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Phytochemical and antioxidant activities of ethno-medicinal plants used by fisher folks of Chilika lagoon for Indigenous Phytotherapy

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Abstract
The present study examined indigenous phytotherapies use by fisher folks, living inside islands of Chilika lagoon. Fishing being the only livelihood, they have to be in salt water of Chilika for a long time and skin disease is the most common disease in this population in all age groups. Being inaccessible to the health care facilities in the mainland, they use frequently leaves of 13 medicinal plants for cure of various ailments. *In vitro* antioxidant activity and phytochemicals were analyzed with standard protocols. Canonical redundancy analysis was carried out to correlate the data obtained. Phytochemical and antioxidant activities of these plants revealed *Heliotropium indicum* and *Plumbago zeylanica* are having very good antioxidant activity. We have identified potential relationships between the medicinal plant species, their phytochemical variables and the ailments for which the local fisher folk uses them, using stepwise forward canonical redundancy analysis.

Keywords: Ethno-medicinal plants, Indigenous phytotherapy, Antioxidant, Canonical correspondence analysis.

1. Introduction
Medicinal plants are an essential part of the traditional health care systems. There are more than 8,000 plant species in South Asia with known medicinal uses [1]. Thus, historically it is evident that South Asia is home to many rich traditional systems of medicine (TSM). Modern allopathic medicine is also using extracts and agents from many medicinal plants. Due to spiral price of modern medicine, government finds it increasingly difficult to meet the cost of pharmaceutical-based health care. Therefore, throughout the region, there is strong and sustained public support for the protection and promotion of the cultural and spiritual values of traditional medicine [1]. In Indian medicine systems, Ayurveda, Siddha and Unani entirely and Homeopathy partially depend either on plant materials or their derivatives for treating human ailments [2]. Right from its beginning, the documentation of traditional knowledge, especially on use of medicinal plants has provided important information for modern drugs [3-4] and even today this area holds much more hidden thesaurus.
Chilika is well known as a Ramsar site, a wetland of International importance and the largest brackish water lagoon of Asia. It has unique biodiversity due to its hydrological regimes. However, until 2002 the phytodiversity of Chilika lagoon was not known. A phytodiversity survey by the Chilika Development Authority (CDA) in 2002 identified 726 plants belonging to 496 genera and 120 families in Chilika (within the water body, including the 20 Islands and shorelines) [5] which represents about one–fourth of the vascular plant species in the Odisha state. The lake is an ecosystem with large fishery resources. It sustains more than 150,000 fisher folk living in 132 villages on the shore and islands. However, how these fisher folks live on different islands utilize this vast plant resources are not known yet. The Odisha state comes under the eastern Indian zone constitutes the major tribal population of India which includes about 65 tribes [6]. The villages in Chilika have dominant fisher community population belong to SC communities such as Keuta, Kandara, Tiara, Nolia, Khatia etc. Fishing is the traditional and primary occupation of these communities. Though they are residing in a tough condition, involving in much physical labor for intensive fishing and have to face much difficulty in every aspect of survival being living in the islands, but they are able to bear up and having good stamina and good health. Intake of antioxidant is reported as a remedy for fatigue and tiredness, but how these fisher folks use the medicinal plants and whether they have

antioxidant activity is not known. Reports reveal that most of the medicinal plants possess antioxidant property, which play an important role in the prevention of various degenerative diseases and have potential benefits to the society.

The main characteristic of an antioxidant is its ability to trap free radicals. Free radicals are molecules containing one or more unpaired electrons in atomic or molecular orbitals. There is increasing evidence that aberrant production of free radicals *in vivo* increases oxidative stress on cellular structures and leads to oxidize nucleic acids, proteins, lipids or DNA, causes change in molecular pathways that underpins the pathogenesis of several important diseases, including carcinogenesis and cancer, cardiovascular diseases, neurodegenerative diseases and in the process of physiological ageing. Therefore, the present investigation was undertaken to study the ethenomedicinal plants of Chilika lagoon and to evaluate their antioxidant potential.

