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Phytochemical analysis of medicinal plants used for treatment of dysentery and diarrhoea by the Paraja Tribe of Koraput, Odisha, India

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Abstract

The present investigation elucidates the ethnomedicinal importance of nine different medicinal plants such as *Holarrhena antidysenterica*. (Roth), *Murraya koenigii* (L.) Spreng, *Thysanolaena maxima* (Roxb.) Kuntze, *Flemingia strobilifera* (L.) W.T. Aiton, *Syzygium cumini* (L.) Skeels, *Helicteres isora* (L.), *Manilkara zapota* (L.) P. Royen., *Plumbago zeylanica* (L.), *Bombax ceiba* (L.) used for treatment of diarrhea and dysentery by the Paraja tribe of Koraput district of Odisha, India. A high degree of informant consensus for each species was observed. The aqueous extract of different plant samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. Phytochemical analysis of these plants revealed that some plants are promising sources of several chemical constituents like alkaloids, flavonoids, glycosides, phenols and antioxidants with free radical scavenging activity in varying proportions and possess anti-diarrhoeal activity. The study suggests that further work should be carried out to elucidate the possible mechanism of action of these extracts against diarrhea and dysentery.

Keywords: Diarrhea, ethnomedicine, phytochemical analysis, Paraja Tribe

1. Introduction

Diarrhea is one of the most common illnesses in all age groups and is a major cause of morbidity and mortality in rural communities [1]. It has long been recognized as an important health problem in developing countries and global threat to human health [2]. Due to the support of national and international organizations for the studies on treatment of diarrhoeal diseases based on indigenous practices, medicinal plants are becoming optimistic source of anti-diarrhoeal drugs [3-5]. Traditional medicine include all kinds of folk medicine, unconventional medicine and indeed any kind of therapeutic method that had been handed down by the tradition of a community or ethnic group [16]. In recent years, special attention is being paid on alternative natural bio remedies to cure the diseases because of their less or no side effects, high efficacy and less cost [6-7].

Koraput district of South Odisha is blessed with rich and diverse cultural heritage and the tribal people possess rich knowledge regarding plants including their usage for treating common ailments [8]. The Paraja tribe is an important primitive indigenous tribal group of Koraput, India and has a sound knowledge of natural herbal medicines for centuries with the knowledge being passed through successive generations. Due to poor sanitation, lack of safe drinking water and unhygienic environment, the incidences of dysentery and diarrhoea are prevalent among the tribe. Because of less accessibility of modern medicines, herbal medicines are the only option available to them for the treatment, as it provides cheap alternative without any known side effects. The traditional medicinal practitioners of Paraja tribe, who are familiar with the herbal medicine, have profound knowledge of herbal preparations used to treat dysentery and diarrhea. There are some reports on the ethno-medical knowledge of Paraja tribes of Koraput [8-10], however, most of these reports are incomplete and inadequate and no report is available about phytochemical evaluation of medicinal plants used by Paraja tribe of Koraput. Owing to the significance in the above context, present study reports the phytochemicals analysis of selected plants used for treatment of diarrhea and dysentery by the Paraja tribe of Koraput.

2. Material and methods**2.1 Study site, documentation and plant identification**

The study was conducted in Koraput district of Odisha, India which lies between 18° 14' to 19° 14' N latitude and 82° 05' to 83° 25' E longitude during 2014-2015. The ethnomedicinal information's were collected from 55 Paraja tribal dominated villages of Koraput through

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interview with local people of Paraja tribe and traditional healers. The information on the medicinal plants, usable plant parts, preparation method for medicine, application mode and dosage were recorded. The selected medicinal plants were collected from the field and identified with the help of flora books [The Botany of Bihar and Orissa by Haines, ^[11] and The Flora of Orissa by Saxena and Brahmam ^[12]].

