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Phytochemical constituents and medicinal applications of *Rauvolfia tetraphylla* L. in the Bhadrak region of Odisha, India

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Abstract
Rauvolfia tetraphylla L., a shrub belonging to the Apocynaceae family, is recognized for its growing importance in both traditional and modern medicine due to its rich assortment of phytochemicals. This study comprehensively examines the qualitative and quantitative attributes of bioactive compounds, encompassing tannin, phenol, alkaloids, saponins, and flavonoids, extracted from the roots of *R. tetraphylla* L. Additionally, it explores the indigenous utilization of this plant. Root samples were collected from three distinct villages (Dobal, Angeipal, and Palikiri) in the Dhamnagar block of Bhadrak district, Odisha, India, and standard procedures were followed for phytochemical assessment. The methanolic extract consistently demonstrated higher values when compared to the aqueous extract across all collected samples from the different villages. Phenol content exhibited variation, ranging from 12.62 to 17.39 mg/g, with the highest concentration (17.39 mg/g) observed in Angeipal village. Similarly, flavonoid content ranged from 28.1 to 217.3 mg/g, with the greatest amount detected in Dobal village (28.1 mg/g). Alkaloid content spanned from 11.24 to 18.42 mg/g, with the highest level found in Palikiri village (18.42 mg/g). In contrast, saponin and tannin content were consistently lower in all samples in comparison to alkaloids, phenols, and flavonoids. A diverse array of secondary metabolites in *R. tetraphylla* highlights its potential in treating conditions such as hypertension, cardiovascular diseases, and inflammatory disorders. Furthermore, its potential as an effective antivenom agent against insect bites, scorpion stings, and snakebites is highlighted.

Keywords: Urinary tract infection, urethra, medicinal plant, *Escherichia Coli* spp.

1. Introduction
Traditional plant-based medical systems have served as the cornerstone of healthcare in numerous global cultures and continue to be the primary source of primary healthcare for over three-quarters of the world's population [1]. The origins of this therapeutic knowledge can be traced to a process of trial and error, with each era discovering, documenting, and passing down the healing properties of specific medicinal plants to successive generations [2]. With the progression of human civilization, written records of various traditional healthcare systems such as Ayurveda, Siddha, and Unani have been developed, primarily grounded in the use of plant-based remedies [3]. Many pharmaceuticals derived from plants remain in demand for the treatment of diverse conditions including congestive cardiac failure, bronchitis, skin allergies, inflammatory disorders, and more [4]. The medicinal value of plants can be attributed to specific chemical compounds that elicit distinct physiological responses in the human body. Among these bioactive substances, alkaloids, flavonoids, tannins, and phenolic compounds stand out as the most significant [5, 6]. Notably, approximately 25% of drugs prescribed worldwide are derived from plants, with 121 of these active compounds currently in use [7]. Out of the 252 drugs categorized as basic and essential by the World Health Organization [8], approximately 11% are derived exclusively from plant sources. Additionally, a substantial number of these essential drugs are synthesized from natural precursors. This highlights the enduring role of medicinal plants in contemporary healthcare [8]. In recognition of their medicinal value, certain Indian postage stamps have featured prominent medicinal plants. The first set of such stamps was issued in 1997, showcasing four distinct medicinal plants: Ghritkumari (*Aloe barbadensis*), Sarpagandha (*Rauwolfia serpentina*), Tulsi (*Ocimum sanctum*), and Haldi (*Curcuma longa*) [9]. This was followed by another set of stamps featuring four more medicinal plants: Brahmi (*Bacopa monnieri*), Guggulu (*Commiphora wightii*), Amla (*Emblica officinalis*), and Ashwagandha (*Withania somniferous*) [10].

Within the diverse landscape of plant families, Apocynaceae has garnered substantial attention in the realm of traditional and modern medicine. This is largely due to the fact that many species within this family produce a milky sap with various pharmaceutical applications [11]. Among the genera within Apocynaceae, *Rauwolfia* stands out as an important one. It has gained significant popularity, primarily because of the presence of pharmacologically valuable alkaloids, with the highest concentrations found in the root bark. *Rauwolfia* encompasses approximately 130 identified species, primarily distributed in tropical and semitropical regions. Among these species, *Rauvolfia tetraphylla* L. has emerged as a vital alternative source of reserpine and other essential alkaloids [12].