2. Materials and methods

2.1. Study area and climate

Chilika, is the largest brackish water lagoon in Asia situated on the east coast of India between $19^0$28' and $19^0$54' N latitude and $85^0$05' and $85^0$38' E longitude (Fig.1). The lagoon is an estuarine one and supports an unique assemblage of marine, brackish water and freshwater species. It is connected with the Bay of Bengal on its eastern side through an outlet which cuts through liner spit that separates the lake from the sea. On the southwestern side, the lake is walled by a range of hills and to the north itself in shallow sedge banks and islands just peeping above the surface. Hemmed in between the mountains and the sea, Chilika spreads itself out into a peer-shaped expanse of water. From about the middle line to the south, enormous rocks and emerald green islands jutting out of the water surface relieve the monotony of the vast expanse of water. Of these Badakuda, Sanakuda, Nalabana, Kalijai, Ghantasila, Chadheiguha, Arakhakuda and Kankadakuda etc. deserve special mention having 726 species of flowering plants belonging to 496 genera and 120 families [5].

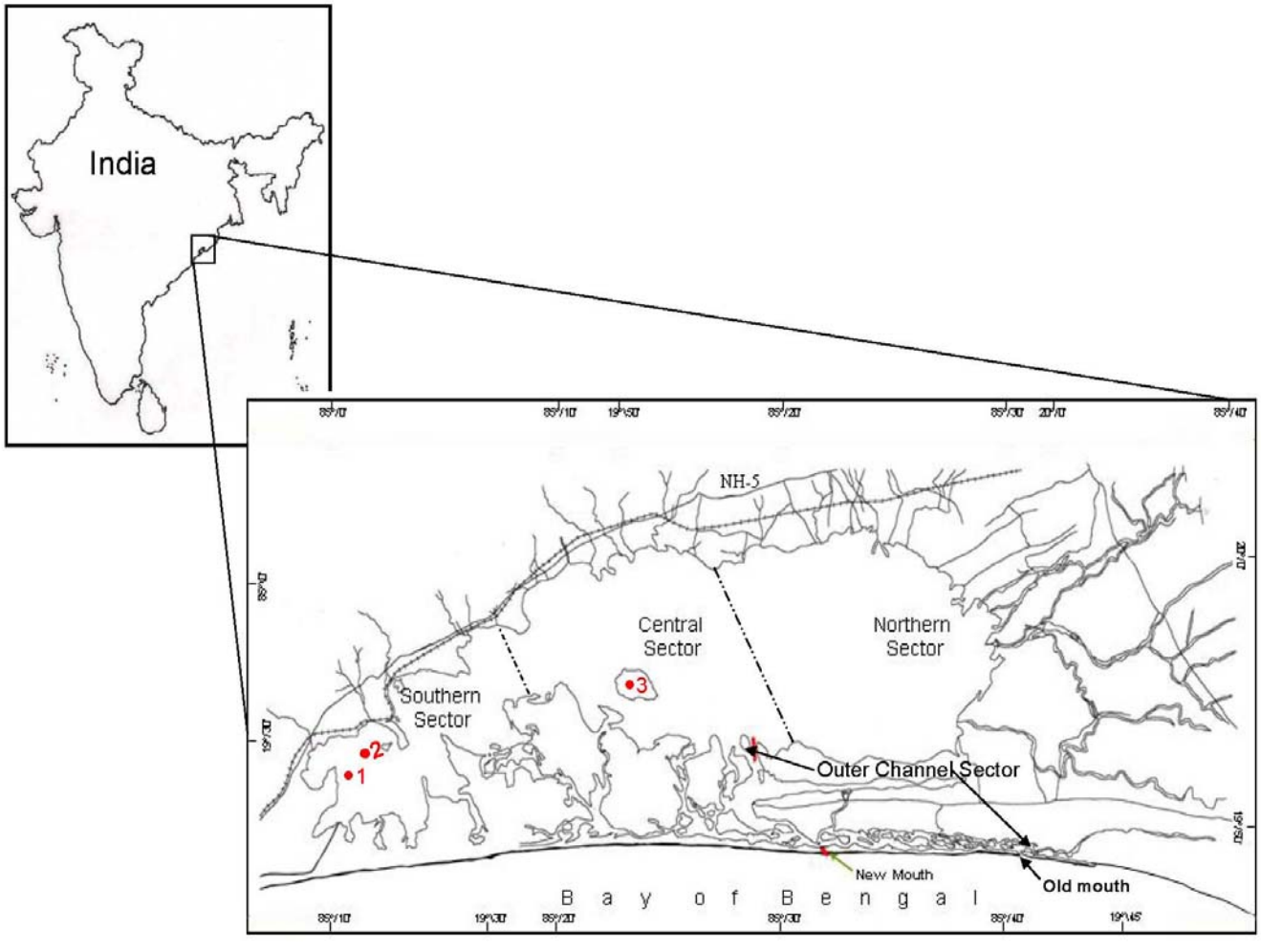

Fig 1: Location map of Chilika lagoon showing collection sites. 1. Bird's Island, 2. Honeymoon Island and 3.Kalijai.

2.2. Data collection and Identification of Plants

The ethnobotanical survey was conducted between February 2012 and January 2013. The information was collected from fisher folks, traditional practitioners, women and village heads. The data were collected using semi-structured questionnaires on types of ailments cured by the traditional use of medicinal plants and plant parts used in curing different ailments as described by Martin (1995) [7]. Cross checking of data was made with the help of group discussions among different age classes of fisher folks. The

surrounding island villages were surveyed with local herbal healers and knowledgeable elders for the identification of various plant species and their traditional uses. The frequency of uses of plants and use in different age groups is also collected. For use of these plants for different diseases in children data has been collected from their mother. Plant specimens were collected for taxonomic identification from three islands namely Birds Island, Honeymoon Island and Kalijai island of Chilika lagoon with the help of local fisher folks. Herbarium of all the specimens collected was prepared and deposited in the herbarium of Department of Botany, Visva-Bharati. All the plant specimens are identified with the help of regional floras [8-9] and finally confirmed by comparing with the authenticated specimens in the Herbarium of Regional Research Laboratory (RRL-B) Bhubaneswar. In this particular study, we selectively chose the leaves of those medicinal plants which are used by the fisher folks for cure of different ailments.