2.2 Preparation of plant extract for phytochemical analysis

The plant samples were air dried for few days and the samples were crushed into powder and stored in polythene bags for use. The plant powder was taken in a test tube and distilled water was added to it such that plant powder soaked in it and was shaken well. The solution was then filtered with the help of filter paper and filtered extract of the selected plant samples were taken and used for further phytochemical analysis ^[13].

For alkaloids crude extract (0.1 ml) was mixed with 2 ml of 1 % HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids. For flavonoid crude extract (0.1 ml) was mixed with 2 ml of 2 % solution of Na OH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids. For phenols and tannins crude extract of 0.1 ml was mixed with 2 ml of 2 % solution of FeCl₃. A blue-green or black colouration indicated the presence of phenols and tannins. For saponins crude extract of 0.1 ml was mixed with 5 ml of distilled water in a test tube and was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins. For glycosides crude extract of 0.1 ml was mixed with 2 ml of chloroform. Then 2 ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring. For steroids crude extract was mixed with 2 ml of chloroform and 2 ml of conc. H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. For terpenoids crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this 2 ml of concentrated H₂SO₄ was added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids ^[14].

2.3 Estimation of total phenols

Total phenol was estimated by following Sadasivam and Manickam ^[14] with some modification ^[5]. The dried plant samples (0.1 g) were mixed with 5 ml 80 % ethanol and kept it for 10-12 h. Then samples were homogenized and centrifuged at 10,000 rpm for 20 minutes. Supernatants were collected and made up the volume to 25 ml with distilled water. Samples (0.1 ml) were taken in various test tubes and 0.5 ml of FC Reagent (Folin Ciocalteu Reagent) was added. One ml of 20 % sodium carbonate (Na₂CO₃) was mixed in each tube. The resulting mixture was allowed to stand at room temperature for 30 minutes and the absorbance was measured at 650 nm against a blank. Total phenolic content was expressed as mg of phenol equivalents per gram of dry weight of the samples by using the standard curve of phenol.

2.4 Estimation of total Flavonoid

Total Flavonoid content was determined by Aluminium Chloride Colorimetric Assay followed by Wadood *et al.* ^[15], with some modification. The dried plant samples (0.1 g) were soaked in 80% ethanol, homogenized and centrifuged at

10,000 rpm for 20 minutes. The crude extracts (0.1 ml) were diluted with 4ml of distilled water. To this 0.3 ml of 5% sodium nitrite (NaNO₂) was mixed. Then 0.3 ml Aluminium chloride (10%) was added and kept for 5 min in the dark for complete the reaction. 1M sodium hydroxide Na OH (2 ml) was added and total volume was made up to 10 ml with distilled water. The absorbance was measured at 510 nm with spectrophotometer. Results were expressed as µg flavonoid/g of dry samples using Catachol as standard.

2.5 Evaluation of total antioxidant capacity

The total antioxidant capacity of the extract was evaluated by the Phospho-molybdenum method with some modification ^[16]. For antioxidant capacity 0.005g of dried extract was added to 25 ml of 70 % ethanol (v/v) and homogenized by vortex agitation. An aliquot of 300 µl of plant extract was mixed with 3 ml of the reagent solution. This solution made of 0.6 M Sulphuric acid (H₂SO₄), 28 mm Sodium Dihydrophosphate Dihydrate (NaH₂PO₄.2H₂O) and 4 mm Ammonium Hepta Molybdate Tetrahydrate [(NH₄)₆Mo₇O₂₄.4H₂O]. These vials were incubated in water bath at maximum 90°C of 90 min. Then samples cooled at room temperature and absorbance was measured at 695 nm against blank. A typical blank solution contained 1ml of reagent solution and appropriate volume of the ethanol 70% (v/v) and it was incubated under the same condition as the rest of samples. Percentage of antioxidant activity as calculated by following equation.

$$\text{Antioxidant activity (\%)} = \frac{(A_{\text{sample}} - A_{\text{blank}}) / A_{\text{ascorbic acid}} - A_{\text{blank}}}{* 100}$$