R. tetraphylla goes by several names in various Indian languages and has earned monikers like Wild Snake Root, Devil Pepper, Four-Leaf Devil Pepper, American Serpent Wood, and Be Still Tree in English. In Hindi, it's known as Vanssarpagandha and Sarpanasini, while in Telugu, it's called Papataku. In Tamil, it's referred to as Pampukaalachchedi, and in Malayalam, it's known as Pampumkolli. The plant is called Dodda Chandrike in Kannada, Barchandrika in Bengali, and Patalagaruda in Odia. Originating from the West Indies and regions in Northern and Southern America, *R. tetraphylla* has successfully naturalized itself in several countries, including India, Pakistan, Sri Lanka, China, Bhutan, Bangladesh, Indonesia, and Myanmar. In India, it is predominantly found in the plains of various states, including Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Bihar, West Bengal, Madhya Pradesh, Jammu and Kashmir, Assam, and Odisha [13]. Extensive literature surveys have revealed that the roots of *R. tetraphylla* have been traditionally used as folk remedies to address a wide range of human ailments, including cholera, eye diseases, fever, hypertension, intestinal disorders, diarrhea, and dysentery. In different regions of Africa, Asia, America, and various Oceanic islands, local populations have harnessed the plant's healing properties [14, 15]. In many parts of India, the root extract is employed as a treatment for snakebites, high blood pressure, stomach discomfort, mental disorders, and even as an antidiabetic agent [16, 17]. Recent scientific investigations have unveiled the antibacterial, anti-inflammatory, insecticidal, and cytotoxic attributes of *R. tetraphylla* [18, 19]. This paper aims to shed light on its phytochemical composition and ethnopharmacological activities. ‘

2. Material and Methods

2.1 Study site

Bhadrak district (20° 43′–21° 13′N and 86° 6′–87° E) with seven blocks (Basudevpur, Bhadrak, Bhandaripokhari, Bonth, Chandbali, Dhamnagar and Tihidi) is located in northeast Odisha (Fig. 1). It spreads over 2505 km^2 with 1.507 million inhabitants (2011 Census). It borders the Balasore district in the north, Jajpur in the south, the Bay of Bengal and Kendrapara district in the east and Koenjhar in the west. The district contributes 1.61% and 3.62% of the state’s territory and population respectively. Rice (*Oryza sativa* L.) is the major cereal crop cultivated by most of the people of the district. The district is located in the deltaic region close to the Bay of Bengal. Obviously, it has all the features of a coastal climate, i.e., saline weather, the influence of coastal wind, thunderstorms during monsoons, dust storms in summer and cyclone proneness.

2.2 Collection of plant

Rauvolfia tetraphylla L. plants were collected from three distinct habitats in the Dhamnagar region: 1. Roadside of village Dobal 2. Grazing field of village Angeipal and 3. Near the rice field of village Palikiri. The collected plants were in their flowering stage, bearing flowers and fruits in a ripened form (Figure 2). The authentication of these plants was conducted following the guidelines in the flora of Odisha [20].

2.3 Preparation of Dried plant materials

R. tetraphylla was brought and kept carefully in the Laboratory of Dept. of Botany, Dhamnagar College, Bhadrak, Odisha. Roots were washed thoroughly under tap water, rinsed with distilled water properly, and kept in hot air for 3 days at 60 °C temperature. The dried roots were molded into pieces and crushed into fine particles (powder) with the help of mortar and pestle.

2.4 Preparation of plant Extract

Methanolic Extract

The root powder of *R. tetraphylla* was measured at 250 grams and packed into a Soxhlet apparatus. Methanol was used as the solvent, and the extraction process was carried out. The resulting extract was filtered through Whatman filter paper no. 1. The methanol in the extract was evaporated at 40 °C using a heating mantle. The dried extract was stored in a desiccator for future use.

Aqueous Extract

10 grams of root powder were soaked in distilled water for 12 hours at room temperature. The aqueous extract was filtered and concentrated to a final volume of 50 ml. This concentrated extract was then subjected to qualitative analysis.

2.5 Qualitative Estimation of Phytochemicals

Qualitative phytochemical analyses of both extracts were conducted following the protocols of Adetuyi and Popoola (2001) [21], Trease and Evans (1989) [22], and Sofowora (1982) [23].

