A COMPARATIVE TAXONOMIC AND PHYTOCHEMICAL STUDY ON *Rauvolfia serpentina* (L.) Benth. ex Kurz AND *Rauvolfia tetraphylla* L. FROM ASSAM (INDIA)

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CERTIFICATE

This is to certify that thesis entitled "A comparative Taxonomic and Phytochemical study on Rauvolfia serpentina (L.) Benth. ex Kurz and Rauvolfia tetraphylla L. from Assam (India)" submitted to the Assam Science & Technology University, Guwahati, for the award of the degree of Master of Science in Botany is a bonafide research work carried out by the student Prarthana Konwar (Roll No. 202820047015) under my guidance and supervision during the period between April 2022 to August 2022 in the Department of Botany. I further certify that no part of this thesis has been submitted anywhere else for the award of any Degree, Diploma, Associateship, Fellowship or other similar titles.

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DECLARATION

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I hereby declare that the work embodied in this thesis entitled "A comparative Taxonomic and Phytochemical study on Rauvolfia serpentina (L.) Benth. ex Kurz and Rauvolfia tetraphylla L. from Assam (India) is a research work done by me under the supervision and guidance of Dr. M. MATHIYAZHAGAN, Associate Professor of Botany, Silapathar Science College, Silapathar. I further declare that this work has not been submitted earlier in full or in parts to any other university for the award of any other Degree, diploma, Associateship, Fellowship or other similar titles.

Date: **PRARTHANA KONWAR**

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Date: **PRARTHANA KONWAR**

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ABBREVIATION

AYUSH-	Ayurveda,Yoga,Unani, Siddha and Homoeopathy.
CARI-	Central Ayurveda Research Institute
GC-MS-	Gas Chromatography- Mass Spectrometry
HPTLC-	High Performance Thin Layer Chromatography
m-	metre
M.W./ Mol.-	Molecular Weight
METL-	Methanolic
mL-	Millilitre
mm-	Millimetre
NIST-	National Institute of Standard and Technology
RT-	Retention Time
WHO-	World Health Organisation

CONTENTS

CHAPTER 1

1. INTRODUCTION

Since time immemorial plants have been exploited by mankind for various purposes such as timber, medicine, food and source of dyes. In certain kinds of formulations, plants have been extensively used to get rid of several ailments by traditional practitioners all over the world as well as by indigenous medicinal systems such as Ayurveda, Siddha, Unani and Traditional Chinese medicine (TCM). Plant based medicines are the primary source of treatment for people living in remote places and having no access for modern medicine. The knowledge of indigenous medicinal practitioners on plants and their healing properties is passed from generation to generation. Plants produce a great number of secondary metabolites (for *e.g.* alkaloids, terpenes and polyphenolic compounds), many of which, are known to possess therapeutic applications. Plant derived chemicals have found distinct place in modern therapy as they have been considered very important leads for modern drug discovery. Compounds such as vincristine, vinblastine, digitalin, digoxin, atropine, camptothecine, morphine, codeine, reserpine, quinine and artemisinin are from plant origin. Plants, either singly or in poly herbal formulations, are being used traditionally worldwide to combat several ailments including microbial diseases, snake bite, skin diseases, diabetes, inflammation and cancer. A vast knowledge on the therapeutic role of plants and their bioactive principles is gathered due to many studies being carried out on medicinal and pharmacological properties of plants (Mahalaxmi et al 2019).

The plant is a biosynthetic laboratory, not only for chemical compounds, but also amultitude of compounds like glycosides, alkaloids etc. These exert physiological andtherapeutic effects. Medicinal herbs constitute an effective source of traditional (Ayurvedic, Unani and Homeopathy) and modern medicine. Plants have been an important source of medicine for thousands of years. There are a number of synthetic medicines which have been derived from medicinal herbs; Digoxin, Aspirin, Ephedrine, Quinine, Vincristine, Vinblastine, Taxol Artemisinin, Hypericin, Silymarin are some examples (Verma and Verma, 2010).

Synthetic drugs are effective in controlling different diseases but these synthetic drugs are out of reach of millions of people (Kumar et al., 2010). Plants with their enormous arrays of secondary metabolites from a reservoir of low molecular weight organic compounds that is mostly untapped as a basis of pharmaceuticals (Srivastava et al., 1996). Medicinally, plants are an important therapeutic aid for a range of ailments (Fakruddin et al., 2012) and plants are used as a source of many potent and powerful drugs in different countries (Srivastava et al., 1996). In contemporary medicines, plants have an extremely important position as the raw material for some important drugs. According to the estimation, an around 70,000 plant species have been used for medicinal purposes. Medicinal herbs provide the starting material for the synthesis of conventional drugs. The curative action of medicinal plant is due to the presence of multifaceted chemical constituents. Regarding the Indian estimation, more than 2500 plant species having medicinal value, Sri Lanka around 1400 and Nepal around 700 (Kumar et al., 2010). The World Health Organization (WHO) approximations that 80% of the inhabitants of some Asian and African countries at present uses herbal medicines for some aspect of primary health care because of superior cultural acceptability, affordability, better compatibility with the human body along with fewer side effects (Jakaria et al., 2015; Dash et al., 2014; Parekh et al., 2005).

1.1 Use of *Rauvolfia* as medicine

Rauvolfia is one of the apocynaceous genera that long has awaited a taxonomic revision. Since the inception of the genus by Plumier in 1703 with two species, the literature has accumulated an abundance of novel specific epithets, based frequently on insufficient material and often due to misconceived synonymy. The 'Kew Index', including all the supplements, lists about 175 names for the world, while the Kew and the Gray Indices together account for about 90 names for the New World alone. An inadequate comprehension of the generic characteristics also has led to the proposal of new genera, such as Ophioxylon,

Cyrtosiphonia, Dissolaena and Heurckla, to include the Asian and New Caledonian Rauvolfias. Bentham and Hooker correctly recognized the synonymy of these genera with Rauvolfia. Plants belonging to other genera, and even other families, in the past have been described as Rauvolfias. Thus Ruiz and Pavon described several species of Citharexylum, a genus of Verbenaceae, as species of *Rauvolfia*.

Genus *Rauvolfia*, belonging to the family Apocynaceae, comprises around 80 species which are distributed in tropical climatic conditions. Traditionally, *R. serpentina* (L.) Benth. ex Kurz, commonly known as *Sarpagandha*, was reported against snakebite, insomnia, melancholia, schizophrenia or more violent mental disorders, diarrhea, dysentery, cholera and colic, scabies, malaria, eye inflammation, etc. (Dey and De, 2010, 2012). *R. serpentina* has an economical importance and the root part of the plant is used in many Ayurvedic poly herbal formations (*Sarpagandha*vati). *R. tetraphylla* L. popularly known as "devil pepper" or "bestill tree" is an endangered woody shrub native in tropical Americas (Faisal et al., 2013). Ethnomedicinal importance of *R. tetraphylla* was found in terms of its use against snakebite, to stimulate uter-ine contraction and to facilitate difficult childbirth cases (Sarmaet al., 1999; Dey and De, 2012). Moreover, *R. serpentina* has been reported for pharmacological properties such as anti-bacterial, anti-inflammatory and cytotoxicity (Dey and De, 2010). *R. tetraphylla*has also been reported to possess antipsychotic (Gupta et al., 2012a), antibacterial activity and anti-inflammatory (Ganga Rao et al., 2012) properties.

Rauvolfia (*R. serpentina*) is an evergreen shrub that is a member of the dogbane or Apocynaceae family (Endress and Bruyns, 2000). 80 to 100 species are included in the *Rauvolfia* genus, and they are native to tropical and subtropical regions of the world, including Europe, Africa, Asia, Australia, and the Central and South Americas (Vakil, 1955). *Rauvolfia serpentina* is native to the moist, deciduous forests of southeast Asia, including India, Burma, Bangladesh, Sri Lanka, and Malaysia (US Dept of Agriculture, 2003). The plant usually grows to a height between 60 and 90 cm and has pale green leaves that are7 to 10 cm long and 3.5 to 5.0 cm wide. The leaves are elliptical or lanceolate shaped and occur in

whorls of 3 to 5leaves. The plant has many shiny, black or purple, round fruits that are approximately 0.5 cm in diameter. It also has small pink or white flowers. The plant has a prominent tuberous, soft taproot that reaches a length between 30 and 50 cm and a diameter between 1.2 and 2.5 cm. (Brijesh, 2011).

R. serpentina was used in folk medicine in India for centuries to treat a wide variety of maladies, including snake and insect bites, febrile conditions, malaria, abdominal pain, and dysentery. It was also used as a uterine stimulant, febrifuge, and cure for insanity. The plant was mentioned in Indian manuscripts as long ago as 1000 BC and is also known as *sarpagandha* and *chandrika* (Yarnell and Abascal, 2001). The genus *Rauvolfia* was named in honor of the16th-century German physician Dr Leonhard Rauwolf, who studied plants while travelling in India. *R. serpentina* was selected for study due to its long, tapering, snake-like roots (Tyler, Brady and Robbers, 1988). The Indian political leader Mahatma Gandhi was known to employ Rauvolfia, reportedly using the root to make a tea that he consumed in the evening to help relax after a busy, over stimulated day (Jerie, 2007). The Indian physician Rustom Jal Vakil is considered responsible for introducing *Rauvolfia* to Western medicine. He collected data on patients treated with *Rauvolfia* for 10 years, from 1939 to 1949. In 1949, he published a watershed paper on the antihypertensive properties of *R. serpentina* in the British Medical Journal (Isharwal, Gupta and Vakil, 2006). He presented his detailed results from treating 50 patients who had high blood pressure with the root of *Rauvolfia*. The results were remarkable and significant. By 1949, more than 90% of Indian physicians were using *Rauvolfia* in the treatment of high blood pressure. After Vakil's original paper, more than 100 scientific articles were published throughout the world (Isharwal, Gupta and Vakil, 2006).

In Ayurveda, *Sarpagandha* is used for the treatment of high blood pressure, insomnia, asthma, acute stomachache and painful delivery and for mental illness such as neuropsychiatric disorders, psychosis, and schizophrenia. The root of *R. serpentina* is the genuine source drug of *Sarpagandha*. It is also used in the treatment of snakebite, insect stings, mental disorders, gastric tumor, general weakness, goiter, hysteria, insomnia, insanity, lipoma, paraplegia, para

typhoid, piles, pneumonia, splenomegaly, stomach disorder, tonsillitis, traumatic wound, tuberculosis, and vertigo (Bindu et al, 2014).

Rauvolfia has been studied for the treatment of mental diseases, including schizophrenia and bipolar disorder, epilepsy and seizures, and of insomnia and sleep problems (Healy and Savage, 1998). One study found *Rauvolfia* to be effective in the treatment of anxiety (Lowinger, 1957). All forms of *Rauvolfia* were used in that study, including reserpine, alseroxylon, and the whole root, and all gave the same results in the control of over anxiety in ambulatory patients. *Rauvolfia* has been studied as a treatment for autistic children between the ages of 3.5 and 9 years (Lehman, Haber and Lesser, 1957). Another study found it to be effective in treatment of delirium tremens in alcohol and drug addicted patients (Avol and Vogel, 1955). The researchers in that study observed a noted decrease in agitation, excitement, and acute hallucinatory episodes. One study found that *Rauvolfia* treated migraine headaches effectively, with a noted improvement in quality of life and a decrease in pain (Friedman, 1955). Another study used *Rauvolfia* to treat angina pectoris in patients with coronary artery disease, finding a decrease in angina symptoms and a prolonged therapeutic effect (Lewis et al, 1956). One-half of the patients in that study went on to develop normal electrocardiograms. In another study, *Rauvolfia* was studied to examine its benefits in improving pruritic and psychogenic dermatosis (Ferrara and Pinkus, 1955). It has also been reported to improve psoriatic outbreaks (Douglas Lobay, 2015).

There is hardly any medicine, herbal or modern, which originated in India more than a century ago and became a subject of intensive research and clinical use for more than two decades ago, in the Western world than *R. serpentina* and some of its alkaloids (Evans, 2009).The root of the *R. serpentina* Benthhas been in use in India for hundreds of years for many unrelated diseases. Vakil, 1949 reviewed the literature on *Rauvolfia* and reported the mention of this plant in an old Hindu manuscript (1000 BC) as well as in the works of Charaka (second century AD) under the Sanskrit name of *Sarpagandha* (Tandon, 2021). According to Wilkins (Wilkins, 1954a, 1954b, 1952, 1953), although an ancient drug, *R.*

serpentina did not receive any notice by clinicians in the USA till 1950, it was in 1952 that CIBA Laboratories (now Novartis) in Switzerland published the first complete report on the isolation of reserpine, its chemistry and pharmacology. It was introduced as the drug Serpasil for the treatment of hypertension, tachycardia and thyrotoxicosis (Wilkins, 1954a, 1954b).

1.2. Chemical Composition

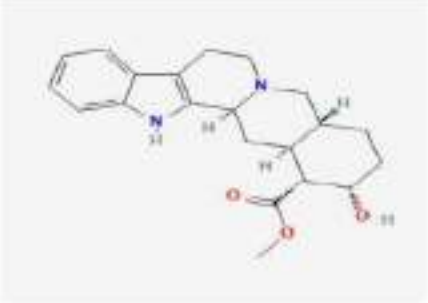
1.2.1. *Rauvolfia serpentina*

The major alkaloid present in root, stem and leaves of the plant is Reserpine varies from 1.7 to 3.0 %. The root barks has more than 90% of the total alkaloids in roots. The minor alkaloids present in the plant are Ajmalicine, ajmaline, isoajmaline, ajmalinine, chandrine, rauwolfinine, renoxidine, rescinnamine, reserpiline, reserpine, reserpinine, sarpagine, serpentine, serpentinine, tetraphyllicine, yohimbine, 3-epi-ayohimbine. The root contains ophioxylin, resin, starch and wax (Vakil,1955). *R. serpentina* is rich in vitamins. Ascorbic acid (vitamin C) was found to be 44.03 m g/100g in *Rauvolfia*, serpentine and Riboflavin, thiamine and niacin were also detected. The presence of phenolic compounds in the plant indicates that this plant may be anti-microbial agent (Harisaranraj R. et al.2009). Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stary F. 1998 and Okwu D.E. and Okwu M.E 2004). Flavonoids, on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Okwu D.E. 2004, Salah N et al.1955 and Del-Rio A. et al.1997). Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from this plant provide anti-inflammatory activity used for the treatment of diseases in herbal medicine (Okwu D.E. 2004). Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. The lower sodium content of serpentine might be an added advantage due to the direct relationship of sodium intake with hypertension on human (Dahl

L.K. 1972). The presence of zinc in the plant could mean that the plant can play valuable roles in the management of diabetes, which result from insulin malfunction (Okaka J.C.and Okaka A.N.O. 2001). This plant is a good source of ascorbic acids, riboflavin, thiamin and niacin (Harisaranraj R. et al.) Natural ascorbic acid is vital for the body performance. Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine, defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular the mouth and gastro-intestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism. This function of ascorbic acid also accounts for its requirement for normal wound healing. As a result of the availability of ascorbic acid in this plant is used in herbal medicine for the treatment of many diseases (Okwu D.E. 2004).

