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Abinash Sahoo
Department of Environmental Science, Fakir Mohan, University, Balasore, Odisha, India

Dr. Ranindra Kumar Nayak
1.) Department of Environmental Science, Fakir Mohan, University, Balasore, Odisha, India
2.) Centre of excellence for Bioresources and management, Fakir Mohan University, Balasore, Odisha, India

Corresponding Author:
Abinash Sahoo
Department of Environmental Science, Fakir Mohan, University, Balasore, Odisha, India

Phytochemical analysis of leaf extracts of mangroves and their associates in Dhamra estuary, Bhadrak, Odisha

Abinash Sahoo and Dr. Ranindra Kumar Nayak

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Abstract
Dhamra Estuary in Bhadrak district of Odisha is endowed with Mangroves, which have been depleted at an alarming rate due to the operation of various biotic factors. The present vegetation is found in denuded condition and are at a great degree of threat due to various anthropogenic activities such as clearing mangrove forests for pisciculture, human habitation and development of port. During the present investigation, 20 species of mangroves and their associates have been reported from different locations of Dhamra estuary. Phytochemical analysis of eight species of mangroves and their associates shows the presence of various phytochemicals such as alkaloids, flavonoids, glycosides, phenols, and tannins etc. that are responsible for their medicinal properties.

Keywords: Mangroves, phytochemical analysis, Dhamra estuary

1. Introduction
Mangroves are salt tolerant plants and grow mainly in brackish water at intertidal zone between land and ocean, Being salt tolerant these are also known as halophytes and can easily be adapted to any unfavorable environmental conditions like flood, cyclone etc. due to their root systems. These plants have a unique salt filtration system with complex root structures to manage salt water inclusion and extreme wave action. These plants make adjustment to low oxygen conditions of water-logged mud [1] but most of them are likely to flourish on the upper water layer of the intertidal zone [2]. The mangroves have special kinds of roots which are known as “Pneumatophores” and “Rhizophores” which helps in respiration as mangroves grow on anaerobic soils. The underwater habitat of their root provide nourishment to environment for juveniles of different kinds of fish species. Physically, they maintain balance between aquatic and terrestrial communities and helps to protect coastal areas from environmental hazards. Mangrove forests mainly contain grasses, ground ferns, epiphytes, palms, shrubs, and trees [3] which can grow both on soil and water due to its amphibian nature [4]. Mangroves has a significant contribution towards the protection of ecological security of coastal areas and became source of income for thousands of fishermen and local people. Since the beginning of human civilization, every part of mangrove plant is being used in medicinal purposes by human beings. Mangrove plants are mostly valuable for their bioactive compounds like antimicrobial and antioxidant compounds. Antioxidants having ability to restrict a particular oxidizing enzymes or reacts with oxidizing agents which causing destruction to other molecules [5]. Cancer causing effects of synthetic oxidants forces scientists to replace these synthetic oxidants with natural oxidants extracted from plant materials [6]. More than 200 biologically active metabolite products have been reported from true mangroves of tropical and subtropical regions [3]. Phytochemicals like Alkaloids, flavonoids, steroids, saponins, tannins and phenols are the important compounds which have a wide range of medicinal properties [7]. Medicines extracted from different parts of mangrove plant were mainly used for the treatment of malaria, dysentery, elephantiasis, ulcers, leprosy, tuberculosis, and various skin diseases [8]. Mangrove plants are huge source of plant secondary metabolites which help the plants to stand in different types unfavourable environment conditions. These secondary metabolites play key role in different types of defence and survival mechanisms. Medicinal properties of mangrove plants having therapeutics ability are used for the treatment of arthritis, asthma, diabetes, inflammation and rheumatism [9]. Mangrove plants can produce substances having different types of biological activities like antioxidant, hepatoprotective mosquito larvicidal, antibacterial, antifungal, antiviral, anti-diarrhoeal, anti-feedant, insecticidal and cytotoxic activity [10, 11].