2.3. Preparation of crude aqueous extract

After proper identification the leaves were collected in sterile polyethylene bags and bring to the laboratory. The leaf samples were washed thoroughly with fresh running water; the healthy leaves were dried at room temperature (25 ± 1 °C) for 3 days to maintain their green color and volatile oils, if present. The leaves were further dried in the oven at 40 °C and put in desiccators for at least 24 h prior to analysis. After drying completely, leaves were grinded to a coarse powder using electric grinder. The coarse powder was subjected to successive extraction in a soxhlet apparatus using distilled water. The aqueous extracts were filtered through whatman No 1 filter paper and concentrate to dry mass with the aid of rotary vacuum evaporator (Buchi type). The aqueous crude extracts were used for the phytochemical screening and assessing the antioxidant activity.

2.4. Phytochemical composition

2.4.1. Preliminary phytochemical screening

The preliminary phytochemical screenings were carried out using standard procedures to identify constituents, as described by Harborne 1984; Trease and Evans 1979; Sofowara 1993 [10-12]. For flavonoids, a few drops of NaOH solution were added to the extract solution (500 µl) followed by dil. HCl. The solution turned yellow and then colorless, indicating the presence of the flavonoid. Presences of alkaloids were tested using 3 reagents namely, Dragendorff's reagent, Wanger's reagent and Mayer's reagent. To the extract solution (500 µl) a few drops of the reagent were added. A reddish brown precipitate indicated the presence of alkaloids. For phenols and tannins to the test solution (500 µl), a few drops of $FeCl_3$ were added. Presence of phenols and tannins were indicated by formation of a blue or blue-green colored solution. Few drops of Na_2HCO_3 were added to the extract solution (500 µl) and shaken for 5 minutes. Formation of froth or lather indicated the presence of saponins. Few drops of aqueous NaOH were added to the extracts (500 µl). Yellow colored solution indicated presence of glycosides. For detection of steroids chloroform was added to the extract solution (500 µl) followed by conc. H_2SO_4 added slowly through the sides of the test tube. The lower sulfuric acid fraction turned brownish yellow and the upper layer turned reddish-orange which indicated presence of steroids.