Chemical structures of some important Alkaloids from *R. serpentina*

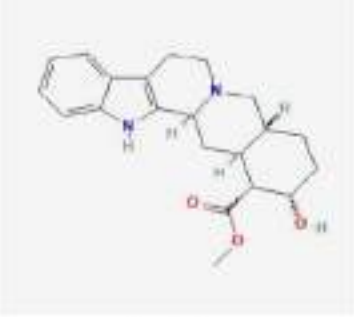
Ajmaline



Yohimbine

Serpentine

Deserpidine



Ajmalicine

Reserpine

1.2.2 *Rauvolfia tetraphylla*:

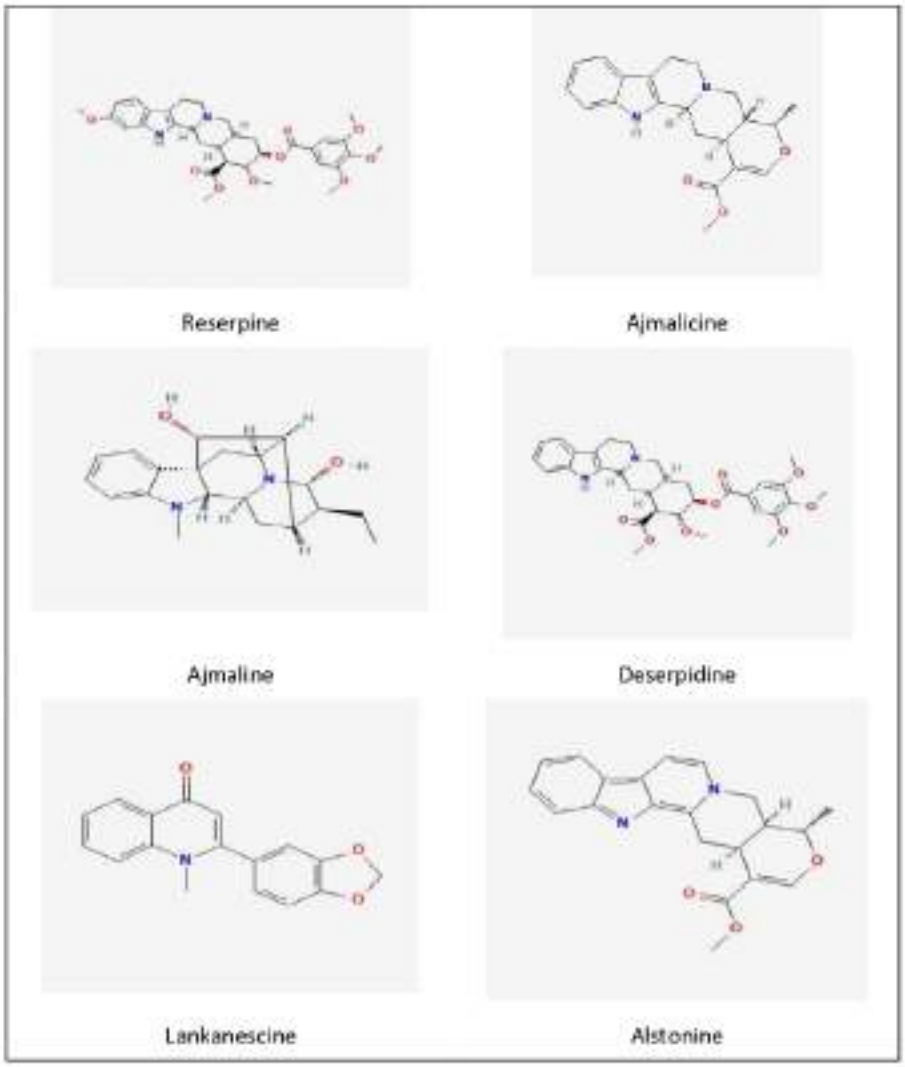
Plants produce a range of chemicals that can be divided into two categories viz. primary and secondary metabolites. These chemicals are known as phytochemicals and most of the secondary metabolites (with complex chemical composition), for e.g., alkaloids, terpenes and polyphenolic compounds, exert multifold effects on the health of human beings besides conferring resistance to plants that produce them against insects, pathogens and herbivores. Secondary metabolites in plants are restricted in distribution within the plant kingdom i.e. some metabolites are present in only one plant species or in a related group of species. Metabolic pathways such as shikimic acid pathway, malonic acid pathway and mevalonic acid pathway are responsible for synthesis of secondary metabolites in plants (Pichersky E and Gang DR.2000, Acamovic T and Brooker JD 2005,Karuppusamy S. 2009, Kennedy DO, Wightman EL.2011, Mazid M et al. 2011, Ribera AE, Zuniga G.2012, Kabera JN et al.2014, Ahmed E et al.2017, Guerriero G et al.2018).*R. tetraphylla* is shown to contain a myriad of secondary metabolites, particularly alkaloids. Reserpine is an important alkaloid found in R. tetraphylla. With the help of chromatographic and spectral analyses, many secondary compounds have been isolated from different parts of R. tetraphylla and their structures have been elucidated (Mahalaxmi et al.2019). Klohs et al.1954isolated reserpine from roots of *R. tetraphylla*. Stoll and Hofmann 1995 had isolated two alkaloids, namely, canescine and pseudo yohimbine from the roots of *R. tetraphylla*. Two ester alkaloids, named as raunescine and isoraunescine, have been isolated from *R. tetraphylla* (Hosansky N, Smith E,1995). An alkaloid designated as Recanescine (that lack the methoxyl group in the C-ll position of reserpine) with sedative and hypotensive property was isolated from *R. tetraphylla* (Neuss et al. 1995 and Hochstein et al.1995). An alkaloid designated as heterophyllin was isolated from roots of *R. tetraphylla* (Hochstein et al.1995). Raunescine (renamed as canembine), an alkaloid, was isolated from roots of *R. tetraphylla* (Bhattacharjee et al.1996). An alkaloid pseudoreserpine possessing hypotensive and sedative activity was isolated from the root of *R.*

tetraphylla (Klohs et al.19957). Djerassi et al. 1957, elucidated the structures of two alkaloids from root of *R. tetraphylla* viz. tetraphylline and tetraphyllicine.

Belikov, 1969 isolated alkaloids viz. ajmaline, ajmalieine, arieine, reserpiline, αyohimbine, sarpagine and reserpine from roots and epigeal parts of *R. tetraphylla*.

A new sarpagine-type alkaloid, named as N (α)- Demethylaccedine, was isolated from stem bark of *R. tetraphylla* (Martinez et al.1989). By HPTLC analysis, reserpine and ajmalicine were identified in root of *R. tetraphylla* (Pandey et al. 2016). Quercetin was identified in the leaves of *R. tetraphylla* by HPTLC analysis (Satyanarayanaet al.2018). Alkaloids viz. 10- methoxytetrahydroalstonine, isoreserpiline, αyohimbine, reserpiline were isolated from chloroform fraction of *R. tetraphylla* leaves by pHzone-refining fast centrifugal partition chromatography (Maurya et al.2013). Six indole alkaloids viz.isoreserpiline, 10methoxytetrahydroalstonine, 11-demethoxyreserpiline, 10-demethoxyreserpiline, αyohimbine and reserpiline have been identified from leaves of *R. tetraphylla* (Gupta et al.2012b). A new labdane diterpene characterized as 3β-hydroxylambda 8(17),13(14)-dien-12(15)-olide was isolated from air-dried stems and branches of *R. tetraphylla* (Brahmachari et al.2011). The study by Panda et al. 2012 revealed a varying concentration of reserpine in different parts of *R. tetraphylla*. Root was shown to contain high concentration of reserpine followed by stem and leaf (Mahalaxmi et al.2019).

Chemical structures of some important Alkaloids from *R. tetraphylla*



1.3 Pharmacology

1.3.1 *Rauvolfia serpentina*

R. serpentina holds an important position in the pharmaceutical world due to the presence of various alkaloids in the oleoresin fraction of the roots. Alkaloids of this plant have a great medicinal importance to treat cardiovascular diseases, high blood pressure, hypertension, arrhythmia, various psychiatric diseases, mental disorders, breast cancer, human promyelocytic leukemia like diseases. Reserpine is the main alkaloid that shows highly complex pattern of activity mainly variation of amine concentration in brain. It is responsible for influencing the concentration of glycogen, acetyl choline, g-amino butyric acid, nucleic acids and antidiuretic hormone. The effects of reserpine include respiratory inhibition, stimulation of peristalsis, myosis, relaxation of nictating membranes and also influences temperature regulating center. It increases the volume and free acidity of gastric secretion. The Pitkriya capsule (Unani formulation) contains arsol (*R. serpentina*) which acts as MusakkinwoMunawwim (sedative and hypnotic), Mudir (Diuretic), Musakkin-e- Asab (nervine sedative) and Mukhaddir (anesthetic). Its various pharmacological activities include anticholinergic, hypotensive, anticontractile, sedative, relaxant, hyperthermic, antidiuretic, sympathomimetic, hypnotic, vasodialater, antiemetic, anti-fibrillar activity tranquilizing agent, anti-arrhythmic, antifungal and nematocidal.*R. serpentina* is believed to have followed pharmacological attributes: (1) By the action on vasomotor center, as it leads to generalized vasodilatation by lowering blood pressure. (2) By depressant action on the cerebral centers as it soothes the general nervous system. (3) It exerts a sedative action on the gastric mucosa and shows stimulating action on the plain musculature of the intestinal tract. (4) It also stimulates the bronchial musculature (Mittal, et al. 2012 and Agrawal, et al. 2013).

The roots of *Rauvolfia* aregenerally used in medicine. *Rauvolfia* is mainly used for the treatment of various central nervous system disorders associated with psychosis, schizophrenia, insanity, insomnia, and epilepsy. Extracts of the roots are valued for the treatment of intestinal disorders, particularly diarrhea and dysentery and also as anathematic. Mixed with other plant extracts, they have been used in the treatment of cholera, colic and fever. The root was believed to stimulate uterine contrition and recommended for use in child-birth in difficult cases. The juice of the leaves has been used as a remedy for opacity of the cornea (Mittal et al. 2012). Extracts of R. serpentina is also useful in treatment of other diseases such as fever, malaria, eye diseases, pneumonia, asthma, AIDS, headache skin disease and spleen disorder (Biradar et al.2016).

Other properties reported for *R. serpentine* are:

Antipsychotic: Historically reserpine has also been used for the treatment of schizophrenia and tardive dyskinesia (Agarwal and Mittal,2013 and Kirtikar and Basu,1993).

Insomnia: *Rauvolfia* is a valuable remedy in treating "insomnia "because of its sedative properties. The very important the *Rauvolfia* plant is highly beneficial in treating insanity. 1 g of powdered root can be taken twice a day with 250 ml of goat's milk, sweetened with sugar candy. Treatment of Hysteria: *Rauvolfia* is useful in treating "hysteria". 1 g of powdered root can be administered thrice with milk. Treatment should be continued till a complete cure is obtained (Agarwal and Mittal,2013 and Kirtikar and Basu,1993).

Febrifuge: It is used as febrifuge or fever relieving drug (Singh et al2009).

Anti-hypertensive: It is also used in treatment for high blood pressure and it has been adapted by medical fraternity in most countries. These alkaloids which have a direct effect on Hypertension have been isolated in it and are widely used by

practitioners of modern medicine. It relieves itching in urticaria (Sen and Bose,1939).

Cancer: It is also used in the treatment of "Breast cancer". Subsequent research and analysis eliminating exclusion bias showed that no increase in the rate of breast cancer occurred in those patients using Rauvolfia (Beljanski and Beljanski, 1986).

1.3.2 *Rauvolfia tetraphylla*

Various parts of *R. tetraphylla* are shown to exhibit a range of pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, cytotoxic, antihemolytic, antihypertensive, anxiolytic, insecticidal, allelopathic, platelet antiaggregant, cardioprotective, antipsychotic and antiparasitic activity. Brief descriptions on pharmacological potential of the plant are given here.

Antibacterial activity: Whole plant as well as different parts such as root, leaf and fruit of *R. tetraphylla* was shown to be effective antibacterial agents. Ethanol extract from *R.tetraphylla* was shown to reveal marked inhibition of gram positive and gram-negative bacteria(Suresh et al.2008). Aqueous extract of *R. tetraphylla* leaves exhibited concentration dependent inhibition of test bacteria viz. *Escherichia coli* and *Klebsiella pneumonia* (Thinakaran et al.2009). Ethanol extract of leaves were effective in causing concentration dependent inhibition of bacteria. *Salmonella typhimurium* and *Micrococcus luteus* were inhibited to highest and least extent, respectively (Nandhini and Bai 2014a). Reserpine, isolated from leaves of *R. tetraphylla*, was found to display inhibitory activity against gram positive and gram-negative bacteria (Abubacker and Vasantha, 2011). Leaf extract of *R.tetraphylla* displayed inhibition of *Staphylococcus aureus* and *Enterobacter faecalis* with marked activity against *S. aureus* (Senthilmurugan et al. 2013). Methanolic leaf extract was shown to be effective against *S. aureus and K. pneumoniae* in a dose dependent manner (Archana and Jeyamanikandan, 2015).

Other activities of *R. tetraphylla* reported are:

Antifungal activity: Several studies conducted on antifungal potential of *R. tetraphylla* revealed its effectiveness against a panel of human and phytopathogenic fungi including dermatophytes and seed-borne fungi. The study carried out by Kumaran and Kannabiran, 2003 revealed mycotoxic effect of ethanol extract obtained from roots of *R. tetraphylla* against the growth of *Colletotrichum capsici*. Aqueous leaf extract of *R. tetraphylla* was effective in causing antifungal activity against *Fusarium indicus* and *Aspergillus flavus* dose dependently (Thinakaran et al.2009). Ethanol extract from leaves was effective against human pathogenic fungi viz. *C. albicans, M. canis, T. rubrum and Cryptococcus* species (Nandhini and Bai 2014a).

Anti-inflammatory activity: Anti-inflammatory activity of various solvent extracts of root bark of *R. tetraphylla* by carrageenan induced rat paw edema model has been reported. Among extracts, hydro-alcoholic and methanol extract displayed significant reduction in paw edema when compared to hexane and ethyl acetate groups (Rao et al.2012).