Alkaloids

200 mg of plant material was boiled in 10 mL of methanol. The mixture was then filtered. To the filtrate, 1% HCl was added, followed by 6 drops of Dragendorff reagent. The formation of a brownish-red precipitate was taken as evidence of the presence of alkaloids.

Saponins

200 mg of plant material was mixed with 5 mL of distilled water. After 0.5 mL of the filtrate was diluted to 5 mL with distilled water, the mixture was shaken vigorously for 2 minutes. The formation of stable foam indicated the presence of saponins.

Flavonoids

To the aqueous filtrate, 5 mL of dilute ammonia solution was added, followed by concentrated H_2SO_4. The appearance of a yellow color indicated the presence of flavonoids.

Tannins

200 mg of root powder was boiled in 10 mL of distilled water. A few drops of $FeCl_3$ were added to the filtrate. The formation of a blue-black precipitate indicated the presence of tannins.

Phenols
10 mg of root extract was placed in a test tube, and a few drops of ferric chloride were added. The formation of a bluish-black color indicated the presence of total phenols.

2.6 Quantitative determination of phytochemicals

Alkaloids
Alkaloid content was measured following the method described by Harborne (1973) [24]. Five grams of dried root powder were dispersed in a 10% acetic acid solution in ethanol to create a suspension. This suspension was maintained at 28°C for 4 hours. The suspension was then filtered through the Whatman Number 42 filter paper. Alkaloids were precipitated by concentrating the filtrate to one-quarter of its original volume and adding drops of concentrated aqueous NH_4OH. The resulting precipitate was washed with 1% ammonia solution and dried at 80°C in an oven. The content of alkaloids was calculated and expressed as mg/g of the sample.

Flavonoids
The flavonoid content was determined using Harborne's (1973) [24] method. Five grams of root powder were boiled in 2M HCl for 30 minutes under reflux. After cooling, an equal volume of ethyl acetate was added dropwise to the filtrate. The weight of the precipitated flavonoids was determined and reported as mg/g.

Tannins
The quantitative estimation of tannins was performed as per the method of Swain (1979) [25]. Finely powdered roots were placed in a beaker containing 20 mL of 50% methanol and heated at 80°C in a water bath for 1 hour with continuous stirring. The extract was quantitatively filtered using double-layered Whatman Number 1 filter paper and rinsed with 50% methanol. One mL of the sample extract was treated with 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% Na_2CO_3 to develop a bluish-green color, which was allowed to stand for 20 minutes. The absorbance was measured at 760 nm, and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0–10 ppm.

Saponins
Saponin analysis was performed according to the method described by Brunner (1984) [26]. One gram of finely powdered sample was stirred in 100 mL of isobutyl alcohol for 5 hours. To the mixture, 20 mL of a 40% saturated solution of magnesium carbonate was added and filtered. To 1 mL of the colorless solution, 2 mL of 5% $FeCl_3$ solution and 50 mL of distilled water were added and left for 30 minutes to develop a blood-red color. The absorbance of the samples along with the standard was read at 380 nm and calculated in mg/g. A standard saponin solution was prepared in the reference range of 0–10 ppm.

Phenols
Phenol content was determined as described by Obodoni and Ochuko (2001) [27]. Five grams of powdered root were boiled with 50 mL of ether for 15 minutes and distributed in a 1:2 ratio (extract: distilled water). To the mixture, 2 mL of ammonium hydroxide followed by 5 mL of pentanol was added and incubated at room temperature for 30 minutes. The absorbance was measured at 505 nm.

2.7 Ethnobotanical Data collection

The data collection process for this study spanned in Dhannagar block of Bhadrak district, Odisha, India. Prior to initiating the fieldwork, the research objectives, methodologies, and potential benefits were clearly communicated to the participants in the local language, Odia. Consent and cooperation were actively sought from the participants before proceeding with the documentation of the plant species they utilized. Data collection was executed through interviews following established protocols [28, 29]. The survey questionnaire employed a semi-structured approach, complemented by open-ended interviews, informal discussions, and direct observations. Interviews were conducted both individually and in group settings, all conducted in the local language, thus providing specific insights into the plants. These insights were subsequently cross-verified for accuracy. Taxonomic details, including vernacular names, botanical names, and information regarding flowering and fruiting seasons, were recorded from the respondents. Moreover, comprehensive data was collected regarding the specific plant parts utilized, their availability, methods of consumption, and potential medicinal applications.