2.4.2. Total phenolic content

Total phenolic content was estimated by the Folin-Ciocalteu method [13]. 0.1 ml of sample was mixed with 2 ml of freshly prepared sodium carbonate (2%) and vortexed vigorously. After 5 min, 100 ml of Folin-Ciocalteu reagent (1 N) were added to the mixture, and incubated for 30 min at room temperature and the absorption spectra were measured in a Shimadzu UV-1800 UV–visible double beam spectrophotometer against a blank at 750 nm. A calibration curve was performed in parallel under the same operating conditions using Gallic acid as a positive control. The results are expressed as mg Gallic acid equivalent per gram of dry extract (mg GAE/g).

2.4.3. Total flavonoid content

The total flavonoid content was determined by a colorimetric method as described by Ardestani and Yazdanparast, 2007 [14]. Each sample (500 µl) was mixed with 2 ml of distilled water and subsequently with 150 µl of a 15 % $NaNO_2$ solution. After 6 min, 150 µl of aluminum chloride ($AlCl_3$) solution (10%) was added and allowed to stand for 6 min. Then, 2 ml of NaOH solution (4%) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then recorded at 510 nm versus blank. Results were expressed as mg catechin equivalent per gram of dry extract (mg CEQ/g).

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging activity

The free radical scavenging activities of the aqueous leaf extract on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined using the method of Blois 1958 [15]. Briefly to 1 ml (100, 200, 300, 400, 500 µg/ml) aqueous leaf extracts, 5 ml of methanol solution of DPPH (0.1 mM) was added, vortexed, followed by incubation at 27 ^{0}C for 20 min. The control was prepared without any extract and absorbance was measured at 517 nm using UV/Vis spectrophotometer. The ability to scavenge DPPH radical was calculated by the following equation: % DPPH radical scavenging = [(Abs $_{Control}$– Abs $_{Sample}$)/Abs $_{Control}$] x 100. The % inhibitions were plotted against respective concentrations used and from the graph IC_{50} value was calculated. The lower IC_{50} indicates higher radical scavenging activity and vice versa.

2.5.2. Determination of superoxide radical scavenging activity

Measurement of superoxide anion scavenging activity was based on the method of Dinis *et al*., 1994 [16]. Superoxide radicals were generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). In brief, 3 ml of sample solutions at different concentrations were mixed with 1 ml of NBT (156 µM) and 1 ml of NADH (468 µM). The reaction started by adding 0.1 ml of phenazine methosulfate (PMS) solution (60 µM) to the mixture. The reaction mixture was incubated at 25 ^{0}C for 5 min, and the absorbance at 560 nm was measured against blank samples. The percentage inhibition of superoxide anion generation was calculated using the formula: %Inhibition of superoxide radical = [(Abs $_{Control}$ − Abs $_{Sample}$)/Abs $_{Control}$] x 100 and IC_{50} was calculated.