Allelopathic activity: Allelopathic effect of aqueous root extract of *R. tetraphylla* in *Cicer arietinum* seeds was reported. Treatment of extract (100mg/ml) was shown to promote germination of seeds, vigor index, and seedling weight. Besides, an increase in total sugar, soluble protein, amino acid, DNA and RNA content was also observed. The study of Sangvikar and Wadje,2012 revealed the positive effect of various solvent extracts of *R.tetraphylla* on seedling emergence of maize (Mandal et al.2013).

Anxiolytic activity: Anxiolytic activity was reported for ethanol extract of *R. tetraphylla* leaves by elevated plus maze model in mice. The extract was shown to exhibit significant anxiolytic activity. Extract treatment resulted in a significant increase in the time spent on open arm, open arm entries % and % time spent (Singh et al. 2017).

Antihypertensive activity: Antihypertensive activity of *R. tetraphylla* in rats was reported. Sodium chloride was administered in animals to elevate systolic, diastolic and mean arterial blood pressure. Administration of methanolic root extract resulted in significant decrease in systolic pressure (Gadhvi et al. 2018).

Antihemolytic activity: Antihemolytic activity of methanol extract of *R. tetraphylla* leaves using cow RBC was reported. The extract was shown to cause concentration dependent inhibition of hemolysis of RBC with IC50 value of 135µg extract/ml (Maheshu et al. 2010).

Anti-venom activity: Aqueous and methanol extracts of *R.tetraphylla* root was screened for venom detoxifying activity in mice against crude venom obtained from Indian cobra. Both extracts did not protect the mice from the lethal dosage of cobra venom (Rajesh et al. 2013).

Cytotoxic activity: The study of Kakad and Dhembare 2014 revealed cytotoxic activity of leaf extract of *R. tetraphylla* against chick embryo fibroblast cell line with cell viability of 50.54%. Behera et al. 2016 evaluated cytotoxic potential of *R. tetraphylla* by brine shrimp lethality assay. Solvent extracts of leaf and fruit displayed dose dependent mortality of shrimps.

Sedative activity: Sedative activity of crude extract obtained from *R. tetraphylla* root bark by rat hole board technique using rats was determined. Extract administration resulted in a significant and dose dependent decrease in the locomotory activity, number of rears and number of head dips in rats. Further, a decrease in faucal boluses was also observed (Madawala et al. 1994).

Antioxidant activity: Various parts viz. leaf, root and fruit of *R. tetraphylla* are shown to exhibit antioxidant activity. Methanol extract obtained from the fruits of

R. tetraphylla was effective in scavenging DPPH radicals in a dose dependent manner (Oza and Solanki,2016)

1.4 Side Effects and Toxicology

Adverse side effects of reserpine include lethargy, sedation, psychiatric depression, hypotension, nausea, vomiting, abdominal cramping, gastric ulceration, nightmares, bradycardia, angina-like symptoms, bronchospasm, skin rash, itching, galactorrhea, breast enlargement, sexual dysfunction, and withdrawal psychosis in 1 case. The most common side effect noted in all patients was nasal congestion, occurring in 5% to 15% of all patients (inchem.org, 2022). After several months of use, mental depression can occur and may persist. With extremely large doses, Parkinson-like symptoms, extrapyramidal reactions, and convulsions can occur. Allergic reactions to *Rauvolfia*, including asthma, are rare.

Adequate doses of reserpine that produce decreased blood pressure will not cause reserpine-induced gastric ulcerations (naturaldatabase.com, 2022). Reserpine has been observed to cause a slight edema in some patients (Krogsgaard, 1957). Possible interactions with other drugs include cardiac glycosides, ephedra, alcohol, antipsychotic drugs, barbiturates, digoxin, diuretics, ephedrine, levodopa, monamine oxidase inhibitors, propranolol, stimulant drugs, and tricyclic antidepressants. *Rauvolfia* may interact with the following lab tests, including tests for corticosteroids, bilirubin, catecholamines, gastric acidity, norepinephrine, prolactin, thyroxine, and vanillylmandelic acid (Yarnell and Abascal, 2001).

From 1959 to 1960, 151 cases of toxicity were reported in the United States from consuming *Rauvolfia*, and only 4% of these cases were in adults (Baumeister et al, 2003; naturaldatabase.com, 2022). Nausea, vomiting, hypotension, sedation, and coma have been described by patients. Also symptoms of bradycardia and facial flushing were reported. Psychiatric depression was most common with doses of reserpine of greater than 0.5 mg per day and was

significantly decreased in a daily dose of less than 0.25 mg of reserpine. Between 1962 and 1965, 225 reports of accidental ingestion were reported in the United States (inchem.org, 2022). Three cases were reported of children between the ages of 30 months and 4 years who ingested reserpine in doses as high as 25 mg. All cases were resolved.

An association does not appear to exist between reserpine and cancer (inchem.org, 2022). No increased risk of birth defects has been shown in female humans who consumed reserpine at any time during their pregnancy. No mutagenic, genotoxic, or recombinogenic effects of reserpine have been demonstrated.

1.5 Aim and objectives of the study

As the drug, *Sarpagandha* has two wild sources, namely. *R. serpentina* and *R. tetraphylla* and both the plants are available in the study area, and also keeping all the above facts in mind, the following objectives are taken for the study:

i) To study Morpho-Taxonomy of *R. serpentina* and *R. tetraphylla* ii) To study Phytochemical composition of *R. serpentina* and *R. tetraphylla*

1.6 Significance of the study

The present study will significantly contribute towards differentiation of *R. serpentina* and *R. tetraphylla* at Morpho-Taxonomy and Phytochemical level as the samples has been taken from same phytogeographic region. The study also clarifies confusions prevailed between raw material collectors to choose the raw material for *Sarpagandha* from wild source.

CHAPTER- 2

2. REVIEW OF LITERATURE

2.1 Description and Distribution

The genus *Rauvolfia* L. belongs to the family Apocynaceae and encompasses herbs or shrubs with leaves in whorls of three or four. The genus *Rauvolfia* is distributed throughout the tropical regions of the earth, and itcontains a variety of alkaloids. The genus includes two very important medicinal species viz. *Rauvolfia serpentina* (L.) Benth. ex Kurz (an Indian species commonly known as*Sarpagandha* in India) and *Rauvolfia tetraphylla* L. Both the species contain bioactive alkaloids having certain biological activities. The roots of *R. tetraphylla* are often used as a substitute of *R. serpentina* for medicinal purpose. *R. tetraphylla* is an endangered species which is known by names viz. Wild snake root, Devil pepper, Four-leaf devil pepper.

Rauvolfia serpentina

R. serpentina is an evergreen shrub that is member of the dogbane or Apocynaceae family. More than 100 species are included in the Rauvolfia genus, and they are native to tropical and subtropical regions of the world, including Europe, Africa, Asia, Australia and the Central and South America. *R. serpentina* is native to the moist, deciduous forests of Southeast Asia, including India, Burma, Bangladesh, Sri-Lanka and Malaysia. *R. serpentina* grows between 40 to 80 cm in height. Leaves are elliptical or lanceolate shaped, usually occurred in whorls of 3 to 5 leaflets together, pale green and 7-10 cm long and 3.5-5.0 cm wide (Lobay 2015). The plant looks attractive due to shining nature of all parts, and fruits are round and 0.5-0.6 cm in diameter. It also has small pink or white flowers. It also has prominent tubers, soft taproot that reaches a length of 30 to 50 cm and diameter between 1.2 to 2.5 cm (Brijesh 2014).

Vernacular names:

Assamese: Sarpagandha Bengali:
Chandra
English: Rauvolfia Root
Gujrati: Sarpagandha
Hindi: Chandrabhaga /Sarpagandha
Kannada: Sutranavi
Malayalam: Amalpori
Marathi: Harkaya
Sanskrit: Chandrika
Tamil: Chevanamalpodi Urdu:
Chota chand

World distribution of *R. serpentina*

Source: https://powo.science.kew.org

Rauvolfia tetraphylla

Rauvolfia tetraphylla is an ever-green shrub covered with short smooth hairs and reaching a height of 4-6 feet. Leaves are unequal, 5-9 x 3-4 cm, ellipticovate, acute at apex, pubescent and usually found in whorls of four. Flowers are cream, off-white colored, about 5mm across, found in terminal corymbose cymes. Calyx lobes are short, ciliate and round. Corolla is approximately 3 mm long, lobes and tubes are short. Drupes are ovoid, 2-seeded, 5-10 mm across, smooth, jointed to the top, purple when ripe. Flowering occurs throughout the year (Kamble et al. 2013; Bhat 2014; Patil 2015). *Rauvolfia tetraphylla* differs from *R. serpentina* in having leaves in whorls of 4 and short corolla tube.

Vernacular names:

Bengali: Bar Chandrika

English: Garden Rauvolfia

Hindi: Barachandrika

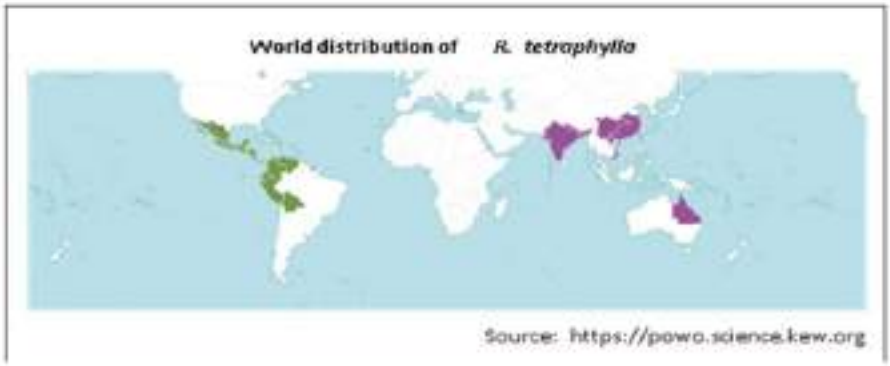
Kannada: Dodda Chandrike

Malayalam: Pumbumkolli

Oriya: Patalagarudi

Telegu: Papataku

Other: Devil Root/American Serpentwood



2.2 Scientific classification

Rauvolfia serpentina:

Kingdom: Plantae

Phylum: Angiosperms

Subphylum: Eudicots

Class: Asteroids

Order: Gentianales

Family: Apocynaceae

Genus: *Rauvolfia*

Species: *serpentina* (L.) Benth ex Kurtz

Specimen of *R. serpentina* at Botanical Survey of India

Source: Botanical Survey of India

Rauvolfia tetraphylla:

Kingdom: Plantae

Phylum: Angiosperms

Subphylum: Eudicots

Class: Asteroids

Order: Gentianales

Family: Apocynaceae

Genus: *Rauvolfia*

Species: *tetraphylla* L.

Specimen of *R. tetraphylla* at Botanical Survey of India

Source: Botanical Survey of India

2.3 Use of *Rauvolfia* as medicine

Rauvolfia serpentina was used by many physiciansthroughout India in the 1940s and then was usedthroughout the world in the 1950s, including in theUnited States and Canada. It fell out of popularity whenadverse side effects, including depression and cancer, became associated with it. Douglas Lobay, 2015 critically examined its adverse side effects, toxicology, andcarcinogenicity and refutes the association between the plant and carcinogenicity and discusses theimportance of correct dosing and of screening patientsto minimize the occurrence of depression. He concludeswith the recommendation of use of low dose *Rauvolfia* (LDR) for suitable patients with hypertension. Theplant provides clinicians with a safe and effectiveadjunct to pharmaceuticals in the treatment of highblood pressure.

A review of current insight to the uses of *Rauvolfia* by Nitin Biradar et al 2016, shows that the use of *Rauvolfia* and treatment for many diseases, its botany, chemistry and mode of action with special emphasis on the plants role in treating high blood pressure and hypertension and also critically examining its adverse side effects, toxicology and carcinogenicity. This review suggested some of the newer use of Rauvolfia serpentina. This can be further studied scientifically and can be used for the benefit of humanity to cure the diseases.

Rajendra Bhanwaria et al 2021, published a paper on Biology, Chemistry and cultivation practices of *Rauvolfia serpentina* and *Rauvolfia tetraphylla* or as distinctive medicinal plant for curing human disease. In this paper Rauvolfia plants serve as an excellent source of various therapeutic agents. Reserpine of *Rauvolfia* seems to be a safe and dominant treatment for the disorder of hypertension. This research work explored the antioxidant as well as the antidiabetic activity of the wild and cultivated variety of *Rauvolfia* plant. The study suggested that the wild source of the plant more potential to exert antioxidant and antidiabetic activity as compared to the cultivated plant.

Mahalaxmi et al 2019, published a comprehensive review on *Rauvolfia tetraphylla*'s ethnobotanical uses, phytochemistry and pharmacological activities.

An intensive literature survey conducted in this review highlighted potential utilization of *R. tetraphylla* ethnomedicinally in different parts of the country. Besides, a vast literature has also revealed pharmacological activities such as antimicrobial, anxiolytic, antioxidant and anti-inflammatory. *R. tetraphylla* is shown to possess similar commercial and therapeutic properties as that of *Rauvolfia serpentina*.

Jakaria et al 2016, discusses pharmacognosy, phytochemistry and pharmacological activities of Rauvolfia tetraphylla in their review to leaf, stem and root of R. tetraphylla were pharmacognostically studied. Preliminary phytochemical study of different extracts revealed that presence of various phytoconstituents like reducing sugars, carbohydrates, alkaloids, amino acids, steroids, tannins, flavonoids, phenols, saponins, fixed oils, fats, gums and mucilages.

Among the bioactive compounds in *Rauvolfia* species indole alkaloids, especially reserpine is most common and this is available in all the three species of *Rauvolfia* found in Assam. As per the estimation Bindu et al 2014, *R. tetraphylla* possesses highest reserpine content comparative to others and the most common Rauvolfia species, R. serpentina reserpine content is comparatively low. Bindu et al 2014, have estimated reserpine content of 5 *Rauvolfia* species collected from Kerala. This study is the first systematic and comparative screening of reserpine among the *Rauvolfia* species in India.

Hussain et al 2015, have conducted phytochemical and GC-MS analysis of n-Hexane extract of R. serpentina from market sample of Kathmandu. Root extract shows the presence of alkaloids, saponins, tannins, flavonoids and phenols. The GC-MS analysis led to identification of 18 compounds; where major constituents were 2-methylheptane (3.48%), cis-1,3-dimethylcyclohexane (13.66%), 2-(propoxymethyl) oxirane (3.45%) Hexylene Glycol (14.28%), 3methylheptan-2-one (15.56%), 2,5- dimethylhexan-3,4-diol (5.70%), 3,4dimethylhexan-2-ol (7.47%), 1,1,2,3-tetramethylcyclopropane (24.33%), Diethyl Phthalate (2.11%) and other chemical constituents with less than 2% peak area.