3. Results

R. tetraphylla (2n = 66) is a small evergreen woody shrub, typically reaching a height of 0.6–1.2 meters (Fig. 2). Its leaves are uneven, whorled, and elliptic-ovate. The flowers are creamy-white or greenish-white and appear in terminal corymbose cymes. The calyx lobes are short, ciliate, and round. The corolla is white, with a urceolate tube. Stamens are inserted at the corolla throat. The ovary is superior, bicarpellary syncarpous, and bilocular, with two ovules in each locule. The fruit, in the form of drupes, is ovoid, smooth, and clusters at the ends of twigs. When ripe, these drupes turn deep red or purple. The present study focused on the aqueous and methanolic root extracts of *R. tetraphylla* from different habitats in the Dhamnagar block of Bhadrak district, Odisha. Both extracts were found to contain various medicinally important phytochemicals, including phenols, tannins, saponins, flavonoids, and alkaloids. The quantitative estimation of phytochemicals revealed significant levels of these compounds in the roots of all varieties (Table 1). Notably, there were significant differences in the phytochemical constituents of *R. tetraphylla* among the various studied regions. The methanolic extract consistently exhibited higher values for these compounds compared to the aqueous extract in all the samples collected from different villages (Table 1; Fig. 3-5). The phenol content ranged from 12.62 to 17.39 mg/g in different locations, with the highest content (17.39 mg/g) observed in village Angeipal. Similarly, flavonoid content ranged from 217.3 to 28.1 mg/g, with the highest content found in village Dobal (28.1 mg/g). Alkaloid content varied from 11.24 to 18.42 mg/g, with the highest amount detected in village Palikiri (18.42 mg/g). On the other hand, there was a significantly lower amount of saponins and tannins in all the samples compared to alkaloids, phenols, and flavonoids (Table 1; Fig. 3-5). The study also noted that the people of the Dhamnagar area traditionally use various parts of *R. tetraphylla* for medicinal purposes. The whole plant is utilized for the treatment of skin diseases, while the root is specifically used for treating snake bites.

4. Discussion

In India, Ayurveda is a traditional medicinal system with a rich history dating back thousands of years. It remains one of the most ancient and enduring healing traditions in the country, grounded in a solid philosophical and empirical foundation [30, 31]. It is not inappropriate to assert that the use of medicines in India has deep roots in the folk medicine practices of ancient times. *R. tetraphylla* plays a significant role in the Indian system of medicine due to its diverse applications, owing to its wide array of secondary metabolites. Advanced chromatographic and spectral analyses have enabled the isolation and elucidation of the structures of numerous secondary compounds from various parts of *R. tetraphylla* [32-34]. The results of qualitative and quantitative phytochemical studies on the root extract of *R. tetraphylla* indicate the presence of alkaloids, flavonoids, phenolics, saponins, and tannins. Notably, the methanolic extract of the root consistently exhibited higher values than the aqueous extract. However, it is worth mentioning that saponins and tannins were found in comparatively lower concentrations. Previous reports on quantitative yield have also consistently shown that *R. tetraphylla* contains substantial amounts of alkaloids, flavonoids, and phenols [35-41]. The roots of *R. tetraphylla* are known to contain approximately 50% of the total alkaloids, including various monoterpene indole alkaloids and the well-known compound reserpine [42]. Several alkaloids have been isolated from the root extract of *R. tetraphylla*, including rauwolscine, yohimbine, ajmalicine, heterophyllin, sarpagine, tetraphylline, serpentine, ajmaline, isoreserpiline, reserpiline, raujemidine, deserpidine, reserpine, and vellosimine, among others [43-50]. Panda *et al.* (2012) [51] reported varying concentrations of reserpine in different parts of *R. tetraphylla*, with the roots containing the highest concentration of reserpine, followed by the stem and leaves. These findings underscore the pharmacological and medicinal significance of *R. tetraphylla*, particularly in the context of traditional Indian medicine.