3. Results and Discussion

The result of the present study on ethno medicinal plants used for treatment of diarrhea and dysentery showed that the Paraja tribe of Koraput have very good knowledge and wisdom on plants and their medicinal importance. The paper provides information about ethnomedicinal and scientific evidences of nine plants being used for treatment of diarrhea and dysentery. The plants are enumerated according to their scientific name, family, mode of preparation and medicinal uses (Table 1). A high degree of informant consensus for each species was observed. In the present study, most of the plants were reported for the first time used against diarrhoea and dysentery. However, no plant was reported as a new medicinal plant as the plants were reported with other use earlier. The decoction of *Helicteres isora* L fruits along with sugar is given orally in treatment of diarrhea and dysentery reported in the present study also reported earlier by Koyas communities of Khammam district of Andhra Pradesh ^[17-18]. The *Murraya koenigii* (L.) is taken as fresh green tender leaves are eaten raw against dysentery and decoction of leaves and petiole is given against diarrhea in Kanyakumari district, Tamil Nadu ^[19]. The paraja tribe using *Syzygium cumini* bark paste orally with water to cure dysentery but the root extract is taken twice daily in empty stomach to cure dysentery and diarrhoea was used by the indigenous community of Bargarh district, Odisha was earlier reported by Sen and Behera ^[20]. The plant species like *Bombax ceiba*, *Plumbago zeylanica*, *Manilkara zapota*, *Flemingia strobilifera*, *Thysanolaena maxima* are the newly reported medicinal plants used by Paraja tribe for the treatment of dysentery and diarrhoea. All the reported plants for treatment of dysentery and diarrhea have not been pharmacologically evaluated yet for their possible anti-diarrhoeal properties. Most of the traditional medicine was prepared by the healers from fresh material collected from the wild. However, in some cases, sun dried

stored plant materials were used. Preparation of paste for the treatment of diseases is a common practice among the other tribal communities in India [4]. In our study, medicines were

also given internally, where paste is highly used for diarrhea and dysentery.

Table 1: List of medicinal plants used by Paraja tribe of Koraput for diarrhoea and dysentery along with their mode of medicinal use.

Scientific Name	Local Name	Family	Parts use	Mode of medicinal use
<i>Holarrhena antidysenterica</i> . (Roth) Wall. ex A.DC.	Kurei	Apocynaceae	Bark	Bark paste is given orally to cure dysentery.
<i>Murraya koenigii</i> (L.) Spreng.	Vurusunga	Rutaceae	Leaves	Green leaves are eaten as raw to cure of dysentery.
<i>Thysanolaena maxima</i> (Roxb.) Kuntze.	Phulo Jhadu	Poaceae	Root	Root paste is taken orally one time a day to cure diarrhoea.
<i>Flemingia strobilifera</i> (L.) W.T. Aiton.	Phool buta	Leguminosae	Tuber	Tuber paste is taken orally to cure from dysentery.
<i>Syzygium cumini</i> (L.) Skeels	Jamukoli	Myrtaceae	Bark	Bark paste is taken orally with water to cure dysentery.
<i>Helicteres isora</i> (L.)	Modimodika	Malvaceae	Fruit	Fruit paste is taken orally to cure chronic dysentery.
<i>Manilkara zapota</i> (L.) P. Royen	Safeta	Sapotaceae	Fruit	The young fruits ground into paste with water and taken orally to cure dysentery.
<i>Plumbago zeylanica</i> (L.)	Swetachitaparu	Plumbaginaceae	Leaves	Leaf paste is taken orally to cure dysentery.
<i>Bombax ceiba</i> (L.)	Simili	Malvaceae	Bark	The bark is crushed and given orally to cure from diarrhea,