2.5.3. Determination hydroxyl ion (OH^-) radicals scavenging activity

The hydroxyl ion (OH^-) scavenging activity was determined according to Klein *et al*., 1981 [17]. Different concentrations of the extracts were added to 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was started by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90 °C for 15 min in a water bath. The reaction was then terminated by the addition of 1 ml of ice-cold TCA (17.5 % w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 l with distilled water) were added and left at room temperature for 15 min. The intensity of the color formed was measured at 412 nm against the blank. Butyl Hydroxyl Toluene (BHT) was considered as the reference standard. The hydroxyl radical scavenging activity is calculated by the formula: % Inhibition of hydroxyl ion scavenging activity =1 – (Difference in ABS of sample/ difference in ABS of blank) X 100

2.5.4. Nitric Oxide (NO) scavenging activity

Nitric Oxide (NO) is a free radical which is an effective inhibitor of several physiological processes such as smooth muscle relaxation and neuronal signaling. The nitric oxide scavenging activity of the selected plants was measured spectrophotometrically following Sreejayan and Rao, 1997 [18]. Sodium nitroprusside (1 ml of 10 mM) was mixed with 1 ml of various concentrations of sample extracts in phosphate buffer (pH 7.4). The mixture was incubated at 25 ^{0}C for 150 min. To 1 ml of the incubated solution, 1 ml of griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1 % naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage inhibition was calculated using the formula: inhibition (%) = [(Abs $_{Control}$– Abs $_{Sample}$)/ Abs $_{Control}$] x 100 and then IC_{50} was calculated.

2.6. Statistical analysis

All the data were expressed as mean ± standard deviation. IC_{50} values were calculated using regression equation in excel program. Statistical analysis was performed using *Student's* t test. The p values less than 0.05 were considered as significant differences.

2.6.1. Canonical correspondence analysis

With the advent of analytical techniques such as redundancy or ordination analyses, complex interactions between community structure and environmental factors can be inferred from what otherwise are cumbersome datasets of descriptive spatial studies, providing the foundation for further experimental probing. We have identified potential relationships between the medicinal plant species, their phytochemical variables and the ailments for which the local fisher folk uses them, using stepwise forward canonical redundancy analysis [19], in which the species data and the phytochemical variables were examined to infer qualitative relationships. The variance partitioning procedure was performed with explanatory variables to remove their effects and to obtain a net effect of an individual factor. Furthermore, a Monte Carlo randomization test with 999 unrestricted permutations was used to determine whether the phytochemical variables had a significant effect on the ailments/uses of these medicinal plants. CCA was used because the species data were in the form of categorical predictors (dummy variables). Standardization by phytochemical parameters (dependent variables) was used because the data analyzed were of various types and units. We performed several CCAs to assess the relative impact of different explanatory variables on the species and the ailments for which it is used. Computations were performed using the computer program CANOCO version 4.5 [20] and the results of multivariate analyses were visualized in the form of a triplot ordination diagram constructed by the Canoco Draw program.

3. Results and discussion

3.1. Ethnomedicinal use of plants

Chilika is a vast lagoon (1165 sq. km) and 150,000 fisher folk living in 132 villages on the shore and islands. There is almost no medical facility and those living in the islands have very limited access to go to the hospital on the mainland. Therefore majority of them depends on the ethnic medicine to cure many ailments. Almost all the peoples in these villages including women and children depend on the fisheries of this lake for their livelihood. For fishing they have to be in salt water always and have many skin diseases besides cases of snake bite, dysentery etc. The fisher folk mostly uses fresh leaf of most of the plants and we have listed 13 medicinal plants of which leaves commonly used by them to cure of various health problems (Table 1). All the taxa are arranged alphabetically by their botanical names with family and frequency of use and their mode of preparation and use have been given. The study reveals the fisher folks have a sound knowledge about the plants and can readily identify and know where it grows. Interestingly, women have more knowledge about the medicinal plants. They collect these plants while collecting firewood, dried them and stored in their houses for future use. In comparison to other tribes, they don't hesitate to share their experiences and with the help of these fisher folks we able to collect most of these plants from the islands which is quite in assessable from the main land. However, they are concerned about the mass collection of these plants by outsider and concern about their conservation. Codes of the medicinal plants and their uses have been provided in the parenthesis which has been used for canonical correspondence analysis. The collected data shows various types of skin diseases are common among the fisher folks. Analysis of data shows they use *Cassia tora* (42.54 %) for itching due to prolonged exposure to salt water, followed by *Centella asiatica* (24.13 %) for skin boils in summer, *Cleome aspera* (18.75 %) for acute infection lead to ulcers and ring worm infections, *Euphorbia hirta* (10.42 %) as antiseptic in wounds and *Ludwigia octovalvis* (4.17 %) mostly aged pupils for the cure of eczema (Fig. 2). We have also analyzed how these plants are used in various age groups. *Centella asiatica* (47.37 %) is the most widely used plants in case of children to treat summer boils (Fig. 2). Being in tropical countries Chilika is having high solar radiation and temperature goes beyond 45 ^{0}C and skin boil is common among children. However, for young men aged 15-40 who are mostly active in daily fishing *Cassia tora* (40 %) is the most frequently use plants for various problems related to skin itching. Young women are also very active in fishing and related fish trading and also use *Cassia tora* (42.11 %) little more than men for skin itching problem. In case of old