The presence of various phytochemicals in the samples under study suggests their potential in treating a wide range of diseases, including antibacterial, antifungal, antioxidant, hypertension, cardioprotective, and anti-inflammatory conditions. Moreover, these phytochemicals may play a crucial role as antivenom agents against insect bites, scorpion bites, and snake bites. To evaluate the anti-inflammatory potential, hydro-alcoholic extracts (70% v/v ethanol) at doses of 200, 400, and 800 mg/kg, as well as methanol, ethyl acetate, and hexane extracts at doses of 100, 200, and 400 mg/kg of *R. tetraphylla* root bark were studied in a Carrageenan-induced rat paw edema model. The thickness of the paw was measured at hourly intervals for six hours. The hydro-alcohol and methanol fractions of *R. tetraphylla* root bark at various doses demonstrated a significant (p<0.001) reduction in paw thickness compared to the vehicle-treated control group [18]. Hypertension is a complex condition that contributes to approximately 40% of cardiovascular mortality, influenced by both genetic and environmental factors [52]. In Ayurveda, Sarpagandha (*Rauvolfia serpentina*) has been employed for centuries to treat high blood pressure. Gadhvi *et al.* (2018) [53] assessed the antihypertensive activity of *R. tetraphylla* root in rats. They found that diastolic, systolic, and mean arterial blood pressure values increased with sodium chloride administration. However, the methanolic root extract produced a significant decrease in systolic pressure. Studies by Sulaiman *et al.* (2020) [54] on an animal model (Wistar albino rats) demonstrated that the root extract of *R. tetraphylla* significantly reduced both systolic and diastolic blood pressure (92 ± 1/121 ± 1). Based on phytochemical and pharmacological evaluations, Sulaiman *et al.* (2020) [54] suggested that *R. tetraphylla* root could serve as a validated substitute for Sarpagandha. Rohela *et al.* (2015) [12] also proposed *R. tetraphylla* L. as an alternative source of reserpine, a well-known antihypertensive compound.

Numerous studies have explored the antifungal properties of *R. tetraphylla* and found it to be effective against a wide range of animal-human and pathogenic fungi, including dermatophytes and seed-borne fungi. Kumaran and Kannabiran (2003) [55] investigated the antifungal efficiency of ethanolic root extracts of *R. tetraphylla*, demonstrating significant inhibitory effects on conidial germination and radial growth of *Colletotrichum capsici*. Rohela *et al.* (2016) [56] reported the antifungal and antibacterial activities of *in vitro*-grown *R. tetraphylla*, including extracts from the root, leaf, and stem. The higher concentration of flavonoids in the root extract indicated strong antioxidant activity. Bonilla *et al.* (2003) [57] highlighted the efficiency of antioxidants and flavonoids in anti-inflammatory activity, suggesting their potential in herbal medicine to treat diseases. Rajesh *et al.* (2013) [58] conducted experiments on mice to screen for venom detoxifying activity using aqueous and methanolic root extracts of *R. tetraphylla* against crude venom obtained from the Indian cobra. Kapoor (2014-15) [37] reported that the methanolic root extract of *R. tetraphylla* exhibited dose-dependent insecticidal activity, particularly against *Musca domestica* larvae. The extract significantly reduced the protein and nucleic acid content of the larvae. Nandhini and Stella Bai (2015) [59] evaluated the cardioprotective activity of *R. tetraphylla* in rats induced with myocardial infarction by isoproterenol hydrochloride. The extract, administered orally, led to a significant reduction in isoproterenol-induced biochemical changes, preserving GSH levels and influencing cardioprotective activity. It also controlled lipid peroxide formation, restored endogenous antioxidants, and preserved the histo-architecture of myocytes. Hernandez-Leon *et al.* (2018) [60] documented the pharmacological efficiency of reserpine, comparing it to the natural resin fraction of Rauwolfia roots. Reserpine is commonly used in the treatment of cardiovascular diseases, vessel diseases, and certain neurological disorders.