An in vitro and in silico approach for identification of bioactive compounds by GC-MS have been conducted along with α- amylase and αglucosidase inhibitory activity of R. tetraphylla and Oroxylum indicum have been done recently by Swargiary and Daimary in 2020. *R. tetraphylla* species is collected from Kokrajhar of Assam. In this study GC-MS analysis revealed four major compounds in R. tetraphylla like (Dimethyl amino)-7-(3-(4-ethylphenoxy)2-hydroxypropyl)-3-methyl-3,7-dihydro-1H-purine-2,6-dione tms (R1), Chlorflurenol (R2), 2-(4-Nitro-pyrazol-1-yl) propionic acid, hydrazide (R3), and 9-(2-Methoxyethyl) carbazole (R4).

Pandey et al, 2016 have conducted a validated and densitometric HPTLC method for the simultaneous quantification of reserpine and ajmalicine in Rauvolfia serpentina and Rauvolfia tetraphylla from the samples of different areas of Punjab and Uttarakhand. HPTLC activities shows that roots of R. serpentina are enriched in the desired constituents and can be preparation of formulations. It was found that the quantity of indole alkaloids in R. tetraphylla was at par to R. serpentina and can be used in the preparation of herbal formulation. HPTLC of methanolic extract of root containing indole alkaloids i.e., reserpine and ajmalicine.

2.4 Substitution of *R. sepentina* with *R. tetraphylla*

Sulaiman et al. 2020evaluated the phytochemistry and biological activities of allied species such as *Rauvolfia tetraphylla* L., *Rauvolfia hookeri* S.R.Sriniv. & Chithra, *Rauvolfia micrantha* Hook.f., and *Rauvolfia verticillata* (Lour.) Baill. and the result indicated that the root of *R. serpentina* is phytochemically similar with that of *R.tetraphylla*.Chemical profiling using HPTLC showed similar chemical profiles for *R.serpentina* and R. tetraphylla. LC/MS characterization of various species showed that most of the active alkaloids are common for both *R. serpentina* and*R. tetraphylla*. Anti-hypertensive activity and analgesic activity were evaluated in experimental animal model. *R. serpentina* and *R. tetraphylla* showed comparatively significant reduction in systolic and diastolic

pressure.Comparable analgesic activity was also shown by *R. serpentina* and *R. tetraphylla*. On the basis of phytochemical and pharmacological evaluation, it was concluded that the root of *R. tetraphylla* can be used as a validated substitute for Sarpagandha.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Plant Material

The samples of *Rauvolfia tetraphylla* for the purpose of taxonomic and phytochemical analysis was collected from Medicinal Plant Garden of North East Herbarium of Ayurveda Research (NEHAR), Central Ayurveda Research Institute, Guwahati (Lat: 26° 06' 57" N; Lon: 91° 46' 50" E) and that of *R. serpentina* was collected from Pub Boragaon, Kamrup (M) district, Assam (Lat: 26° 06' 52" N; Lon: 91° 46' 41" E). The collection was done in the month of May, 2022.

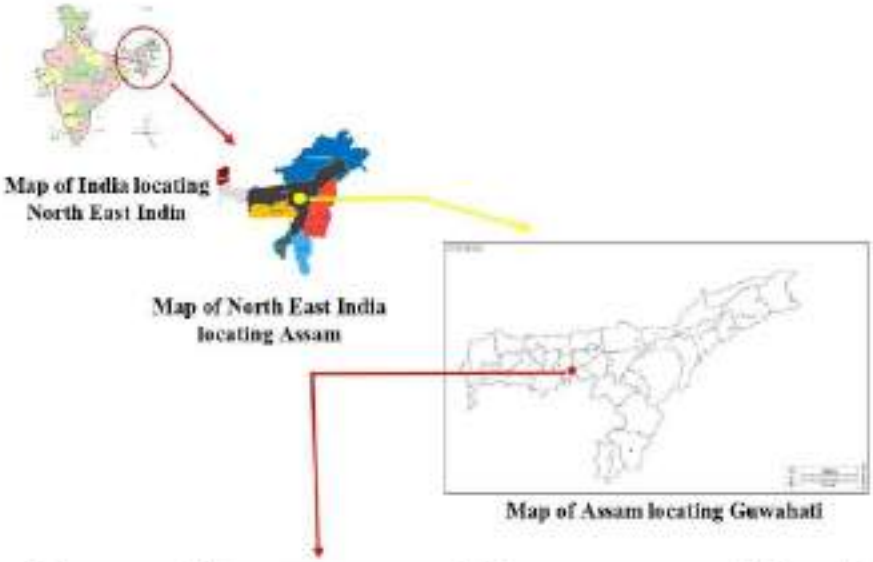
The plant specimens were identified at North East Herbarium of Ayurveda Research (NEHAR), CARI, Guwahati, Kamrup (M) district, Assam where voucher specimens (NEHAR- **7979** and NEHAR- **7980**) are deposited for future reference along with in the Herbarium of Silapathar Science College, district Dhemaji, Assam.

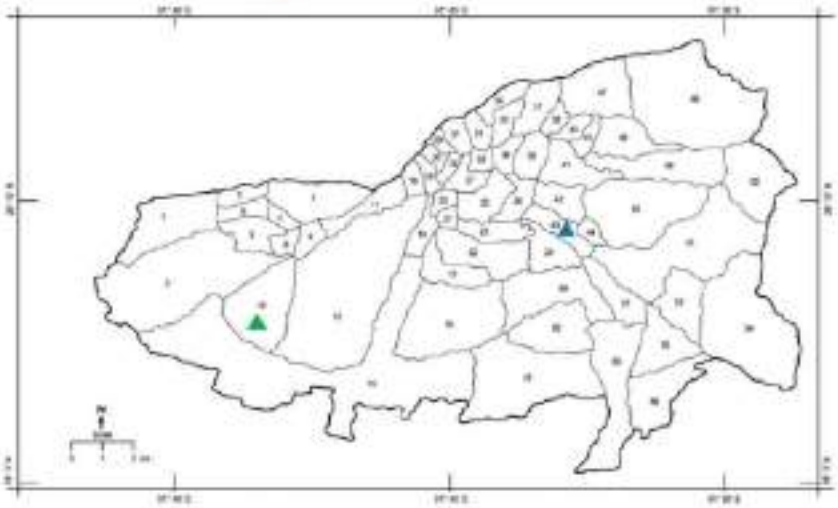
3.2 Morphological and Taxonomical studies

R. serpentina and *R. tetraphylla* were screened in wild and collected for morphological studies. Both species were identified, illustrated and taxonomic details were compared with the help of relevant literature like Biradar et al (2016), Bhanwaria et al. (2021), Mahalaxmi et al. (2019). Taxonomic keys of both species have been made. The plant specimens were collected, properly pressed, dried and processed as herbarium following the standard method of herbarium preparation.

Morphological observations were made to record characteristics of Root, stem, leaves, flowers and fruits for enumerating these attributes for comparison. Photographic evidences of the observation are recorded in Photo plates.

SAMPLE COLLECTION AREA





Map of Guwahati, Assam, locating the area of specimen collection

▲ Collection site of *R. serpentina*

▲ Collection site of *R. tetraphylla*

3.3 Anatomical studies

The anatomical studies were performed for various parts (flower, leaf, stem, roots) of both plants under study by taking hand cut sections. For the stomata studies the peels of fresh leaves were taken and microscopic photographs were taken.

The sections were stained with safranin and fast green to visualize the anatomical structures. The sections and peels were observed under light microscope and the photographs were taken.

In leaf venation study, one to several leaves were collected into petri dishes and immersed in 10% NaOH at room temperature until they became translucent. This step took one to three weeks, depending on species; thick, leathery or stone-like leaves took longer. Then the leaves were washed twice with water and bleached in 50% commercial bleach for 10 minutes. After another two washing processes, the leaves were dehydrated for 10 minutes successively in 50% and then 75% ethanol. Leaves were then stained by 1% Safranin in 95% ethanol for 20 minutes. Stained leaves were washed for a minute or two, depending on leaf thickness, with absolute ethanol to de-stain non vascular tissues. Photographs of venation patterns were taken at a range of magnifications (Ghalia and Sehaan,2020).

3.4 Phytochemical study

The roots of both the samples were collected and washed thoroughly with running tap water to remove surface dirt and dust. Then the materials were dried under shade for about 30 days. The shade dried root samples were chopped and formed into fine powder by using a mixer grinder. The fine powder form of root samples were used for extract preparation by using the Soxhlet apparatus. 10 gm of powdered root samples were filled in the thimble and 250 mL of methanol was used for methanolic extract preparation. Then thimbles were placed in the Soxhlet extractor. The apparatus was fitted below with round bottom flask that contained the methanol solvent. Then the whole setup was heated with the mantle. This process was allowed to run for 8 hours (8 cycles). The temperature was

maintained at 42 – 50 °C. The extracts obtained were filtered by using Whatman's No.-1 filter paper and concentrated by using rotary evaporator to remove the solvents. After complete evaporation, each of the solvent extract was weighed, preserved at -4 °C .

3.4.1 GC MS Analysis

GC MS Analysis was carried out using CAIF facility of Guwahati Biotech Park Incubation Centre at Guwahati Biotech Park, Guwahati, Assam.

Gas Chromatography–Mass Spectrometry (GC- MS) is the logical combination of two techniques namely Gas Chromatography and Mass Spectrometry, where gas chromatography is used for separation properties and mass spectrometry is for the detection of compound based on their mass, combination of these techniques helps to identify the different component of the test sample.

GC-MS analysis of the extracts of the samples were carried out with Perkin Elmer (USA) GC-MS instrument, Model: Clarus 680 GC & amp; Clarus 600C MS comprising a liquid auto-sampler. The Software used in the system is TurboMass Ver. 6.4.2. The peaks were analyzed using data analysis software NIST-2014. The capillary column used is 'Elite- 5MS' having dimensions- length- 60 m, ID- 0.25 mm and film thickness- 0.25 µm and the stationary phase is 5% diphenyl 95% dimethyl polysiloxane.

GC-Protocol: Helium gas (99.99%) was used as carrier gas (i.e mobile phase) at flow rate of 1 ml/min. An injection volume of 1 µl was employed in splitless mode. Injector temperature is 280°C and ion-source temperature 180°C. The oven temperature was programmed at 60°C (for 1 min), with an increase at the rate 7°C/min to 200°C (hold for 3 min) then again increased at rate of 10.C/min to 300.C (bold for 5 min). The total run time is ~ 39min. Solvent delay was kept for 7 minutes.

MS Protocol: Mass Spectra was taken in Electron Impact positive(EI+) mode at 70 eV. A solvent delay of 8 min was there for MS scan. Mass range i.e m/z range is 50-

600 amu. Interpretation of the peaks appeared in the GC Chromatogram were done by library search of the mass spectrum of corresponding peaks using the database software of National Institute Standard and Technology-2014 (NIST2014). The mass spectrums of the unknown components were compared with the spectrum known components of NIST library and the compounds were identified with name, molecular weight, empirical formula etc.

3.4.2 Bioactivity reported for Major phytochemicals

Bioactivity reported for Major phytochemicals present in roots of *R. 32erpentine* and *R. tetraphylla* are reviewed from secondary sources and reported in tables separately for *R. 32erpentine* and *R. tetraphylla*.

3.4.3 Glimpses of field and laboratory work.

Field collection of sample

Field collection of sample

Taxonomic work on sample

Taxonomic work on sample

During laboratory work

During illustration of sample

Experimental study sample

Perkin Elmer GC-MS instrument

With the team of Central Ayurveda Research Institute, Guwahati

CHAPTER 4

4. RESULTS AND DISCUSSION

The results of the taxonomic studies including Morphology and Anatomical studies are enumerated in Tables for comparison and preparation of Taxonomic key.

4.1 Comparative Morphological Study of *R. serpentina* and *R. tetraphylla*

Characters	*R. serpentina*	*R. tetraphylla*
Habit	It is an erect, pubescent, perennial shrub.	A small, glabrous, perennial much-branched woody shrub.
Leaves	In whorls of 3, thin, lanceolate, acute.	Leaves in whorls of 4, ovateelliptic.
Inflorescence	Irregular corymbose cymes, white or pink, often tinged with violet.	Flowers greenish-white or creamy-white in umbellate cymes.
Stem	Usually un-branched, slender, green in colour.	Usually un-branched, slender, straw colored
Root	Tuberous with pale brown cork.	Tuberous with pale yellow cork.
Fruit	Drupe, single or didynamous, shining black.	Drupes ovoid, deep red or purple when ripe

Photo Plate 1:

A comparative morphological study of *R. serpentina* and *R. tetraphylla*

R. serpentina	*R. tetraphylla*
	
Habit	**Habit**
	
Leaf	**Leaf**
	
Flower	**Flower**

R. serpentina	*R. tetraphylla*
	
Stem	**Stem**
	
Roots	**Roots**
	
Drupes	**Drupes**

4.2 Taxonomic key

i	Leaf lateral veins 5–20 pairs, arcuate ascending from leaf base and descending towards the apex; Ovaries and fruit of connate carpels, often only at base; Leaves ovate or elliptic, tomentose when young; corolla tube 2–3 mm; fruit entire at apex .....*R. tetraphylla*
ii	Leaves narrowly elliptic or obovate, glabrous, 7–20 cm, membranous; corolla tube 10–20 mm; fruit forked at apex; petiole 10–15 mm; inflorescence solitary; peduncle, pedicel, calyx, and corolla red or reddish; corolla lobes 1.5–3.5 mm; stamens inserted at middle of corolla tube .....*R. serpentina*

Photo Plate 2:

Herbarium sheets of *Rauvolfia serpentina* prepared under study

Herbarium sheets of *Rauvolfia serpentina* prepared under study

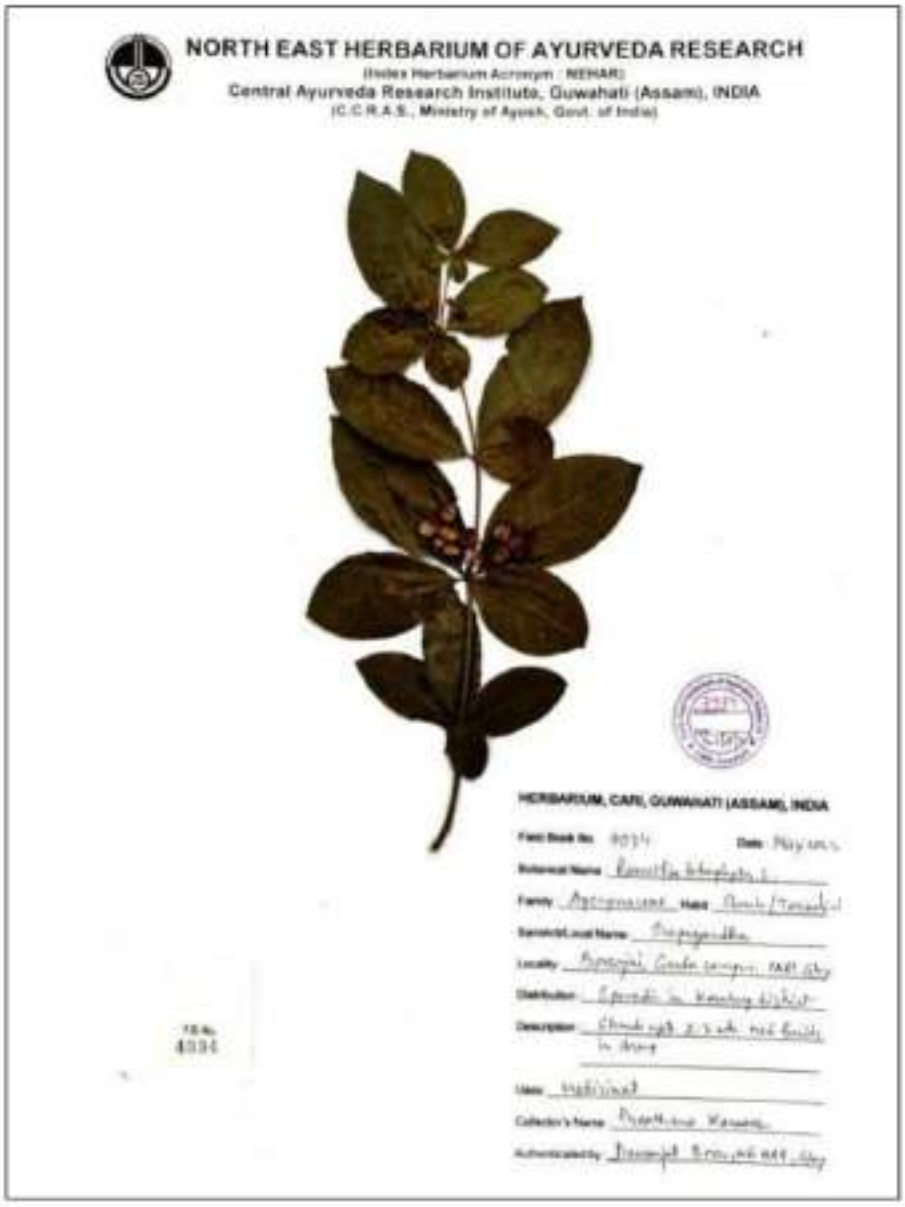
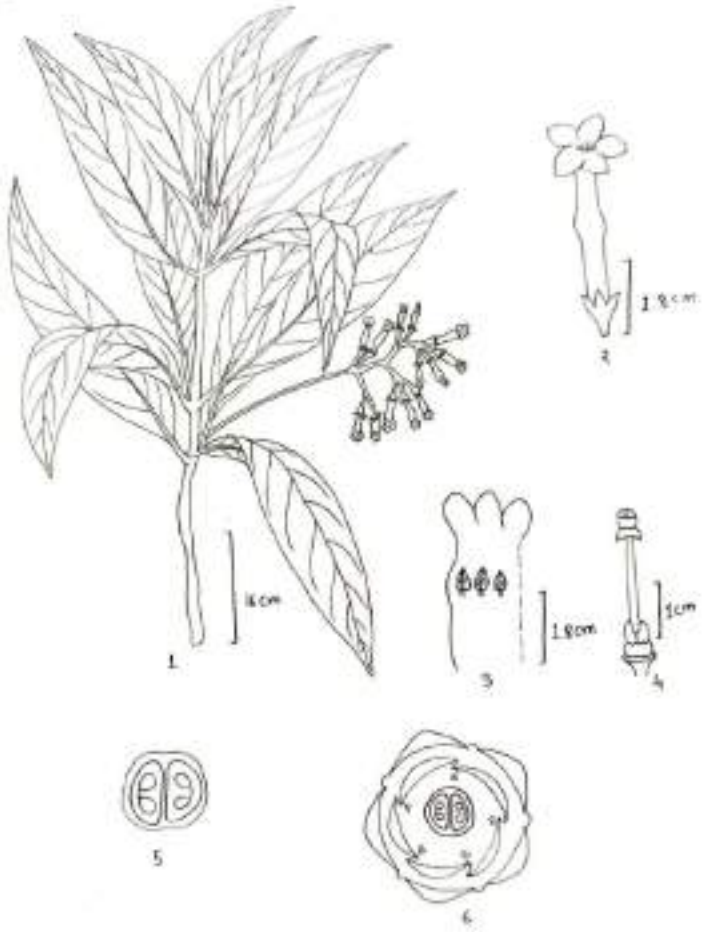


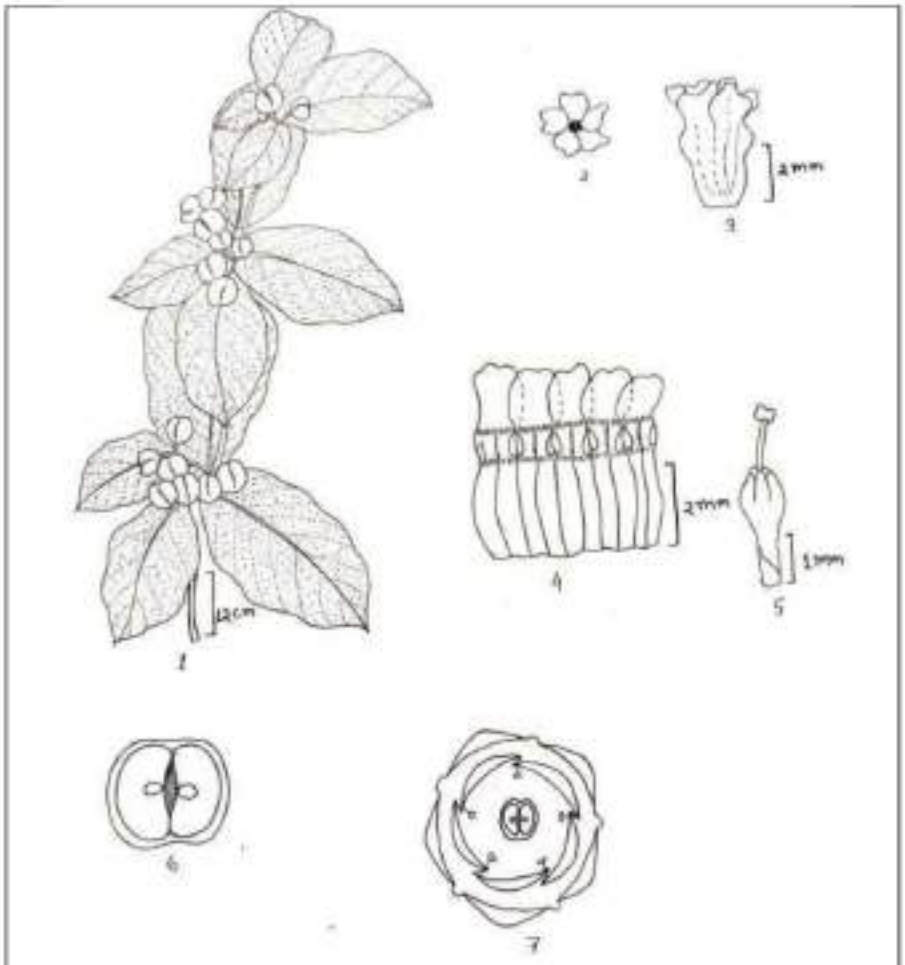
Photo Plate 3:

Illustrations of *Rauvolfia serpentina* and *R. tetraphylla*



R. serpentina

1: Flowering twig; 2: Flower; 3: Anthers; 4: Gynoecium; 5: Ovary; 6: Floral Diagram



R. tetraphylla

1: Flowering twig; 2: Flower; 3: Anthers; 4: Gynoecium; 5: Ovary; 6: Floral Diagram

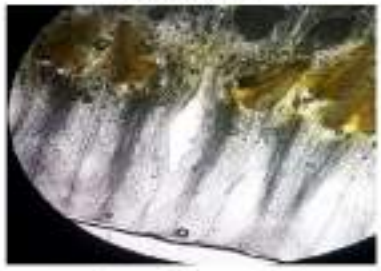
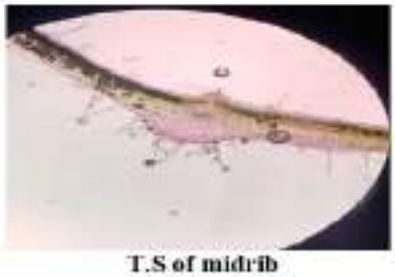
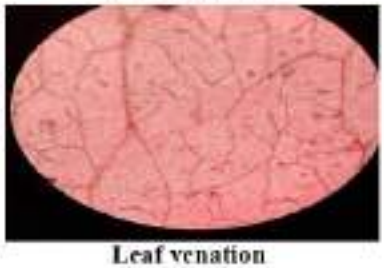
4.3 Comparative Anatomical Study of *R. serpentina* and *R. tetraphylla*

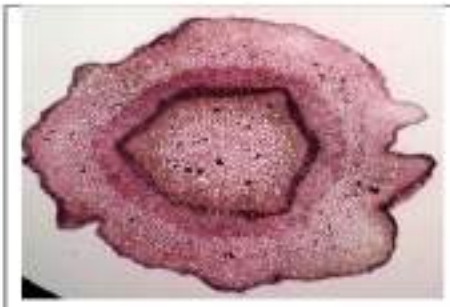
Characters	*R. serpentina*	*R. tetraphylla*
Gynoecium	Carpels-2, have a filiform style and large bifid stigma; a bilocular ovary has two ovules in each locule.	The ovary is superior with bicarpellary, syncarpous,
Androecium	Stamens-5, in epipetalous condition are enclosed within the dilated portion of the corolla tube.	Stamens-5, epipetalous
Ovary	Ovary have two chambers or locules i.e., bilocular and contains two ovule in each locule. Placentation is axile.	Ovary have two chambers or locules i.e., bilocular and contains one ovule in each locule. Placentation is axile.
Leaf	Trichomes are absent in Epidermis.	Upper epidermis was devoid of stomata and it had numerous uniseriate, multicellular trichomes.
Stomata	Stomata are paracytic that possess one or more pairs of lateral subsidiary cells oriented parallel with the guard cells.	Stomata are of both paracytic and anomocytic type. Gourd cells are surrounded by the same size, shape arrangements as the rest of the epidermal cells.

Leaf venation	Cross-Venulate i.e., small veins connecting secondary veins.	Reticulatei.e., smaller veins forming a network.
Stem	Stem lack of trichomes	Stem views the single layer of epidermis with uniseriate, multicellular trichomes
Root	Root shows thick striated bark and uniformly wide, white or pink coloured phellodenn-bast region devoid of mechanical tissue. The secondary xylem is characterized by straight rays, close, concentric growth rings and centric xylem plate.	Root shows hard, narrow outer bark with few indistinct striations and brown, hard, wide phellodenn-bast region. The secondary xylem has nimerous large vessels, curved rays and somewhat excentric xylem plate.

Photo Plate 4:

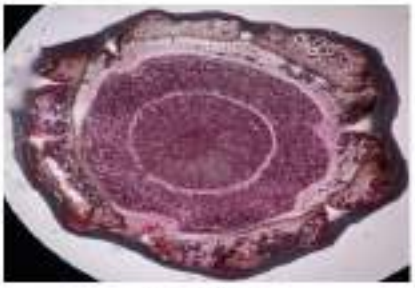
Comparative anatomical study of *R. serpentina* and *R. tetraphylla*

R. serpentina	***R. tetraphylla***
	
Gynoecium	**Gynoecium**
	
Epipetalous anther	**Epipetalous anther**
	
T.S of midrib	**T.S of midrib**
	
Leaf venation	**Leaf venation**



T.S of Stem

T.S of Stem



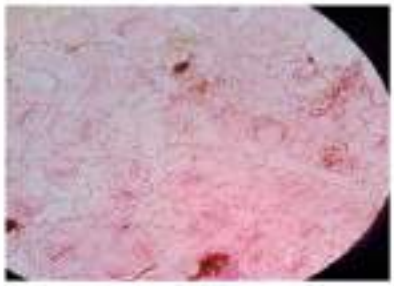
T.S of Root

T.S of Root

T.S of Ovary

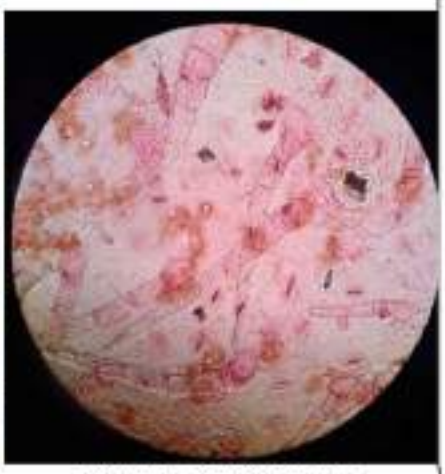
T.S of Ovary

Stomata



Stomata

Absence of Trichome in
R. serpentina



Presence of Trichome in
R. tetraphylla

4.4 Phytochemical analysis

The major phytochemicals reported in the root extract are emmerated in the following Tables followed by Figures depicting their presence in GC-MS study. Name of chemicals present is mentioned along with Molecular formula, molecular weight, %peak area and Retention time during the study.