age persons eczema is a major problem and they use *Ludwigia octovalvis* (43.79 %) to cure it (Fig. 2).

Documentation of ethno botanical/ethno pharmacological information is important with respect to know the human–plant relationship [21], understand human–biodiversity relations [22], implement general policy and program [23] and evaluate livelihood, monetary benefits and marketing patterns [24-25]. Besides, fulfilling the herbal needs, medicinal plants are also of commercial values. Although, several research articles were available regarding ethno botanical attributes of plants in India [26-32] but, there is an ever-increasing demand of beyond the anthropological significance of ethno botanical studies of rural/tribal communities. Since these fisher folks lived in the islands which is difficult to assess from the main land, their traditional knowledge of utilizing the plants were not known and this is the first report from these communities. In addition to sources of drugs in modern medicines [33], plants had played a vast role in the treatment of human ailments globally. Such investigation may lead to the discovery of novel bioactive molecules that will help to evaluate the potency of indigenous herbal drugs.

Table 1: Ethnomedicinal use of plants by the fisher folks of Chilika lagoon

S. No	Name of the plant	Family	Islands	Ailments/uses	Frequency
1.	*Alstonia scholaris* (Linn.) R.Br. (Code-AS)	APOCYNACEAE	Bird's Island	The latex of leaf is applied on breast nipples to induce lactation of mother after child birth (Code- LCB)	++
2.	*Andrographis paniculata* (Burm.f.) Wall. ex Nees (Code- AP)	ACANTHACEAE	Honeymoon Island	Decoction of leaf taken orally before bed against round worm (Code- RW)	+++
3.	*Cassia tora* Linn. (Code- CT)	CAESALPINIACEAE	Kalijai	Crude extract of leaves used to cure itching which occur due to salt water during fishing (Code- SD)	++++
4.	*Centella asiatica* (Linn.) Urb. (*Hydrocotyle asiatica* Linn.). (Code- CA)	APIACEAE	Honeymoon Island	The fresh leaves are applied over affected areas to cure skin boils in summer. Fresh leaves are also consumed raw to cure acidity and indigestion. (Code- SD)	+++
5.	*Cleome aspera* Koenig ex DC. (Code- CAS)	CAPPARACEAE	Honeymoon Island	Crude extract of the leaf locally applied to the affected area for 3-5 days to treat ulcers and ring worm infection. (Code- RW & SD)	++
6.	*Clerodendrum viscosum* Vent. (Code- CV)	VERBENACEAE	Honeymoon Island	Young leaves mixed with coconut and taken orally to cure dysentery (Code-DY)	+++
7.	*Coccinia grandis* (Linn.) Voigt (Code- CG)	CUCURBITACEAE	Bird's Island	Leaf crushed and the juce applied directly in the ear for earache. (Code- EA)	++++
8.	*Eclipta prostrata* (Linn.) Linn. (Code- EP)	ASTERACEAE	Kalijai	Crude leaf extract taken orally 2-3 times to cure Jaundice (Code- JA)	
9.	*Euphorbia hirta* Linn. (Code- EH)	EUPHORBIACEAE	Kalijai	Crude extract of the leaf is used as antiseptic against wound (Code-SD & AN)	++
10.	*Heliotropium indicum* Linn. (Code- HI)	BORAGINACEAE	Kalijai	Juice of the fresh leaf is given as an antidote for poison consumed (Code- ANT)	+++
11.	*Ludwigia octovalvis* (Jacq.) Raven (Jussiaea suffruticosa Linn.) (Code- LO)	ONAGRACEAE	Kalijai	The paste of leaf applied externally to cure eczema (Code- SD)	++++
12.	*Plumbago zeylanica* Linn. (Code- PZ)	PLUMBAGINACEAE	Honeymoon Island	Leaf paste used as antidote in snake bite. Leaf paste is applied on the wound and taken orally for immediate cure. (Code – ANT)	++++
13.	*Vitex negundo* Linn. (Code- VN)	VERBENACEAE	Honeymoon Island	Decoction of young leaf taken orally for cure headache and sinus problem (Code – HA)	

