is perhaps even more justified by the significant antioxidant activity of its different plant pars. Bark, edible parts, seed oils, and pericarp of the plant have all showed antioxidant properties in a number of established antioxidant assays. While fractions of the stem bark have been certainly proven to have free radical scavenging activity by four different assays (Santhanam et al., 2013), the other plant portions have given antioxidant activity in only one or two essays. Further validation of these antioxidant activities would require testing the plant extracts using multiple assays.

In the antimicrobial tests, *Z. rhetsa* bark, root, seeds, and pericarp extracts showed moderate MIC range against a number of different Gram (+) ve and Gram (-) ve bacterium as well as some fungi and algae. Methanol extracts of the bark were also investigated to contain significant anti-inflammatory effects, and the researchers were also able to determine the pathway of this anti-inflammatory action (Thu et al., 2010). However, the tests were all *in vitro*, and we propose future *in vivo* anti-inflammatory assays to be conducted to validate these results. Mixed results were seen in the cytotoxicity tests where the bark extract and its constituents seemed to be ineffective against stomach cancer and human dermal fibroblast (HDF) cell lines but highly effective against B16-F10 melanoma cell lines. This result is significant as it explores the dermo protective potentiality of *Z. rhetsa* in skincare products such as sunscreens. Seed essential oils of *Z. rhetsa* seemed to be quite promising in reducing diarrheal episodes in mice in a number of studies. As for the other activities such as antinociception, insecticidal, anthelmintic, and spasmolytic- we believe that to scientifically confirm them, a few more different assays must be explored before they can be considered in therapeutic preparations.

Lastly, *Z. rhetsa* fruits can be considered as a potential source of α-linolenic acid (ALA) (Naik, 2015). Further research is required to explore this perspective and utilizing fruits as a food supplement directed to reduce the risk of cardiovascular diseases. The prospect of utilizing essential oil collected from *Z. rhetsa* in perfumery needs to be explored further.

Limitations of the study

This systematic review on the traditional uses, phytochemistry, and pharmacology of *Z. rhetsa* has certain limitations. We focused on reviewing the pharmacology but did not report on the pharmacokinetics and toxicology profile of the plant parts. Without the associated pharmacokinetic profile of each isolated compound or plant extract, it is difficult to make decisions on their potential as future leads for drug discovery. Meta-analysis was not conducted for this study either. Without a detailed meta-analysis, it is difficult to concretely determine whether the biological activities we reported exist and if they are significant enough or not.

Future perspectives

Z. rhetsa has been used by tribal healers for many years, and numerous chemicals constituents have been isolated from different parts of this plant. It can be said without a doubt that *Z. rhetsa* possess various important pharmacological properties. However, there are still several challenges that need overcoming before it can be considered for potential drug development and clinical trials.

First of all, many of the pharmacological studies of *Z. rhetsa* were done with crude extracts and preparations, which is not sufficient to pinpoint the bioactive compounds responsible for each specific property. Therefore, bioactivity-guided activity testing of the isolated compounds is required to identify potential lead compounds. Secondly, not enough information exists regarding the molecular mechanism of action of some of the isolated compounds. Future studies need to be focused on determining the molecular pathway behind each activity, including host receptor interactions, which would help in the drug design process. Third, we found insufficient pharmacokinetic data available on *Z. rhetsa* when conducting or systematic review. Toxicity studies were also few and far between. More extensive pharmacokinetic studies need to be conducted, and toxicity studies have to be performed on a cellular and molecular level before clinical studies can be considered. Finally, the *Zanthoxylum* genus has a very rich biodiversity, and morphological similarity exists between its different species. This may lead to difficulty in the identification of *Z. rhetsa* species. So, it is important to establish a unified international evaluation system for ease of identification. Strategies for improving the quality of cultivated plants and the efficiency of extraction processes also need to design.

CONCLUSION

The present review provides an updated and structured compilation of the available scientific evidence on the ethnopharmacology of *Z. rhetsa*. It is apparent from the experimental data that *Z. rhetsa* is rich in nutrients and bioactive substances, which is of great interest to researchers. It is expected that this review will persuade to augment the bioactivity guided research and identify potential lead molecules.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Aziz F	Rahman FI	Huque S	Ether SA	Hasan CM	Ahsan M
Concepts or ideas	x	x				
Design	x	x	x			
Definition of intellectual content					x	x
Literature search			x	x		
Experimental studies						
Data acquisition						
Data analysis	x	x	x	x	x	X
Statistical analysis						
Manuscript preparation	x	x	x	x		
Manuscript editing					x	x
Manuscript review	x	x	x	x	x	x

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