Dhamra Estuary in Bhadrak district of Odisha is endowed with Mangroves, which have been depleted at an alarming rate due to the operation of various biotic factors. Past floristic studies conducted by earlier workers reveals that mangroves were much rich different coastal regions of Odisha [12-15]. The present vegetation is found in denuded condition and are at a great degree of threat due to various anthropogenic activities such as clearing mangrove forests for pisciculture, human habitation and development of port.

2. Objective: To carry out the qualitative phytochemical evaluation from different extracts of the leaves of mangrove plants and their associates in Dhamra estuary.

3. Study area: Odisha is one of the South-East states of India. It is located between the parallels of 17.49^0 N and 22.34^0 N latitude and meridians of 81.27^0 E and 87.29^0 E longitudes. It is bounded by the Bay of Bengal in the east, Chhattisgarh on the west and Andhra Pradesh on the south. It extends over an area of 155,707 km^2. Bhadrak district is situated in the north-eastern part of Odisha and lies between latitudes of 20.45^0 N & 21.14^0 N and longitudes of 86.17^0 E & 86.59^0 E. It is bounded in the north by Balasore, in the south by Kendrapara and Jajpur, in the west by Keonjhar districts and in the east by Bay of Bengal. The geographical area of the district is 2788 km^2. Dhamra Estuary is formed by the confluence of the Brahmani and Baitarani rivers. It is situated between latitude of 20.47^0 N and longitude of 84.54^0 E. The study area includes the Mangrove forests of Dhamra Estuary in the Bhadrak district of Odisha (Figure 1).