++++ – very common; +++ – common; ++ – common to restricted area or age group; + – rare.

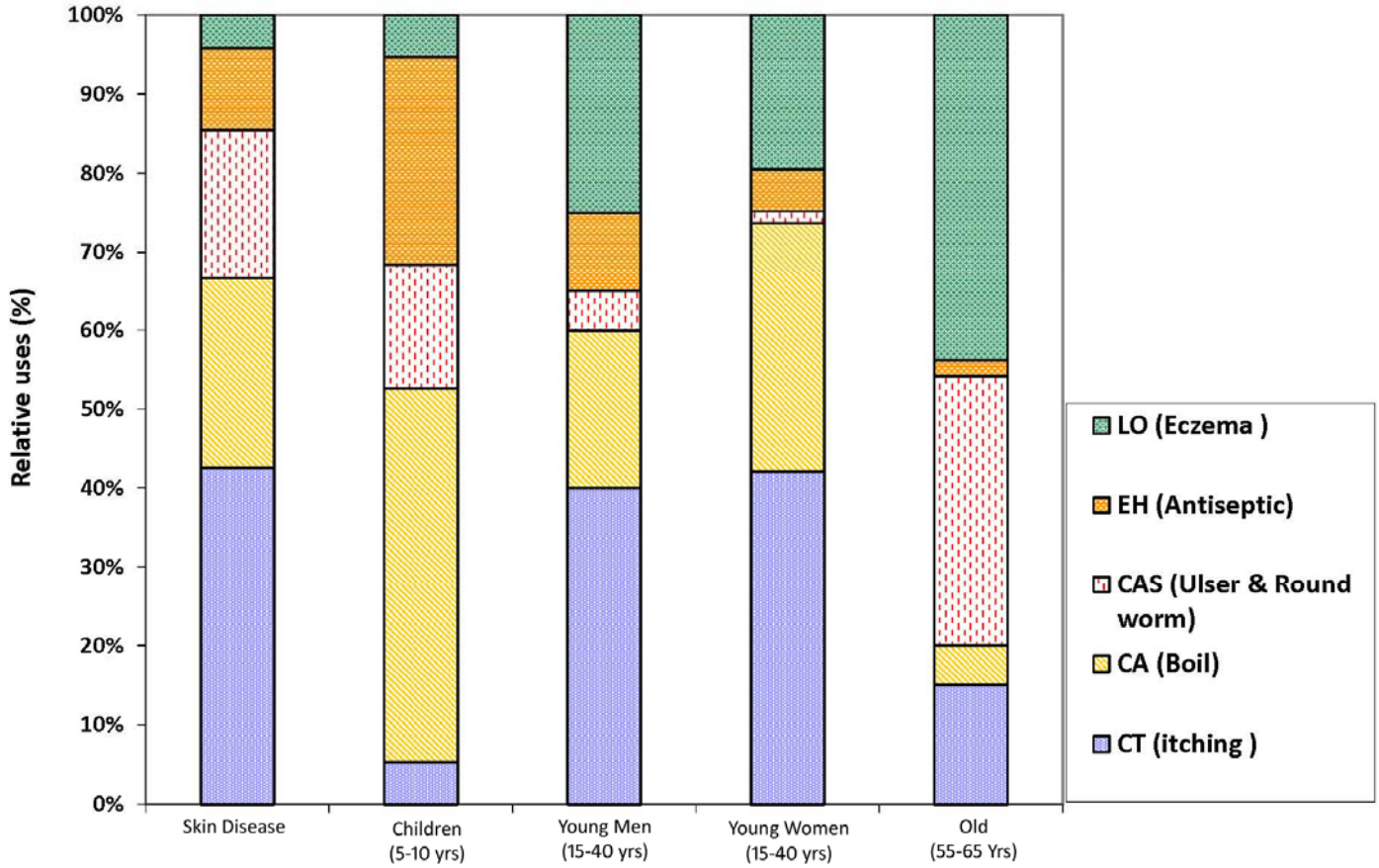

Fig 2: showing % use of plant for skin diseases and use in various age group

3.2. Phytochemical screening

Preliminary phytochemical screening of aqueous leaf extract of 13 ethnomedicinal plants of Chilika lagoon revealed the presence of various bioactive components like flavonoids, alkaloids, phenols, tannin, saponin, glycosides, and steroids of which flavonoids, phenols and tannins were prominent in all the studied medicinal plants. The result of phytochemical test has been summarized in the Table 2. In comparison to other phytochemicals, glycosides are present in minor quantity in the aqueous extract and were found only in *Cassia tora, Coccinia grandis, Heliotropium indicum* and in *Plumbago zeylanica*. Amongst the chemical classes present in medicinal plant species, alkaloids stand as a class of major importance in developing new drugs because alkaloids own a great variety of chemical structures and have been identified as being responsible for the pharmacological properties of the medicinal plants [34]. In the present study, we find good concentrations of alkaloids in the aqueous extract of leaves of *Andrographis paniculata, Cassia tora, Centella asiatica, Euphorbia hirta* and *Heliotropium indicum*. Among all the studied medicinal plants in *Heliotropium indicum* almost all the phytochemicals are found in good concentration.

