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Indigenous knowledge and phytochemical analysis of potential medicinal plants of Kuldiha wildlife sanctuary in Odisha, India

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Abstract
Kuldiha wildlife sanctuary in the Balasore district of the state of Odisha in India is endowed with rich biodiversity including medicinal plants. People of various ethnic groups in this region have rich indigenous knowledge about the medicinal plants, which are used for the treatment of various ailments. During the present investigation, ethno-medicinal studies have been conducted in different locations of Kuldiha wildlife Sanctuary and its adjoining regions. Phytochemical analysis of some potential medicinal plants reveals the presence of some phytochemicals such as alkaloids, flavonoids, terpenoids, glycosides, phenol, tannin, and saponin which are responsible for their medicinal properties and provide evidence for the validation of the ethno medicinal data for further use. Further studies on these plants' quantitative phytochemical analysis and antimicrobial activities will provide much useful information for their applications in the herbal drug industry.

Keywords: Indigenous knowledge, medicinal plants, Kuldiha wildlife sanctuary

Introduction
Kuldiha wildlife sanctuary in the Balasore district of the state of Odisha in India has rich biodiversity including medicinal plants. The sanctuary is spread across a 272.75 km^2 area. It is famous for the Mayurbhanj elephant reserve that spreads across Similipal, Kuldiha, and Hadgarh wildlife reserve. Kuldiha elephant reserve is locally known as the Tenda elephant reserve. The sanctuary has been declared an ecological sensitive zone on August 2, 2013, by the government of India. It is a mixed deciduous forest dominated by the Sal trees and various types of medicinal plants.

The forests of Kuldiha wildlife sanctuary possess the characteristics of tropical deciduous forests [1]. The Sanctuary is mainly covered by the plant species *Shorea robusta* followed by others including *Dalbergia latifolia*, *Gardenia latifocia*, *Kydia calycina* etc. It also provides shelter to a wide variety of birds and animals including tigers, elephants, giant squirrels, hornbill, hill myna, peafowl, etc.

From time immemorial, Medicinal plants have been the fundamental unit of the traditional medicine system in Kuldiha Wildlife Sanctuary and its adjoining regions. This area is inhabited by a large population of various tribes such as Bathudi, Bhumij, Ho, Kolha, Munda, Santal, Shabar. From time immorally, people of these ethnic groups have been utilizing various plants and plant products for the treatment of different diseases. People of this region have rich knowledge of traditional medicine, especially in folk medicine. The impact of biotic factors such as urbanization, and industrialization which have caused the loss of forests have resulted in the loss of biodiversity as well as indigenous knowledge in different regions of Odisha [2-10]. Phytochemical analysis of ethno-medicinal plants as well as their antimicrobial activities provides much relevant information for the authentication of the medicinal properties of these plants [11-12].

Materials and Methods
Study area
The present investigation involves ethno medicinal study in different locations of Kuldiha wild life Sanctuary and its adjoining regions. Kuldiha wild life Sanctuary is situated between 21°20′31″ to 21°29′08″ N latitude and 86°25′23″ to 86°44′56″ E longitude in the southern part of Balasore district of the state of Odisha in India (Fig.1).

Chotnagpur plateau and Similipal biosphere reserve through Sakhuapada and Natohill ranges. The maximum temperature of this region in the warmest month is 42 °C and the minimum temperature in the coldest month is 6 °C (Rout *et al*. 2018) [9]. It is considered the tropical moist deciduous forest possessing average rainfall of 1568 mm. Gadasahi forest is linked to this region in the south west region. Major prominent hills surrounding the region includes Asta pahar, Devgiri pahar, South Ranga Matia, Kolia parbat having an extension of 423 m, 682 m, 629 m, and 495 m respectively. Rock of the Sanctuary includes rhodolite, pyrogens granites, charnokite, and pyrogens granites. There are two reservoirs in Kuldiha. The Russia reservoir in the northwest is over river Tangana and Sindhua reservoir in the southeast is over Uastal nala. These two reservoirs have helped the crop and climate regime of the region.