Table 1: Major phytochemicals present in roots of *R. serpentina*

SL No	Name of Compound	Molecular formula	Molecular weight	% Peak area	Ret. Time
1	THYMINE	$C_5H_6N_2O_2$	126	1.30	12.85
2	3-ACETYLTHYMINE	$C_7H_8N_2O_3$	168	1.30	12.85
3	MOLINATE	$C_9H_{17}NOS$	187	1.30	12.85
4	1,3-DIOXEPANE, 2-PENTADECYL-	$C_{20}H_{40}O_2$	312	1.75	14.34
5	N-(1-METHOXYCARBONYL-1-METHYLETHYL)-4-METHYL-2-AZA-1,3-DIOXANE	$C_9H_{17}NO_4$	203	1.75	14.34
6	4-HEPTANOL, 4-PROPYL-	$C_{10}H_{22}O$	158	1.75	14.34
7	OCTANOIC ACID, 2-HEXYL-	$C_{14}H_{28}O_2$	228	1.75	14.34
8	1-ETHYL-2-HYDROXYMETHYLIMIDAZOLE	$C_{18}H_{16}N_2O_2P$	126	2.93	16.008
9	5-HYDROXYMETHYLFURFURAL	$C_6H_6O_3$	126	2.93	16.008
10	TRANS-2-METHYL-4-N-PENTYLTHIANE, S, S-DIOXIDE	$C_{10}H_{20}O_2S$	218	1.23	19.034

11	CHLOROACETIC ACID, TETRADECYL ESTER	$C_{16}H_{31}ClO_2$	290	1.23	19.034
12	TRANS-2-METHYL-4-N-BUTYLTHIANE, S, S-DIOXIDE	$C_{10}H_{20}O_2S$	204	1.23	19.034
13	CIS-2-METHYL-4-NPENTYLTHIANE, S, S-DIOXIDE	$C_{11}H_{22}O_2S$	218	1.23	19.034
14	3-TRSNS-(1,1-DIMETHYLETHYL)-4-TRANS-METHOXYCYCLOHEXANOL	$C_{11}H_{22}O_2$	186	13.09	20.54
15	TRIDECANE, 2,2,4,10,12,12-HEXAMETHYL-7-(3,5,5-TRIMETHYLHEXYL)-	$C_{28}H_{58}$	394	13.09	20.54
16	OXIRANE, [(DODECYLOXY)METHYL]-	$C_{15}H_{30}O_2$	242	13.09	20.54
17	3, CIS-(1,1-DIMETHYLETHYL)-4, CISMETHOXYCYCLOHEXANOL	$C_{13}H_{22}O_2$	186	13.09	20.54
18	3',5'-DIMETHOXY ACETOPHENONE	$C_{10}H_{12}O_3$	180	2.52	22.36
19	4-METHYL-2,5-DIMETHOXYBENZALDEHYDE	$C_{10}H_{12}O_3$	180	2.52	22.36
20	3-TERT-BUTYL-4-HYDROXYANISOLE	$C_{11}H_{16}O_2$	180	2.52	22.36
21	2-AMINO-1-(3,4-DIMETHOXYPHENYL) ETHANONE	$C_{10}H_{13}NO_3$	195	2.52	22.36

22	CIS-1-CHLORO-9-OCTADECENE	$C_{18}H_{35}Cl$	286	1.01	31.859
23	OLEIC ACID	$C_{18}H_{34}O_2$	282	1.01	31.859
24	Z, Z-6,28-HEPTATRIACTONTADIEN-2-ONE	$C_{37}H_{70}O$	530	1.01	31.859
25	2,6,10,14-TETRAMETHYL-7-(3-METHYLPENT-4-ENYLIDENE) PENTADECANE	$C_{25}H_{48}$	348	1.01	31.859
26	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	$C_{28}H_{56}O_2$	438	1.76	24.976
27	BETA. -D-MANNOFURANOSIDE, 1-O-(10-UNDECENYL)-	$C_{17}H_{32}O_6$	332	1.76	24.976
28	I-PROPYL 10-METHYL-DODECANOATE	$C_{16}H_{32}O_2$	256	1.76	24.976
29	I-PROPYL HEXACOSANOATE	$C_{29}H_{58}O_2$	438	1.76	24.976
30	EICOSANOIC ACID	$C_{10}H_{40}O_2$	312	2.32	29.523
31	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	$C_{38}H_{68}O_8$	652	2.32	29.523
32	N-HEXADECANOIC ACID	$C_{16}H_{32}O_2$	256	2.32	29.523
33	TETRADECANOIC ACID	$C_{14}H_{28}O_2$	228	2.32	29.523
34	D-MANNITOL, 1-O-(22-HYDROXYDOCOSYL)-	$C_{28}H_{58}O_7$	506	1.55	36.086
35	METHYL 2-HYDROXY-EICOSANOATE	$C_{21}H_{42}O_3$	342	1.55	36.086
36	D-MANNITOL, 1-O-(16-HYDROXYHEXADECYL)-	$C_{22}H_{46}O_7$	422	1.55	36.086

Figure 1: **GC-MS Chromatogram (main) of root methanolic extract of *R. serpentina***

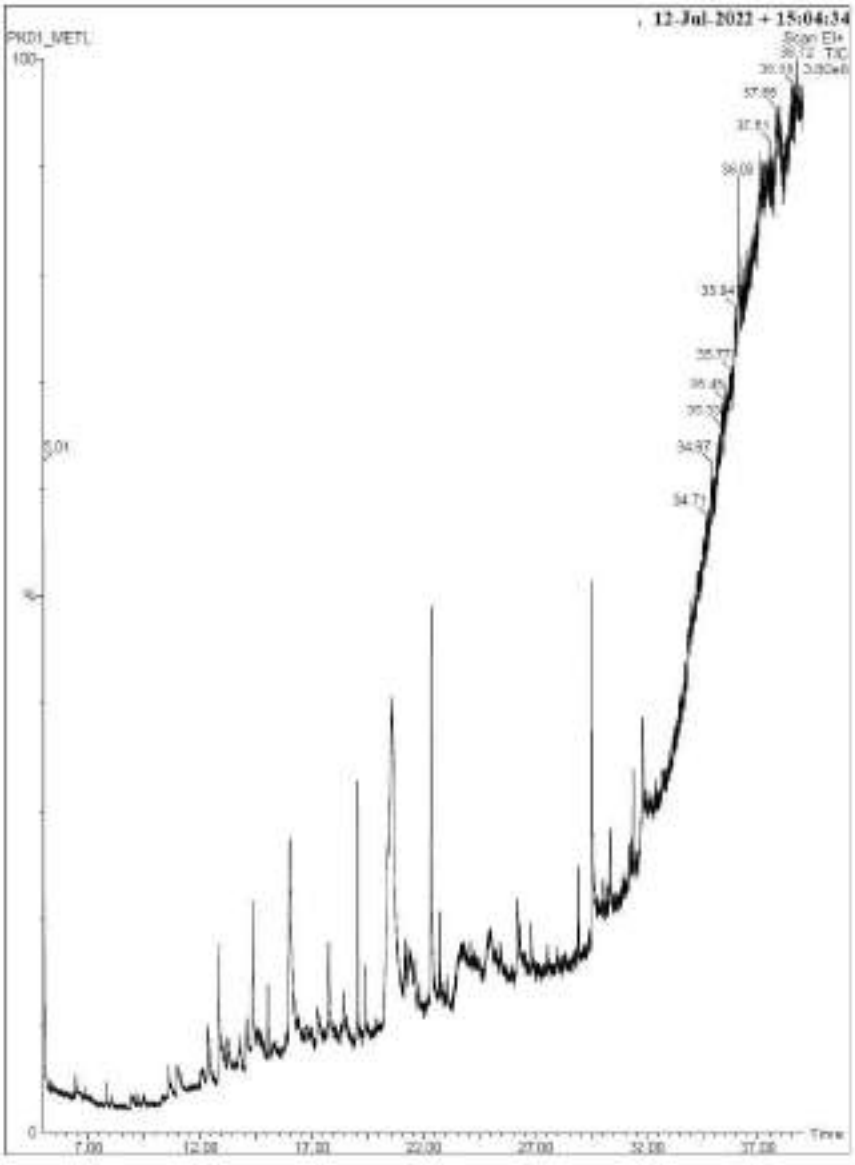


Figure 2: **GC-MS Chromatogram (extended) of root methanolic extract of *R. serpentina***

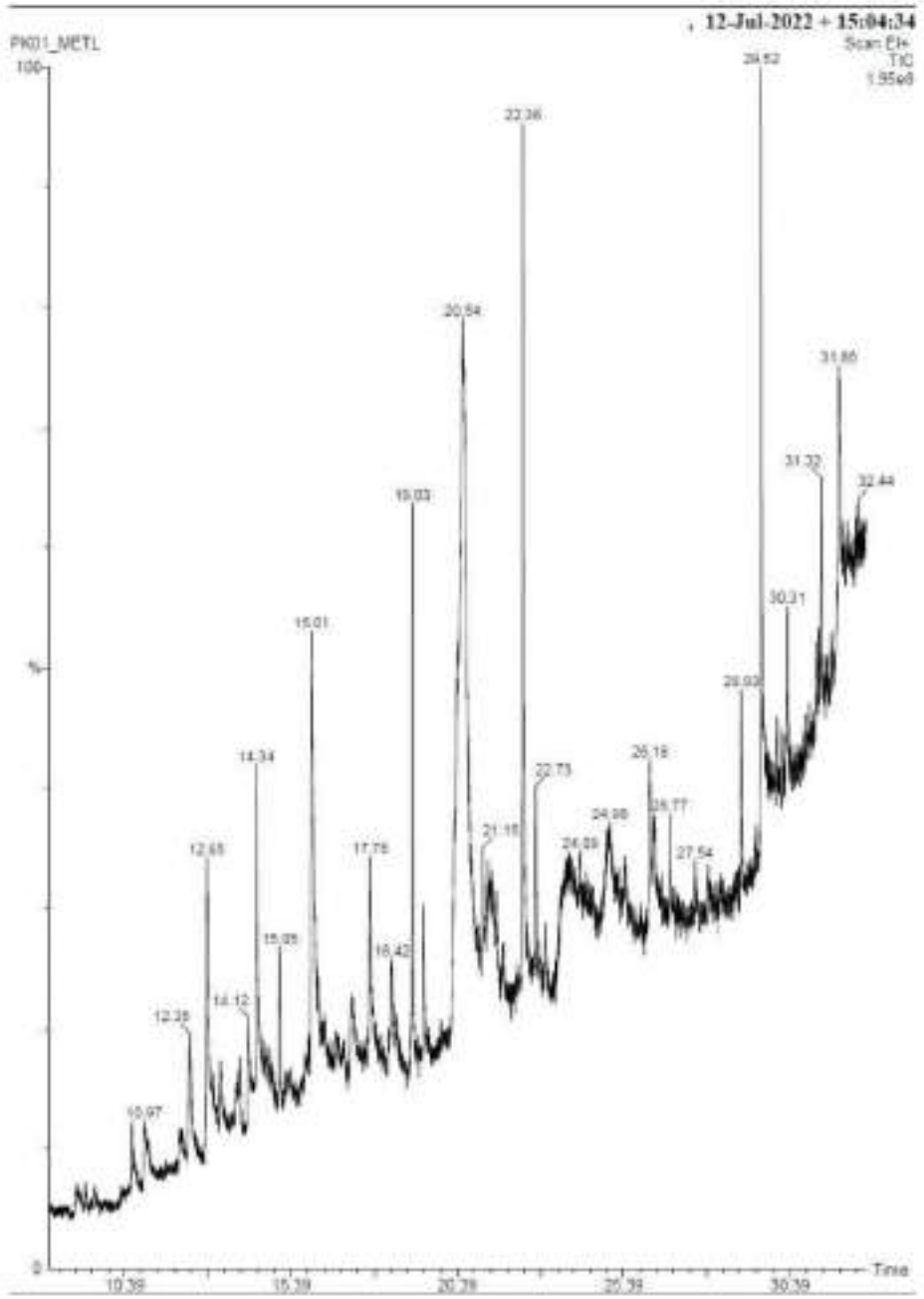
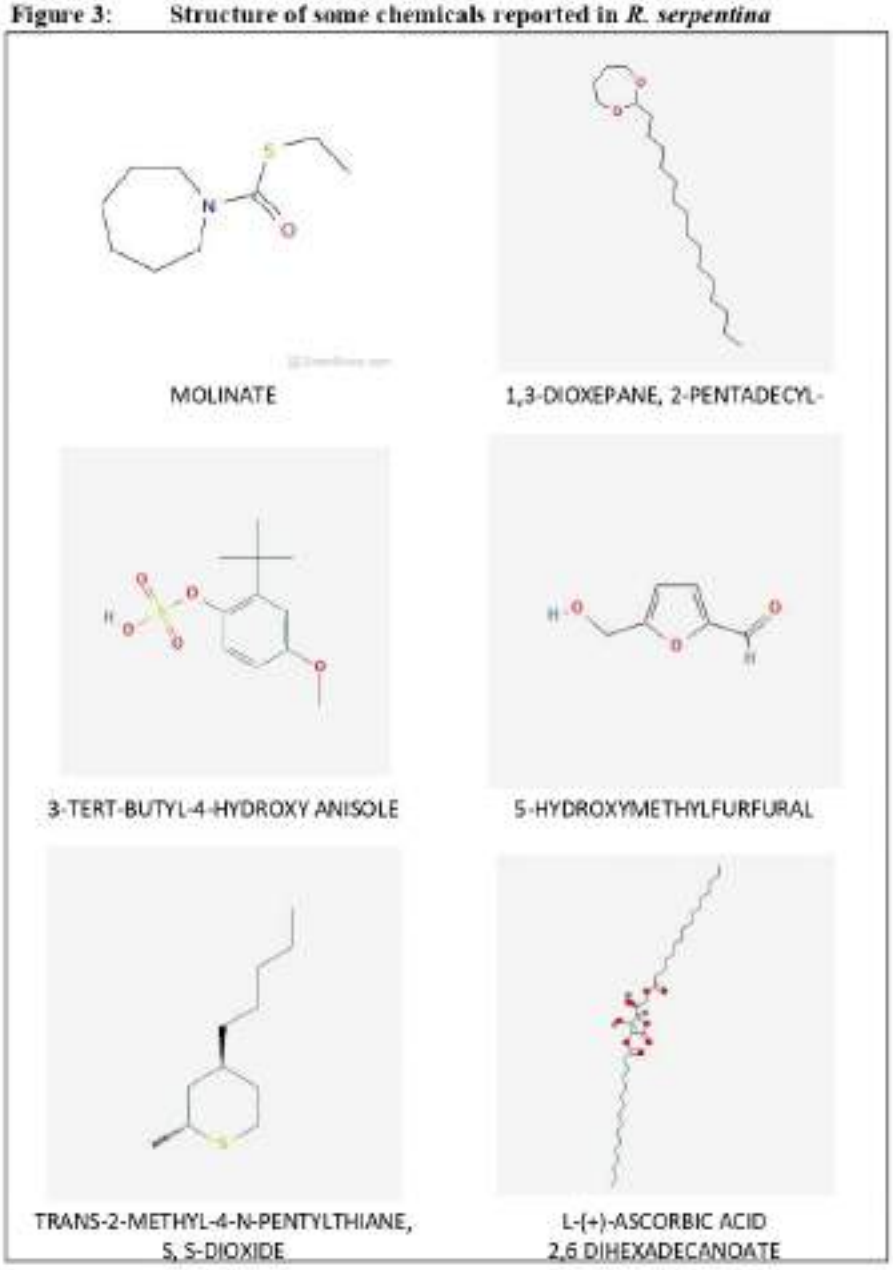