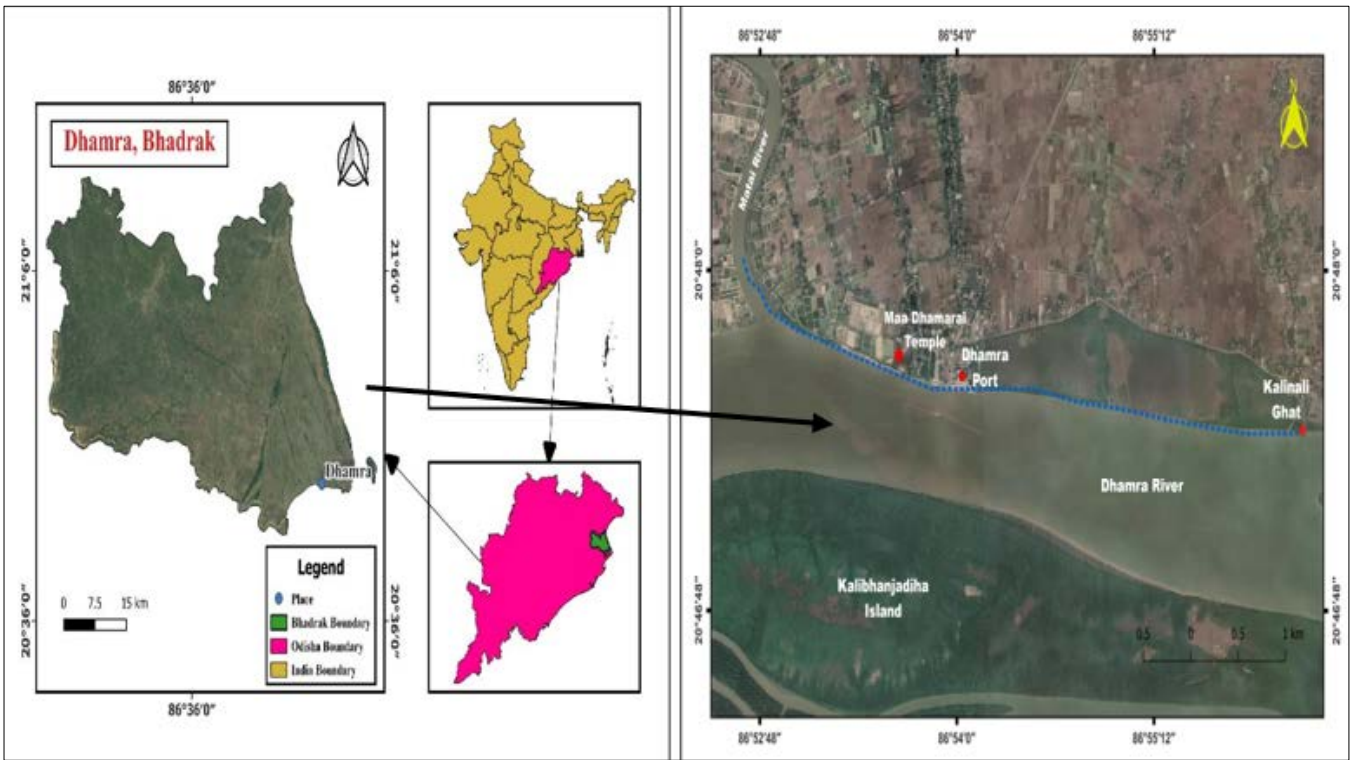


Fig 1: Mangroves of Dhamra Estuary, Bhadrak, Odisha.

4. Materials and Methods

The present research has been undertaken to study the diversity and phytochemical analysis of mangroves of Dhamra estuary in Bhadrak district of Odisha. The materials and methods involve the following steps.

A. Plant collection

Plant species were mostly collected in flowering and fruiting conditions and with reproductive characters which are required for the proper identification.

B. Local names

Vernacular names as used by the local people have been recorded during the field visit and plant sample collection.

C. Plant identification

All the specimens have been collected and identified basing on personal observations and critical study of various morphological, eco-physiological and medicinal characters of the specimens. The specimens were identified with the help of Flora books *viz*. The Botany of Bihar and Orissa (Haines, 1921-25) [16] and the Flora of Orissa (Saxena and Brahmam, 1994-96) [17].

D. Phytochemicals analysis

The phytochemical analysis has been done as following steps:

I. Extraction of plant leaves: Near about 6gm of air-dried powdered plant material of leaves were taken in three different conical flasks containing 40ml of three different solvents i.e. ethanol, n-hexane and distilled water and was properly stoppered with cotton wool. Then the mixture was kept on orbital shaker for 48 hours with 150 rpm at room temperature. Then the extracts were filtered with Whatmann No.1 filter paper and the supernatant was collected in air tight containers and was stored at 4^0c. The tests for different phytochemicals were carried out for all the 3 different types of extracts.

II. Test for alkaloids: 1ml of plant extract was taken in a test tube and 2 ml of 1% HCL was added. Then 6 drops of both Mayer's reagent and Dragendroff's reagent were added to it. An organic precipitate was formed which indicated the presence of alkaloid in the sample.

III. Test for flavonoids: About 10 drops of aqueous plant extract was taken in a test tube and 5ml of dilute ammonium solution was added to it. Then little amount of concentrated sulphuric acid (H_2SO_4) was added slowly to the sides of test tube. A yellow colour was observed which confirmed the presence of flavonoids and it disappeared on standing.

IV. Test for glycosides: 1ml of plant extract was taken in a test tube and was treated with 2ml of glacial acetic acid with one drop of ferric chloride solution. Then 1ml of concentrated Sulphuric acid (H_2SO_4) was added over it gently in the sides of the test tube. A brown ring of the interface was formed which indicated the presence of a deoxysugar of glycosides. A violet ring might appear below the brown ring whereas in the acetic acid layer, a greenish ring might form just gradually through thin layer.

V. Test for phenols: 2ml of plant extract was taken in a test tube and 3ml of ethanol was added to it. Then a pinch of $FeCl_3$ was added to it. A greenish yellow colour was formed which indicated the presence of phenols.

VI. Test for tannins: 1ml of plant extract was taken in a test tube and then a few drops of 1% of lead acetate were added to it. A yellow precipitate was formed which the presence of tannins detected.

VII. Test for saponins: 3ml of plant extract with 20ml of dist. H_2O was shaken in a measuring cylinder for about 15 minutes. A 1cm layer of foam was formed which indicated the presence of Saponins.

VIII. Test for proteins: 1ml of plant extract was taken then few drops of nitric acid were added by the sides of the test tube and observed for till the formation of yellow colour.