The utilization of *R. tetraphylla* for the treatment of skin diseases and snake bites in the study area aligns with similar findings from other studies [61-64]. Moreover, people of the Rajshahi district have employed *R. tetraphylla* as an antidote to poison, while the Jaunsari tribes have used *R. tetraphylla* roots to address nervous disorders, fever, epilepsy, anxiety, and intestinal disorders [65]. Khatun *et al.* (2013) [66] reported the ethnobotanical practices in the Chuadanga and Jhenaidah districts, where traditional medicinal practitioners (Kavirajes) incorporated *R. tetraphylla* stem and root, particularly the inner portion, in snakebite treatment. The inner stem portion, when applied as a paste, was used to treat the affected area. Additionally, approximately 100 grams of *R. tetraphylla* leaves, root, and stem powder were administered orally to counteract the effects of venom. The plant was also cultivated near homes as a snake repellent. Balasubramaniam and Murugesan (2004) [67] reported ethnobotanical knowledge among the inhabitants of the Velliangiri hills in Coimbatore, mentioning the use of the root of *R. tetraphylla* for various ailments.

Table 1: Quantitative estimation of phytochemicals of *Rauvolfia tetraphylla* L.

Sl. No	Phytochemicals in *R. tetraphylla* (Root)	Root extract of *R. tetraphylla* L. from village Dobal		Root extract of *R. tetraphylla* from village ngeipal		Root extract of *R. tetraphylla* from village Palikiri	
		Aqueous extract (mg/gm)	Methanolic Extract (mg/gm)	Aqueous Extract (mg/gm)	Methanolic Extract (mg/gm)	Aqueous Extract (mg/gm)	Methanolic Extract (mg/gm)
1	Phenol	8.36	16.39	13.48	17.39	8.36	12.63
2	Tannins	3.38	5.36	4.21	5.39	4.86	7.38
3	Saponins	4.76	6.73	4.38	5.83	3.38	7.34
4	Flavonoids	22.4	28.1	15.2	18.3	13.4	17.3
5	Alkaloids	8.41	11.26	9.4	11.24	12.31	18.42