Figure 3: Structure of some chemicals reported in *R. serpentina*



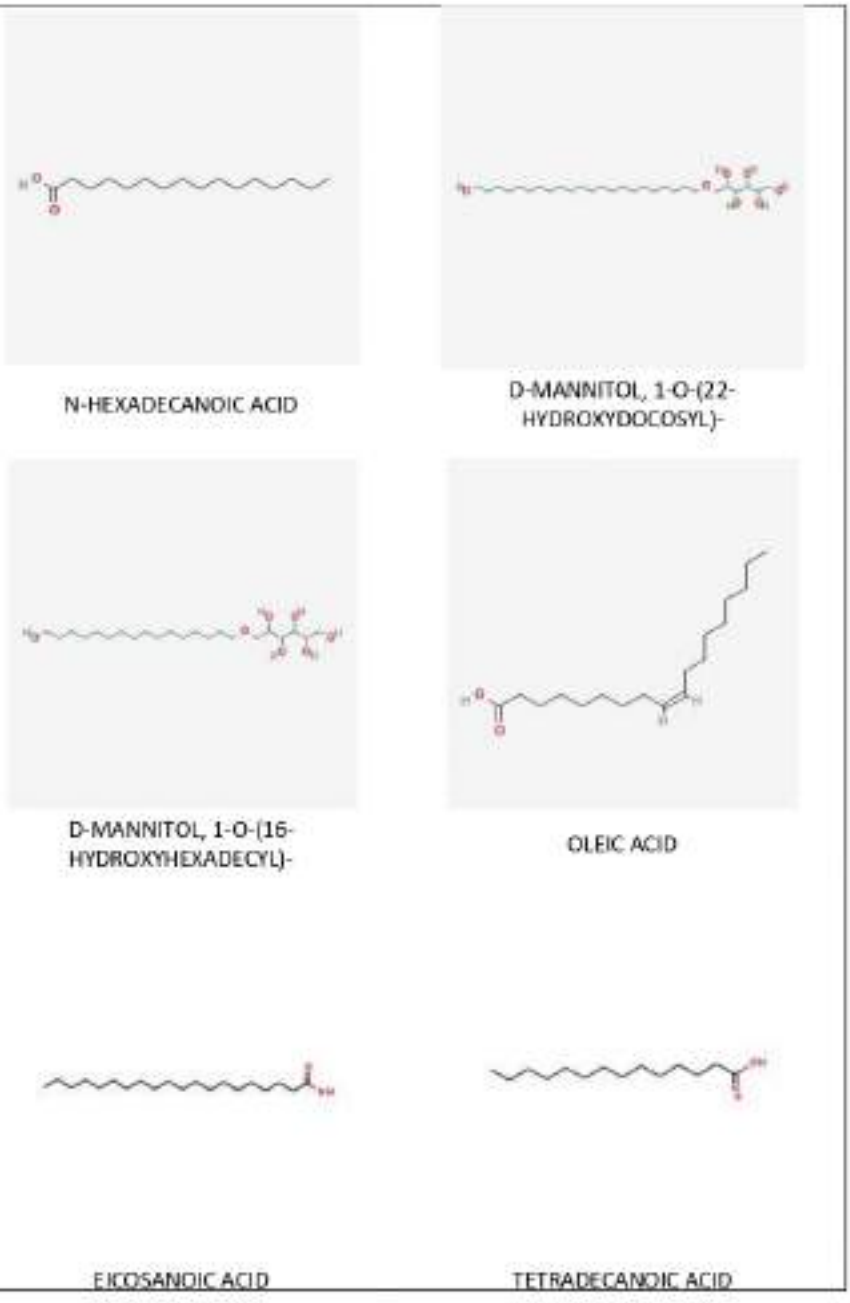

N-HEXADECANOIC ACID
D-MANNITOL, 1-O-(22-HYDROXYDOCOSYL)-
D-MANNITOL, 1-O-(16-HYDROXYHEXADECYL)-
OLEIC ACID
EICOSANOIC ACID
TETRADECANOIC ACID

Table 2: Major phytochemicals present in roots of *R. tetraphylla*

SL No	Name of Compound	Molecular formula	Molecula r weight	% Peak area	Ret. Time
1	1-NONYLCYCLOHEPTANE	$C_{16}H_{32}$	224	0.41	31.33
2	13-METHYLTETRADEC-9-ENOIC ACID METHYL ESTER	$C_{16}H_{30}O_2$	254	0.41	31.33
3	METHYL 11-DOCOSENOATE	$C_{23}H_{44}O_2$	352	0.41	31.33
4	OLEIC ACID	$C_{18}H_{34}O_2$	282	0.41	31.33
5	1,3-DIOXOLANE, 4-ETHYL-5-OCTYL-2,2-BIS(TRIFLUOROMETHYL)-, TRANS-	$C_{15}H_{24}F_6O_2$	350	1.74	5.01
6	1,3-DIOXOLANE, 4-ETHYL-5-OCTYL-2,2-BIS(TRIFLUOROMETHYL)-, CIS-	$C_{15}H_{24}F_6O_2$	350	1.74	5.01
7	2-N-HEXYLTHIOLANE, S, S-DIOXIDE	$C_{10}H_{20}O_2S$	204	1.74	5.01
8	16-HYDROXYHEXADECANOIC ACID	$C_{16}H_{32}O_3$	272	1.74	5.01
9	PROPANOIC ACID, 3,3'-SULFONYLBIS-	$C_9H_{10}O_5S$	210	3.06	5.42
10	HEXA-1,3,5-TRIYNE	C_6H_2	74	3.06	5.42
11	1,2,3,4-TRIDECANETETROL	$C_{13}H_{28}O_4$	248	3.06	5.42
12	1,2,3,4-TETRADECANETETROL	$C_{14}H_{30}O_4$	262	3.06	5.42
13	PHENOL, 2,6-DIMETHOXY-	$C_8H_{10}O_3$	154	1.41	18.40
14	1-SILACYCLO-2,4-	$C_5H_6Cl_2Si$	96	1.41	18.40

	HEXADIENE				
15	1-SILACYCLOHEXA-2,5-DIENE	C_5H_8Si	96	1.41	18.40
16	TRIFLUOROMETHYL T-BUTYL DISULFIDE	$C_5H_9F_3S$	190	16.37	20.56
17	METHYL 2,6-ANHYDRO-ALPHA -D-ALTROSIDE	$C_7H_{12}O_5$	176	16.37	20.56
18	PROPANAMIDE, 3-CYCLOPENTYL-N-METHYL-	$C_9H_{17}NO$	155	16.37	20.56
19	1,2,4,5-CYCLOHEXANETETROL, (1. ALPHA.,2.ALPHA.,4. ALPHA.,5. BETA.)-	$C_6H_{12}O_4$	148	16.37	20.56
20	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	$C_{29}H_{58}O_2$	438	6.68	24.91
21	BETA-D-MANNOFURANOSIDE, 1-O-(10-UNDECENYL)-	$C_{17}H_{32}O_6$	332	6.68	24.91
22	3-O-METHYL-D-GLUCOSE	$C_7H_{14}O_6$	194	6.68	24.91
23	D-FRUCTOSE, 1,3,6-TRIDEOXY-3,6-EPITHIO-	$C_6H_{10}O_3S$	162	6.68	24.91
24	EPI-INOSITOL	$C_6H_{12}O_6$	180	1.80	5.13
25	MUCO-INOSITOL	$C_6H_{12}O_6$	180	1.80	5.13
26	CIS-INOSITOL	$C_6H_{12}O_6$	180	1.80	5.13
27	INOSITOL	$C_6H_{12}O_6$	180	1.80	5.13
28	1-ETHOXY-2,4-HEXADIENE	$C_8H_{14}O$	126	1.33	16.05
29	2-AZIRIDINONE, 1-TERT-BUTYL-3-(1-METHYLCYCLOHEXYL)-	$C_{13}H_{23}NO$	209	1.33	16.05
30	2-ETHOXY-3,5-HEXADIENE	$C_8H_{14}O$	126	1.33	16.05

31	1-CYCLOHEXYL-1-(4ETHYLCYCLOHEXYL) ETHANE	$C_{16}H_{30}$	222	1.33	16.05
32	3-TERT-BUTYL-4-HYDROXYANISOLE	$C_{11}H_{16}O_2$	180	2.43	22.36
33	4-METHYL-2,5-DIMETHOXYBENZALDEHYDE	$C_{10}H_{12}O_3$	180	2.43	22.36
34	2',4'-DIMETHOXYACETOPHENONE	$C_{10}H_{12}O_3$	180	2.43	22.36
35	ETHANONE, 1-(3,4-DIMETHOXYPHENYL)-	$C_{10}H_{12}O_3$	180	2.43	22.36
36	I-PROPYL 10-METHYL-DODECANOATE	$C_{16}H_{32}O_2$	256	2.32	23.94
37	N-CAPRIC ACID ISOPROPYL ESTER	$C_{13}H_{26}O_2$	214	2.32	23.94
38	3, CIS-(1,1-DIMETHYLETHYL)-4,CISMETHOXYCYCLOHEXANOL	$C_{13}H_{22}O_2$	186	2.32	23.94
39	I-PROPYL HEXACOSANOATE	$C_{29}H_{58}O_2$	438	2.32	23.94

Figure 4: **GC-MS Chromatogram (main) of root methanolic extract of *R. tetraphylla***

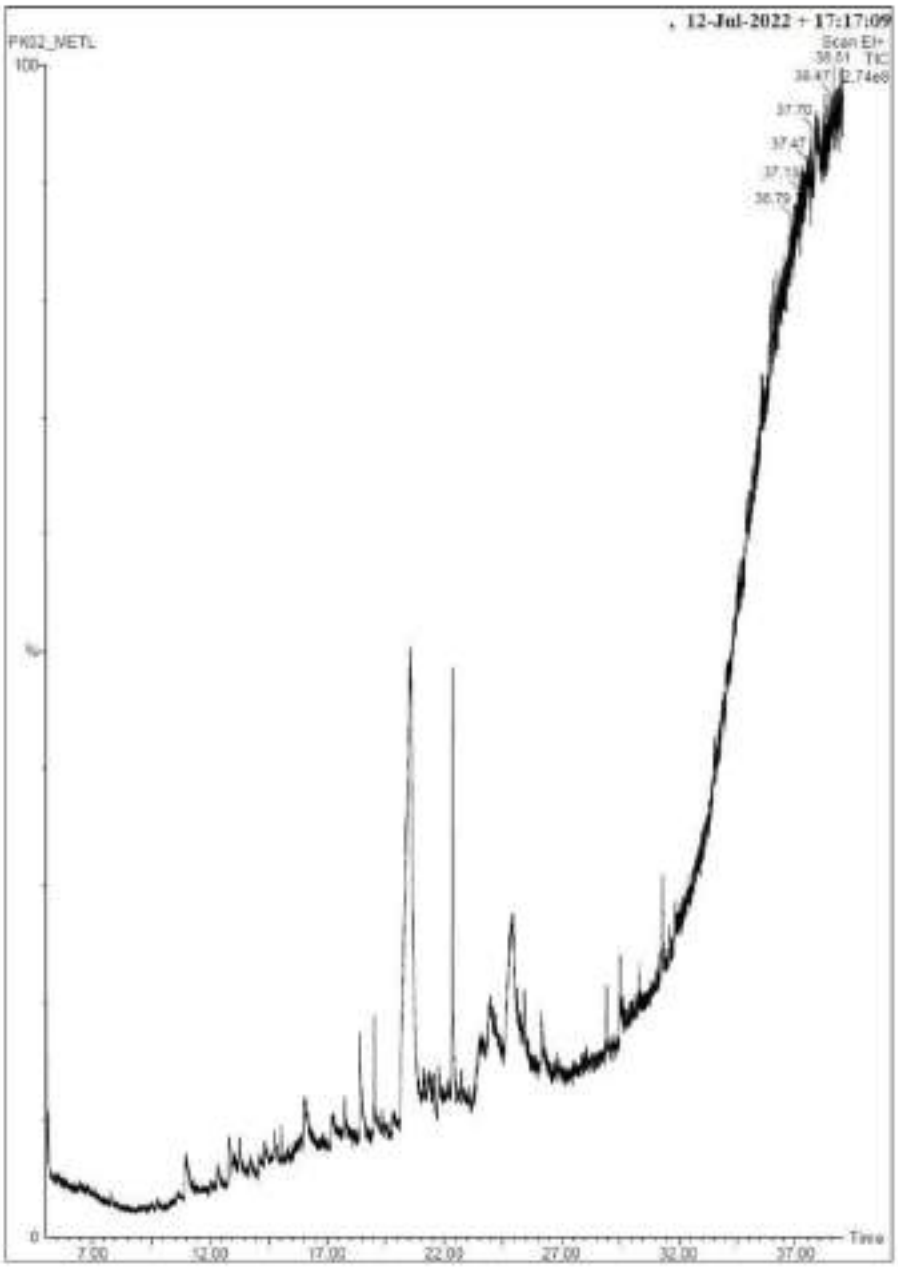


Figure 5: GC-MS Chromatogram (extended) of root methanolic extract of *R. tetraphylla*