IX. Test for terpenoids (Salkowski test): 0.5ml of the aqueous plant extract was taken in a test tube which was mixed with 2ml of chloroform and then 3ml of concentrated H_2SO_4 was added carefully to the sides of test tube to form a layer. In the interface a reddish-brown colour was formed which indicated the presence of terpenoids.

5. Results and Discussions

In the present investigation, 20 species of mangroves and their associates have been found from different parts of Dhamra estuary (Table-1).

Table 1: Mangroves reported from different locations of Dhamra Estuary, Bhadrak district, Odisha

S. No.	Plant Name	Family Names	Odia Names	Type of Plant
1.	*Acanthus ilicifolius* L.	Acanthaceae	Harakancha	Shrub
2.	*Avicennia alba* Blume	Avicenniaceae	Kala bani	Tree
3.	*Avicennia marina* (Forsk.) Vierh.	Avicenniaceae	Singala bani	Shrub
4.	*Avicennia officinalis* L.	Avicenniaceae	Dhala bani	Tree
5.	*Caesalpinia bonduc* (L.) Roxb	Caesalpiniaceae	Gila	Shrub
6.	*Caesalpinia crista* L.	Caesalpiniaceae	Nentei	Herb
7.	*Clerodendrum inerme* (L.) Gaertn.	Verbenacea	Chiani	Shrub
8.	*Derris trifoliata* Lour	Fabaceae	Kala katira nai	Shrub
9.	*Excoecaria agallocha* L.	Euphorbiaceae	Guan	Shrub
10.	*Hibiscus tiliaceus* L.	Malvaceae	Bania	Tree
11.	*Ipomoea pes-caprae* (L.) R. Br.	Convolvulaceae	Kansari lata	Shrub
12.	*Kandelia candel* L.	Rhizophoraceae	Sinduka	Tree
13.	*Phoenix paludosa* Roxb.	Arecaceae	Hentala	Shrub
14.	*Porteresia coarctata* Roxb.	Poaceae	Dhani dhana	Grass
15.	*Rhizophora apiculata* Blume.	Rhizophoraceae	Rai	Tree
16.	*Rhizophora mucronata* Lamk.	Rhizophoraceae	Rai	Tree
17.	*Sesuvium portulacastrum* L.	Aizoaceae	Goda bani	Herb
18.	*Suaeda maritima* L.	Chenopodiaceae	Giria saga	Shrub
19.	*Tamarix troupii* Hole.	Tamaricaceae	Jagula	Shrub
20.	*Thespesia populnea* L.	Malvaceae	Habali	Tree

Some species are found in denuded conditions in these areas due to biotic interferences such as Pisciculture, human habitation etc. (Figure 2).