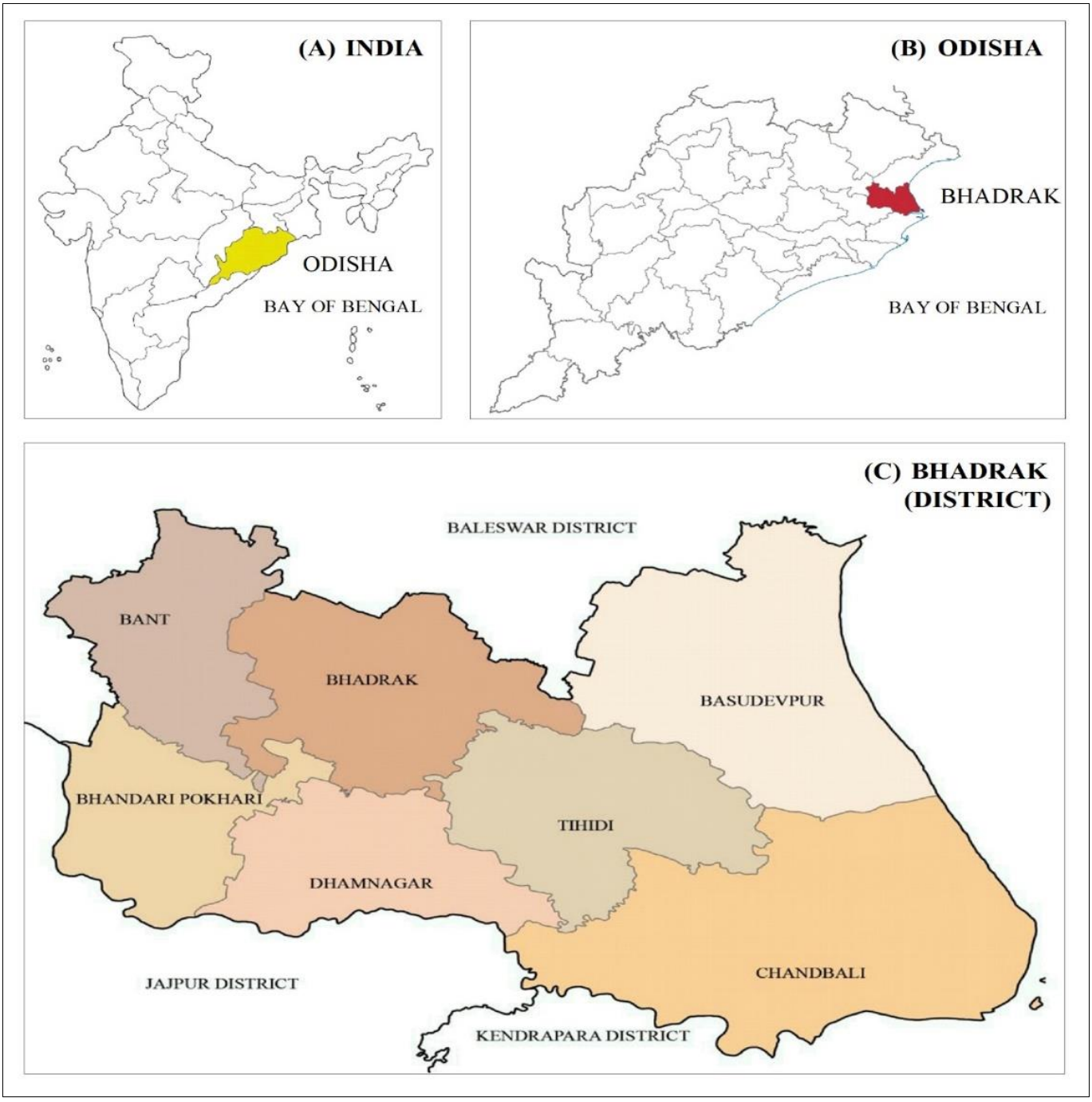

Fig 1: (A) Location of Odisha state in the eastern region of India (B) map of the Odisha state showing Bhadrak district and (C) study area showing different blocks of the Bhadrak district.

Fig 2: *Rauvolfia tetraphylla* L. plant and root.

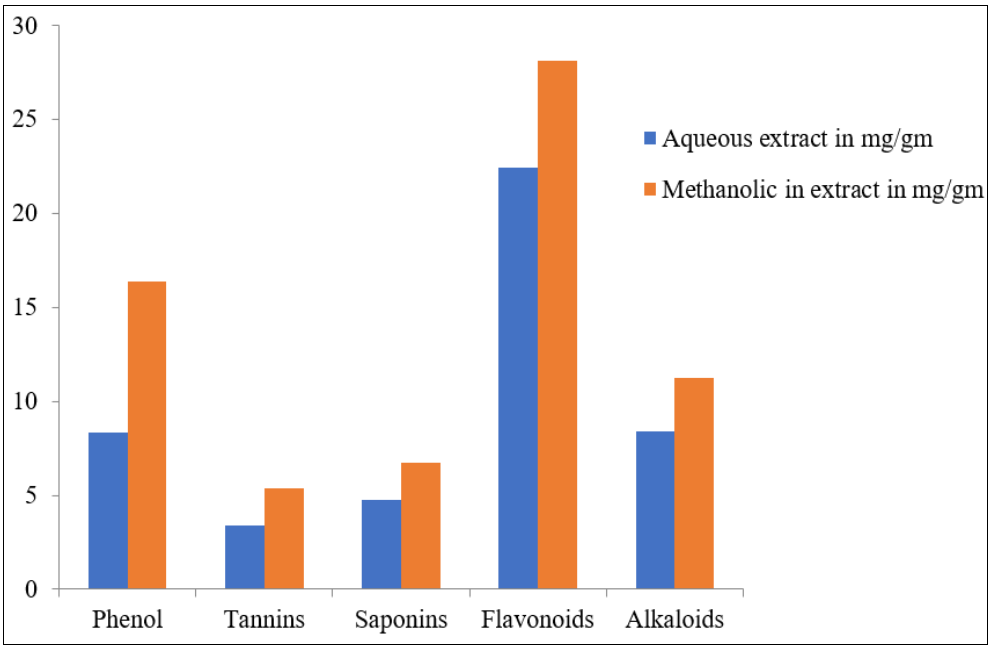

Fig 3: Phytochemicals in root extract of *R tetraphylla* L. from village Dobal.