Phytochemical analysis of nine medicinal plants used for treatment of diarrhea and dysentery by the Paraja tribal communities of Koraput were studied for the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, terpenoids, and alkaloids which have possess anti-diarrhoeal activity presented in Table 2. It was observed that all the plants such as *Holarrhena*

antidysenterica, *Murraya koenigii*, *Thysanolaena maxima*, *Flemingia strobilifera*, *Syzygium cumini*, *Helicteres isora*, *Manilkara zapota*, *Plumbago zeylanica*, *Bombax ceiba* contain flavonoid, terpenoid and phenol but alkaloids are present only in 6 plants. Saponins were found to be present in *Murraya koenigii*, *Flemingia strobilifera*, *Syzygium cumini*, *Helicteres isora*.

Table 2: Phytochemical analysis of medicinal plants used by Paraja tribes for the treatment of diarrhoea and dysentery (- = absent, + = present, ++ = strongly present)

Medicinal plants	Alkaloid	Flavonoid	Steroid	Terpenoid	Glycoside	Saponin	Tanin	Phenol
<i>Holarrhena antidysenterica</i>	+	+	+	+	+	-	++	+
<i>Murraya koenigii</i>	+	++	++	+	-	++	++	++
<i>Thysanolaena maxima</i>	++	+	-	++	-	-	-	+
<i>Flemingia strobilifera</i>	++	+	-	++	++	+	+	+
<i>Syzygium cumini</i>	-	+	+	+	-	+	++	++
<i>Helicteres isora</i>	++	+	-	++	+	+	+	+
<i>Manilkara zapota</i>	-	+	-	+	++	-	-	+
<i>Plumbago zeylanica</i>	-	+	+	+	++	-	++	++
<i>Bombax ceiba</i>	+	+	-	+	+	-	-	+

The results of quantitative analysis of total phenols, flavonoid and total antioxidant capacity in the medicinal plants was summarized and shown in Table 3. The content of phenols,

flavonoid and total antioxidant capacity was found to be more in *Holarrhena antidysenterica* followed by *Flemingia strobilifera*.

Table 3: Quantitative phytochemical analysis of total phenol, flavonoid and total antioxidant capacity of medicinal plants used for the treatment of diarrhoea and dysentery. Data are the mean of three replications replication \pm standard deviations.

Scientific name	Phenol (mg g ⁻¹ dry wt.)	Flavonoid (μ g g ⁻¹ dry wt.)	Total antioxidant capacity (%)
<i>Holarrhena antidysenterica</i>	25.56 \pm 0.05	13.45 \pm 2.05	23.87 \pm 0.26
<i>Murraya koenigii</i>	17.13 \pm 0.28	04.05 \pm 0.49	03.17 \pm 0.09
<i>Thysanolaena maxima</i>	03.80 \pm 0.28	04.00 \pm 1.56	06.85 \pm 0.53
<i>Flemingia strobilifera</i>	24.53 \pm 0.66	08.75 \pm 2.62	21.33 \pm 0.08
<i>Syzygium cumini</i>	13.33 \pm 0.47	06.25 \pm 1.34	07.73 \pm 0.00
<i>Helicteres isora</i>	19.99 \pm 0.09	08.35 \pm 0.64	05.67 \pm 0.08
<i>Manilkara zapota</i>	13.23 \pm 0.42	05.15 \pm 1.63	04.80 \pm 0.18
<i>Plumbago zeylanica</i>	05.65 \pm 0.49	04.70 \pm 0.42	04.11 \pm 0.17
<i>Bombax ceiba</i>	12.46 \pm 0.28	06.35 \pm 0.07	06.81 \pm 0.12

Phytochemical analysis of these plants revealed that some plants are promising sources of several chemical constituents like alkaloids, flavonoids, glycosides, phenols and antioxidants with free radical scavenging activity in varying proportions and possess antidiarrhoeal activity. The present

information may serve as a baseline data to initiate further research for discovery of new compounds and biological activities of these potential plants. Further research on these plants may provide some important clues for development of new drugs for dysentery and diarrhea.

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