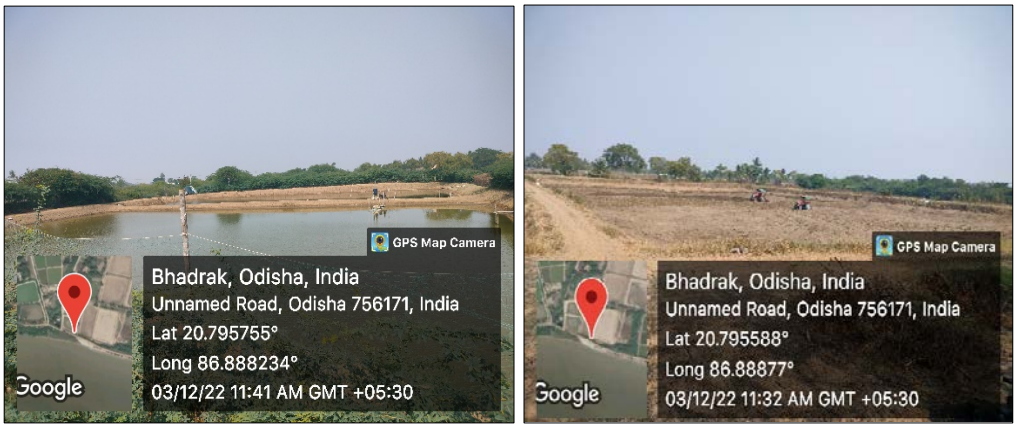
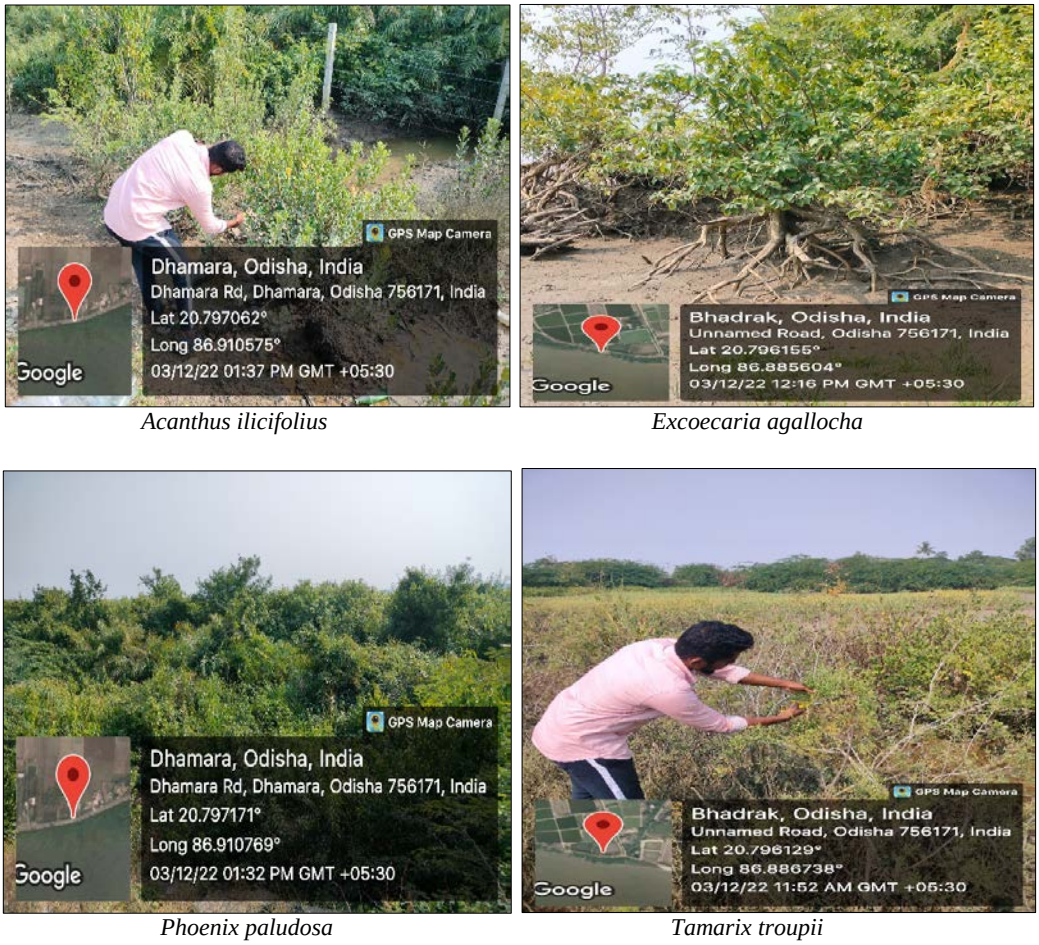


Fig 2: Pisciculture activities in Dhamra Estuary, Bhadrak District, Odisha.

It has been observed that, these mangrove plants share a good number of potential medicinal properties and have other socio-economic importance (Figure 3).



Acanthus ilicifolius *Excoecaria agallocha*

Phoenix paludosa *Tamarix troupii*

Fig 3: Distribution of Mangroves of Dhamra Estuary in Bhadrak District, Odisha

The result of phytochemical analysis of some potential mangrove plants and associates such as *Acanthus ilicifolius*, *Avicennia alba*, *Avicennia officinalis*, *Avicennia marina*, *Excoecaria agallocha*, *Tamarix troupii*, *Phoenix paludosa* and *Thespesia populnea* shows the presence of phytochemicals which includes alkaloids, terpenoids, flavonoids, tannins, proteins, saponins, glycosides and phenols which are responsible for their antimicrobial and medicinal properties (Table 2).