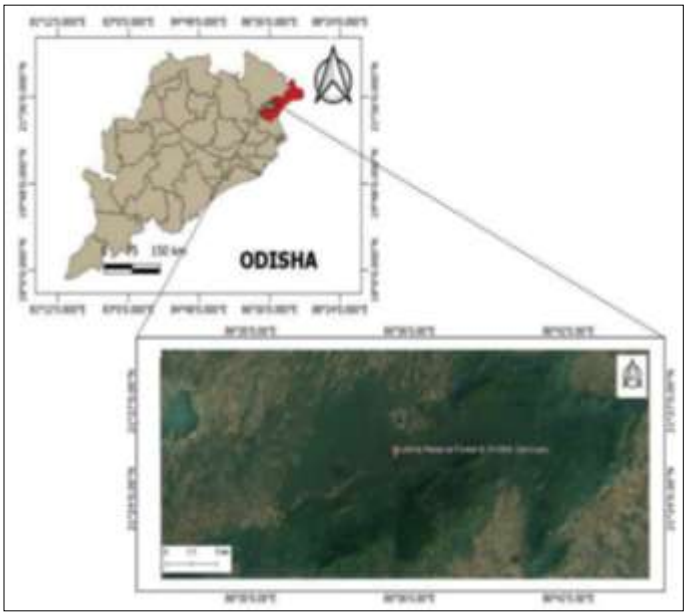

Fig 1: Map of Kuldiha Wildlife Sanctuary

Plant collection and preservation

Regular field trips have been conducted in different seasons of the year during 2017-2022 to various locations of the study region to know the distribution pattern and natural habitat of medicinal plants. The plant species collected have been identified with the help of regional flora books [13-14]. Herbarium samples have been collected from different locations in the study areas and the herbarium specimens have been prepared for future reference followings standard methods [15].

Method of data collection

The ethnobotanical data have been collected from the local ethnic groups and Vaidyas. The local elderly people with specialized knowledge of medicinal plants were consulted during the field survey. A number of group discussions have been done among the local people during the period of investigations. The data have been collected through interviews and personal interaction. As far as possible, the data have been verified by criss-cross checking method to confirm the authenticity of the information.

Phytochemical analysis

Phytochemical analysis has been done by standard procedures with slight modifications as follows [16, 17].

Extraction of plant materials

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. acetone, methanol, and distilled water, and was properly stoppered with cotton wool. Then the mixture was kept on an orbital shaker for 48 hours at 150 rpm at room temperature. Then the extracts were filtered with Whitman No.1 filter paper and the supernatant was collected in air-tight containers and stored at 4^0c. The tests for different phytochemicals were carried out for all the 3 different types of extracts.

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 alkaloids in the sample.

Test for Flavonoids

About 10 drops of aqueous plant extract were taken in a test tube and 5ml of dilute ammonium solution was added to it. Then the 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.

Test for Terpenoids

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 the test tube to form a layer. In the interface, a reddish-brown color was formed which indicated the presence of terpenoids.

Test for Glycosides

1 ml of plant extract was taken in a test tube and was treated with 2 ml 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 a thin layer.

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.

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 and the presence of tannins was detected.

Test for Saponins

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

Results and discussion

Ethno botanical surveys have been conducted in different regions of Kuldiha Wildlife Sanctuary, Odisha. The study has been carried to assess the medicinal uses of plants to meet the primary health care of needs of the local or tribal people. The herbarium specimens have been prepared following standard methods. The impacts of various biotic factors which are responsible for habitat destruction have been assessed and the present statuses of different medicinal plants have been evaluated.

It has also been observed that some plants are found to be very effective for the treatment of various diseases in Kuldiha Wildlife Sanctuary and its adjoining regions. The potential medicinal plants with respective scientific and local names along with their family and parts (root, stem, leaves, rhizome, roots) used for the treatment of various diseases have been recorded in the table (Table-1)

Phytochemical analysis of five potential medicinal plants *viz. Azadirachta indica* A. Juss. *Citrus aurantifolia* (Chris.)Sw., *Curcuma longa* L., *Emblica officinalis* Gaertn. And *Tinospora cordifolia* (Willd.)Miers (fig.2) which are primarily used for the treatment of some common diseases by the local ethnic population reveals the presence of various types of phytochemicals such as alkaloid, flavonoid, terpenoid, glycosides, phenol, tannin, saponin which are responsible for their medicinal properties and these phytochemicals provide evidence for the validation of the ethno medicinal data obtained from the study area for further use (Table-2).

Table 1: Ethnomedicinal uses of potential Medicinal plants of Kuldiha Wildlife Sanctuary