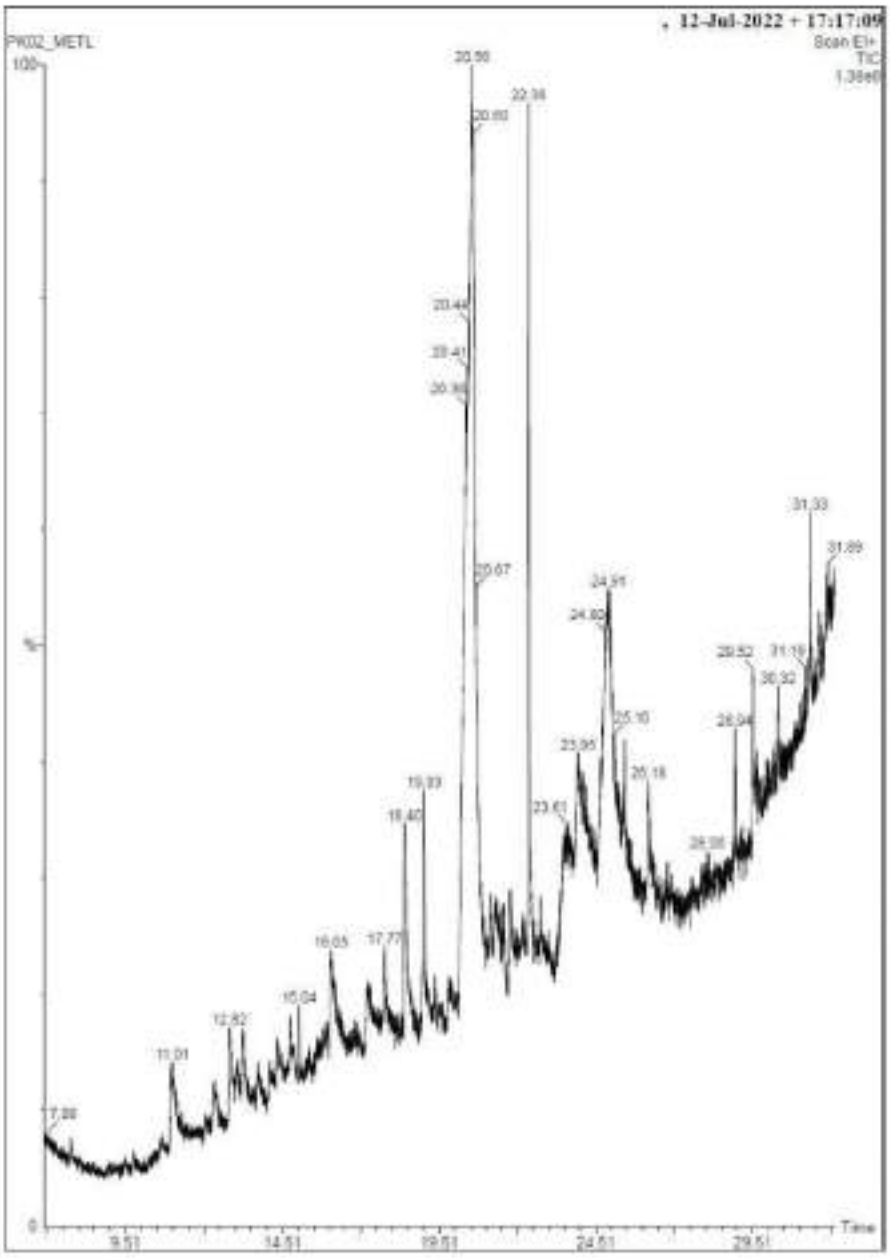
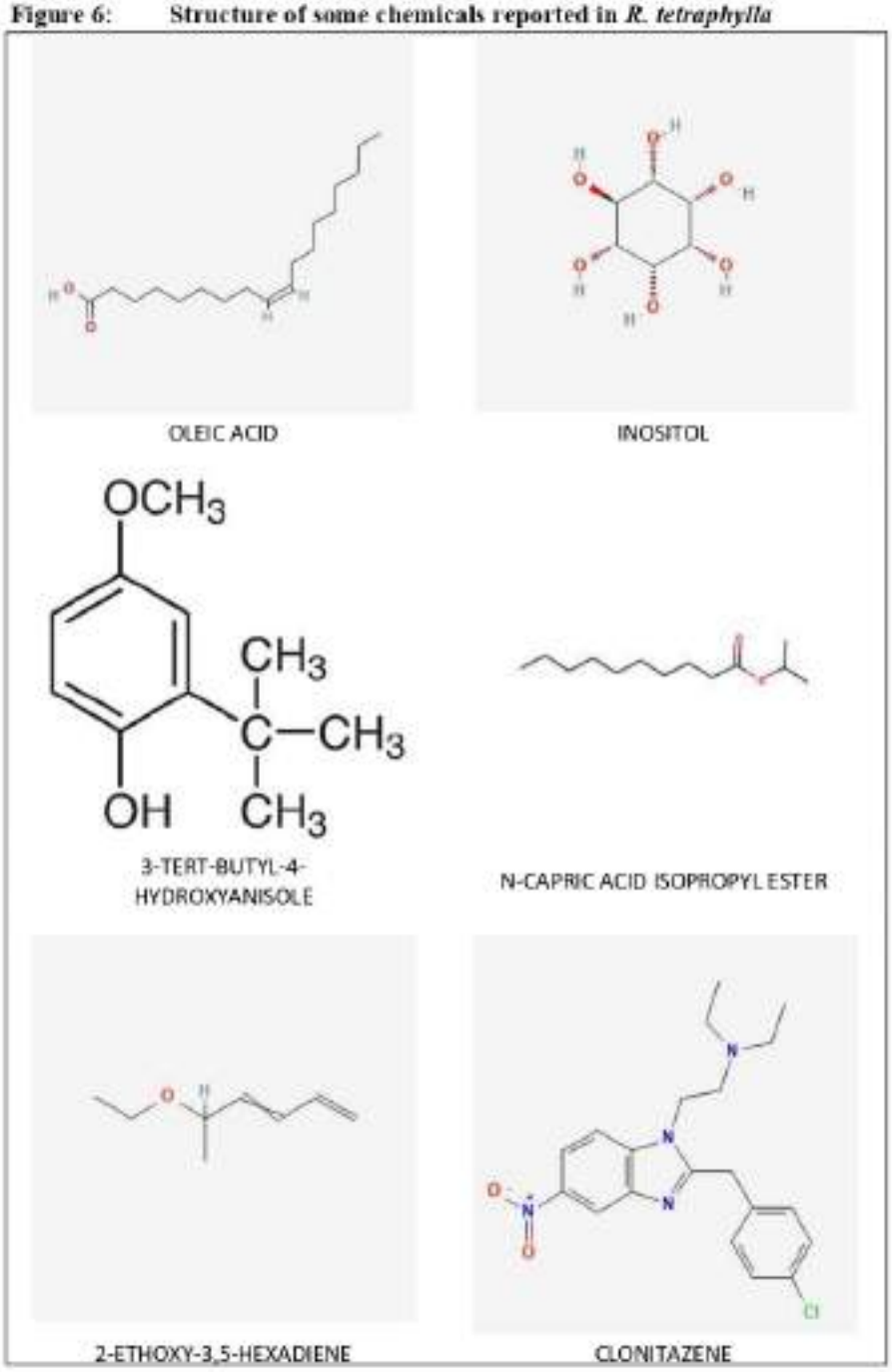
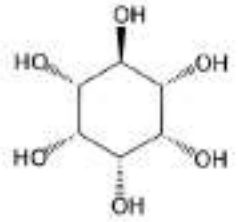


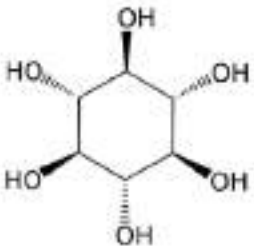
Figure 6: Structure of some chemicals reported in *R. tetraphylla*





EPI-INOSITOL

EICOSEN-1-OL,CIS-9-



SCYLLO INOSITOL

4.5 Bioactivity reported for Major phytochemicals Bioactivity reported for Major phytochemicals present in roots of *R. serpentina* and *R. tetraphylla* are reviewed from secondary sources and reported in the following Tables for *R. serpentina* and *R. tetraphylla*.

Table 3: Bioactivity reported for Major phytochemicals present in roots of *R. serpentina* (from secondary sources)

Sl.	Compounds Name	Molecular Formula	Mol. Wt.	% Peak Area	Ret. Time	Pharmacological Properties
1	Molinate	$C_9H_{17}NOS$	126	1.30	12.85	Antispermatogenic, herbicide, agrochemical (Kushwaha 2019)
2	1,3-DIOXEPANE, 2PENTADECYL-	$C_{20}H_{40}O_2$	312	1.75	14.34	Anti-proliferative (Kareem et al, 2016)
3	3-TERT-BUTYL-4-HYDROXYANISOLE	$C_{11}H_{16}O_2$	180	2.52	22.36	Anti-oxidant (Matsuoka, Atsuko, et al.1996)
4	5-HYDROXYMETHYLFURFURAL	$C_6H_6O_3$	126	2.93	16.008	Stop neuron apoptosis (Mohammad M et al.2022)
5	TRANS-2METHYL-4-NPENTYLTHIANE, S, S-DIOXIDE	$C_{10}H_{20}O_2S$	218	1.23	19.03	Nitric oxide synthase inhibitor, stimulates sympathetic nervous system

						(Mohammad M et al.2022)
6	OLEIC ACID	$C_{18}H_{34}O_2$	282	1.01	31.859	Antimicrobial, Antifungal, anticonvulsive activity, Antiadhesive, Antiallergic, Antianalgesic, Antiatherosclerosis, Anesthetic, Antihelmenthic, Antianxiety, Antibacterial, Antiberiberi, Antibiotic, Anticancer, Anticonvulsant, Antidiabetic, Antidiarrheic, Antifertility, Antigastric, Antiinammatory, Antiobesity, Antioxidant, Antiulcer, Antituberculosic, Anticold, Antihepatotoxic and Antiviral

						(Ralte, Laldinfeli, et al.2022)
7	EICOSANOIC ACID	$C_{20}H_{40}O_2$	312	2.32	29.523	Reduced heart diseases, kidney and liver function, blood clotting (Ralte, Laldinfeli, et al.2022)
8	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	$C_{38}H_{68}O_8$	652	2.32	29.523	Antiallergic, Antianemic, Antianxiety, Antibacterial, Antibronchitic, Anticancer, Anticarcinogenic, Anticataract, Anticoagulant, Anticonvulsant, Antidiabetic, Antidiarrheic, Antifatigue, Antifertility, Antigastric, Antiinflammatory, Antimalarial, Antioxidant, Antistress, Antiulcer, Antiatherosceleroti c, Anticold,

						Antiglaucomic, Antihepatic, Antihypertensive, Antiplague, Antiproliferant, Antiprotozoal, Antiseptic, Antistroke, Antituberculic, Antitumor (Ramya et al. 2015)
9	N-HEXADECANOIC ACID	$C_{16}H_{32}O_2$	256	2.32	29.523	Anti-oxidant, hypocholesterolemic, antiandrogenic (Kavitha S et al. 2014)
10	TETRADECANOIC ACID	$C_{14}H_{28}O_2$	228	2.32	29.523	Anti-virulence (JuárezRodriguez, Martha Maria, et al)
11	D-MANNITOL, 1-O-(22-HYDROXYDOCOSYL)-	$C_{28}H_{58}O_7$	506	1.55	36.086	Role in abiotic and biotic stress (Wakai et al. 2013)
12	D-MANNITOL, 1-O-(16-HYDROXYHEXADECYL	$C_{22}H_{46}O_7$	422	1.55	36.086	use for acute traumatic brain injury (Stoop et al. 1996)

Table 4: Bioactivity reported for Major phytochemicals present in roots of *R. tetraphylla* (from secondary sources)

SL. NO.	Compounds Name	Molecular Formula	Mol. Weight	% Peak Area	Ret. Time	Pharmacological Properties
1	OLEIC ACID	$C_{18}H_{34}O_2$	282	0.41	31.33	Antimicrobial, Antifungal, anticonvulsive activity, Anti-adhesive, Antiallergic, Antianalgesic, Antiatherosclerosis, Anesthetic, Antihelmenthic, Antianxiety, Antibacterial, Antiberiberi, Antibiotic, Anticancer, Anticonvulsant, Antidiabetic, Antidiarrheic, Antifertility, Antigastric, Anti-inammatory, Antiobesity, Antioxidant, Antiulcer, Antitubercellosic, Anticold, Antihepatotoxic and Antiviral (Ramya et al. 2015)

2	INOSITOL	$C_6H_{12}O_6$	180	1.80	5.13	Effective treatment of PCOS(Awuchi et al.2019)
3	3-TERTBUTYL-4HYDROXYANISOLE	$C_{11}H_{16}O_2$	180	2.43	22.36	Anti- oxidant (Matsuoka, Atsuko, et al.1990)
4	N-CAPRIC ACID ISOPROPYL ESTER	$C_{13}H_{26}O_2$	214	2.32	23.94	Flavour and fragrance agent
5	2-ETHOXY-3,5-HEXADIENE	$C_8H_{14}O$	126	1.33	16.05	Anti- microbial
6	EPI-INOSITOL	$C_6H_{12}O_6$	180	1.80	5.13	an artificial stereoisomer of myoinositol (Einat et al. 2004)
7	EICOSEN-1-OL, CIS-9-	$C_{20}H_{40}O$	296	1.33	16.05	Antibacterial (Dehpour et al.2012)
8	SCYLLO INOSITOL	$C_6H_{12}O_6$	180	1.80	5.13	Use in the treatment of Alzheimer Disease (Hunger et al. 1957)
9	CLONITAZENE	$C_{20}H_{23}ClN_4O_2$	386	16.37	20.56	Opioid analgesic (Hunger et al. 1957)

CHAPTER 5

5. SUMMARY AND CONCLUSION

Both the species of *Rauvolfia* commonly known as *Sarpagandha* belonging to the family Apocynaceae are distributed in tropical climatic conditions and traditionally used against snakebite, insomnia, melancholia, schizophrenia or more violent mental disorders, diarrhea, dysentery, cholera and colic, scabies, malaria, eye inflammation, etc. There is hardly any medicine, herbal or modern, which originated in India more than a century ago and became a subject of intensive research and clinical use for the presence of variety of alkaloids and medicinally important phytochemicals. The roots of *R. tetraphylla* are often used as a substitute of *R. serpentina* for medicinal purpose and therefore to study Morpho-Taxonomy and to study phytochemical composition of *R. serpentina* and *R. tetraphylla* the present study was conducted.

In *R. serpentina*, leaves in whorls of 3, thin, lanceolate and acute; inflorescence irregular corymbose cymes, white or pink, often tinged with violet; and roots having tuberous with pale brown cork. Whereas, in *R. tetraphylla*, leaves in whorls of 4, ovate-elliptic; flowers greenish-white or creamy-white in umbellate cymes; and roots having Tuberous with pale yellow cork which are very distinct characters to be considered for identification in field.

Also, *R. tetraphylla* bears trichomes in leaves, whereas *R. serpentina* lacks any trichome in leaves.

With regards to major phytochemicals reported in the present study, only two major chemicals are found to be common for both *R. serpentina* and *R. tetraphylla*. Among these, 3-tert-butyl-4-hydroxyanisole is reported to have Antioxidant activities (Matsuoka, Atsuko, et al.1996) and very recently Laldinfeli, et al.2022 reported Olecic acid present in both the species of *Rauvolfia* are showing Antimicrobial, Antifungal, anticonvulsive activity, Anti-adhesive, Antiallergic,

Antianalgesic, Antiatherosclerosis, Anesthetic, Antihelmenthic, Antianxiety, Antibacterial, Antiberiberi, Antibiotic, Anticancer, Anticonvulsant, Antidiabetic, Antidiartheic, Antifertility, Antigastric, Anti-inammatory, Antiobesity, Antioxidant, Antiulcer, Antitubercellosic, Anticold, Antihepatotoxic and Antiviral activities.

Also, as the phytochemicals of specimens shows variation with previously reported results, further detail study is needed in this regard.

Conclusion

The present study significantly contributes towards differentiation of *R. serpentina* and *R. tetraphylla* at Morpho-Taxonomy level and distinguishes both the species with respect to phytochemicals present in respective roots. As the samples has been collected from same phytogeographic region, this comparative phytochemical study also clarifies confusions prevailed between both the species both at raw material collection stage and at phytochemical level.

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