The results reveal that in both the fraction ethanol and n-hexane, alkaloid is present in 7 species except *Excoecaria agallocha*, flavonoid is present in 6 species except *Tamarix troupii* and *Avicennia officinalis*, glycoside is present in 7 species except *Excoecaria agallocha*, phenols are present in *Avicennia marina*, *Thespesia populnea*, *Excoecaria agallocha* and *Acanthus ilicifolius*, tannin is present in 7 species except *Phoenix paludosa*, saponin is found to be present in 7 species except *Thespesia populnea*, protein and terpenoid are present in all above the mentioned species (Table 2).

In distilled water fraction alkaloid is found in *Avicennia alba*, *Avicennia officinalis*, *Avicennia marina* and *Thespesia populnea*, flavonoid is present in all 8 species, glycoside is found in 6 species except *Avicennia officinalis* and *Avicennia marina*, Phenol is present in *Avicennia officinalis* and *Avicennia marina*, tannin is present in 7 species except *Avicennia alba*, saponin is identified in 5 species except *Excoecaria agallocha*, *Thespesia populnea* and *Tamarix troupii*, protein is present in 6 species except *Acanthus ilicifolius* and *Avicennia alba* and terpenoid is identified in all 8 species (Table 2).

Table 2: Phytochemical analysis of mangroves of Dhamra Estuary in Bhadrak, Odisha

SL No	Name of the plants	Plant parts	Fraction	Alkaloid	Flavonoid	Glycoside	Phenol	Tannin	Saponin	Protein	Terpenoid
1	*Avicennia alba* Blume.	Leaves	Ethanol	+	+	+	-	+	+	+	+
			n-hexane	+	+	+	-	+	+	+	+
			Distilled water	-	+	+	-	+	+	-	+
2	*Avicennia marina* (Forsk.) Vierh.	Leaves	Ethanol	+	+	+	+	+	+	+	+
			n-hexane	+	+	+	+	+	+	+	+
			Distilled water	+	+	-	+	+	+	+	+
3	*Thespesia populnea*	Leaves	Ethanol	+	+	+	+	+	-	+	+
			n-hexane	+	+	+	+	+	-	+	+
			Distilled water	+	+	+	-	+	-	+	+
4	*Phoenix paludosa*	Leaves	Ethanol	+	+	+	-	-	+	+	+

			n-hexane	+	+	+	-	-	+	+	+
			Distilled water	-	+	+	-	+	+	+	+
5	*Excoecaria agallocha*	Leaves	Ethanol	-	+	-	+	+	+	+	+
			n-hexane	-	+	-	+	+	+	+	+
			Distilled water	-	+	+	-	+	-	+	+
6	*Tamarix troupii*	Leaves	Ethanol	+	-	+	-	+	+	+	+
			n-hexane	+	-	+	-	+	+	+	+
			Distilled water	-	+	+	-	+	+	+	+
7	*Acanthus ilicifolius*	Leaves	Ethanol	+	+	+	+	+	+	+	+
			n-hexane	+	+	+	+	+	+	+	+
			Distilled water	-	+	+	-	+	+	-	+
8	*Avicennia officinalis*	Leaves	Ethanol	+	-	+	-	+	+	+	+
			n-hexane	+	-	+	-	+	+	+	+
			Distilled water	+	+	-	+	+	+	+	+

6. Conclusion

The mangrove ecosystem of Dhamra Estuary provides exclusive and expensive resources that are essential for human survival from both ecological and economic point of view in this area. From the present study of the mangroves of Dhamra Estuary, it has been observed that there is no growth of mangrove cover in the study areas due to both the anthropogenic activities and natural calamities. So, it would be the right decision to promote mangrove research and development in this region. In the present study it has been found that mangroves of Dhamra estuary have been subjected to severe biotic pressures. It contains many medicinal plants having some important phytochemicals which are responsible for their antimicrobial and medicinal properties. So, *in situ* conservation of mangroves and their associates should be promoted in this region. Hence, conservation of the existing mangrove vegetation of Dhamra estuary and restoration in the degraded land should be done for the protection of coastal environment in this region.

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