Sl. No.	Botanical Name	Family	Local Name	Method of use
1	*Aegle marmelos* L.	Rutaceae	Bela	The Fruit act as a tonic for the heart and brain, and helps in the treatment of constipation, and diabetes.
2	*Anogeissus latifolia* Roxb.	Combretaceae	Dhaura	The powder of the bark is applied externally as a pest to treat wounds and localized swelling.
3	*Azadirachta indica A.* Juss	Meliaceae	Nimba	Leaves are used to reduce inflammation of the gastrointestinal tract, which helps reduce a series of diseases like constipation, and stomach ulcers.
4	*Citrus aurantifolia* (Chris.)Sw.	Rutaceae	Kagaji Lembu	The fruit rinds are used for colds, sore throat bronchitis, and arthritis.
5	*Curcuma longa* L.	Zingiberaceae	Haladi	The rhizome is used for the treatment of skin diseases, wound healing, and liver ailments
6	*Dalbergia latifolia* Roxb.	Fabaceae	Shishu	The wood and bark are used in the treatment of blood diseases, burning sensations, dysentery, dyspepsia, and skin alignment.
7	*Diospyros embryopteris* Pers.	Ebenaceae	Mankada Kendu	The fruit is beneficial in treating dysentery, gonorrhea, and leprosy.
8	*Emblica officinalis* Gaertn.	Euphorbiaceae	Anla	The fruit possesses anti–diabetic, hypolipidemic, anti–microbial, anti-inflammatory, antioxidant, hepatoprotective, and anti-emetic activities.
9	*Ficus religiosa* L.	Moraceae	Aswastha	The leaves bark and ripe fruits are used for treating asthma.
10	*Ocimum sanctum* L.	Lamiaceae	Tulasi	The juice of leaves is used for the treatment of common c fever ever, cough, and bronchitis
11	*Pongamia glabra* Vent.	Fabaceae	Karanja	The leaf is used as a digestive and laxative and to treat inflammation and wound.
12	*Madhuca longifolia* Koenig.	Sapotaceae	Mahula	The leaves are used for the treatment of diabetes, bronchitis, intestinal diseases, and dermatopathy.
13	*Saraca asoca* Roxb.	Caesalpiniaceae	Ashok	The bark is help in the treatment of dyspepsia, fever, and burning sensation.
14	*Shorea robusta* Gaertn.	Dipterocarpaceae	Sal	The resins obtained from the tree give relief from skin disorders, pain in the nervous, burning injury, and diarrhea.
15	*Tectona grandis* L.	Verbenaceae	Sagwan	The wood powder is vermifuge and promotes digestion and is effective in relieving bilious headaches and toothaches, reducing inflammations and eruption in the skin.
16	*Terminalia chebula* Retz.	Combretaceae	Harida	The dried fruit is taken to increase appetite and act as a digestive aid, liver stimulant, stomachic, gastrointestinal prokinetic agent, and mild laxative.
17	*Terminalia tomentosa* Roxb.	Combretaceae	Asan	The bark of the plant is astringent and useful for the treatment of ulcers, fractures, hemorrhages, and diarrhea.
18	*Tinospora cordifolia* (Willd.)Miers	Menispermaceae	Giloy	Juice of stem is used for the treatment of constipation, vomiting, and jaundice.
19	*Withania somnifera* (L.)Dunal	Solanaceae	Ashwagandha	The paste prepared from its leaves is used for curing inflammation of tubercular glands.
20	*Zingiber officinale* Rosc.	Zingiberaceae	Ada	The Rhizome is used for the treatment of cough, sore throat, arthritis, and muscular aches.

Fig 2: Potential medicinal plants of Kuldiha wildlife sactuary (a) *Azadirachta indica* A. Juss (b) *Citrus aurantifolia* (Chris.)Sw. (c) *Curcuma longa* L. (d) *Emblica officinalis* Gaertn. (e) *Tinospora cordifolia* (Willd.)Miers

Table 2: Phytochemical analysis of common medicinal plants used by the local people of Kuldiha wildlife sanctuary

Sl. No.	Scientific Name/Family/ Local Name	The plant part used for analysis	Solvent	Phytochemicals						
				Alk	Flv	Terp	Gly	Phe	Tan	Sap
1	*Azadirachta indica A.* Juss Meliaceae Nimba	Leaf	Distilled water	+	+	+	-	+	+	+
			Methanol	+	+	+	+	+	+	-__
			Ethanol	+	+	+	+	-	+	-
2	*Citrus aurantifolia* (Chris.)Sw. Rutaceae Kagaji Lembu	Fruit	Distilled water	+	-	+	+	+	+	-
			Methanol	-	+	+	+	+	+	+
			Ethanol	+	-	+	+	+	+	-
3	*Curcuma longa* L. Zingiberaceae Haladi	Rhizome	Distilled water	+	+	+	+	-	+	+
			Methanol	+	+	+	+	+	-	+
			Ethanol	+	+	+	+	-	+	-
4	*Emblica officinalis* Gaertn. Euphorbiaceae Anla	Fruit	Distilled water	+	+	+	+	-	-	-
			Methanol	+	-	+	+	-	+	-
			Ethanol	+	+	+	+	-	+	+
5	*Tinospora cordifolia* Willd.) Miers Menispermaceae Gundichanati	Whole Plant	Distilled water	-	+	+	+	-	+	+
			Methanol	-	-	-	+	-	+	-
			Ethanol	+	+	+	+	+	+	+

N.B.
+: Presence of a particular phytochemical
- : Absence of a particular phytochemical
Alk: Alkaloid, Flv: Flavonoid, Terp: Terpenoid Gly: Glycosides, Phe: Phenol, Tan: Tanin, Sap: Saponin

Conclusion

During the present investigation, it has been observed that the people of various ethnic groups in and around Kuldiha Wildlife Sanctuary have rich indigenous knowledge regarding the use of various medicinal plants and plant products for the treatment of various diseases. Phytochemical analysis of some potential medicinal plants mentioned above reveals the presence of various compounds such as alkaloids, flavonoids, terpenoids, glycosides, phenol, tannin, saponin, etc. which are responsible for their medicinal properties. Further studies on the quantitative phytochemical analysis and antimicrobial activities of these plants will provide much useful information for the authentication of this indigenous knowledge and its applications in the herbal drug industry.

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