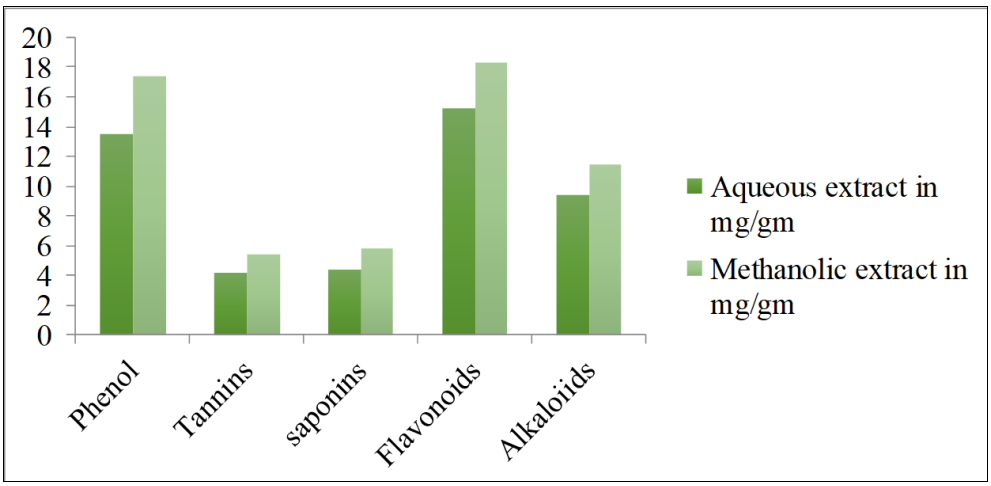

Fig 4: Phytochemicals in root extract of *R tetraphylla* L. from village Angeipal.

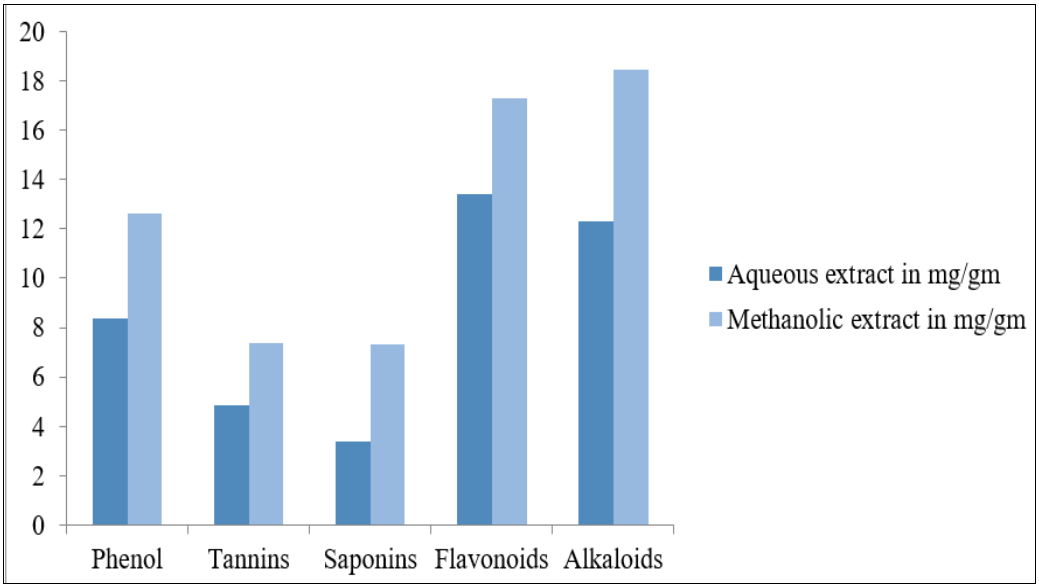

Fig 5: Phytochemicals in root extract of *R tetraphylla* L. from village Palikiri.

5. Conclusion

R. tetraphylla, a botanical treasure trove, boasts a myriad of traditional applications stemming from its rich phytochemical makeup. This plant presents a wide range of potential health advantages, serving as an antifungal, antioxidant, anti-inflammatory, and even a potential remedy for snakebites and skin conditions. It straddles the realms of pharmacology and traditional medicine, showing promise in treating cardiovascular and neurological disorders. These findings harmonize with ancient healing practices that have revered *R. tetraphylla* for generations. The plant's bioactive compounds hint at its potential in developing valuable drugs and therapies. However, while traditional wisdom and ethnobotanical studies offer valuable insights, further scientific exploration is essential to elucidate its mechanisms of action and unlock its medicinal potential. This research could revolutionize healthcare, pharmacology, and drug development while underscoring the importance of preserving traditional knowledge for future generations. The holistic exploration of *R. tetraphylla* offers an exciting journey at the intersection of ancient wisdom and modern science.

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