The total phenolic content and total flavonoid content of the aqueous extract of the leaves of the studied medicinal plant are given in Fig. 3. The results show maximum phenolic content in *Heliotropium indicum* (213.06 ± 3.02 mg GAE/g), followed by *Centella asiatica* (198.11 ± 1.56 mg GAE/g) and in *Coccinia grandis* (191.07 ± 3.62 mg GAE/g). Phenolic compounds are known to be powerful chain breaking antioxidants and are important constituents of plants. Phenolic compounds may contribute directly to antioxidative action. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1.0 gm daily from a diet rich in fruits and vegetables [35]. The antioxidant properties of phenolic compounds are due to their redox properties, which can play a vital role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposed peroxides [36]. Good quantities of flavonoids are also found in the studied medicinal plants of Chilika. Flavonoids content of *Plumbago zeylanica* (150.05 ± 1.68 mg CE/g) was maximum among the studied plant followed by in *Ludwigia octovalvis* (147.02 ± 1.11 mg CE/g), *Alstonia scholaris* (143.14 ± 2.43 mg CE/g) and *Heliotropium indicum* (139.18 ± 1.2.12 mg CE/g). It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [37-38] Since we got good phenolic and flavonoid content in these studied plants, therefore, we carried out the antioxidant activity of the aqueous leaf extract and correlate it to the aliments for which it was used using Canonical correspondence analysis.

Table 2: Phytochemical properties of selected medicinal plants of Chilika lagoon

Sl No.	Plant sp.	Flavonoids	Alkaloid s	Tannin	Saponin	Glycosides	Phenols	Steroids
1.	*Alstonia scholaris*	+	+	+++	+	+	+++	+++
2.	*Andrographis paniculata*	+	+++	+	++	+	+	++
3.	*Cassia tora*	+++	+++	++	+	+	++	+
4.	*Centella asiatica*	++	+++	+	+++	-	+++	++
5.	*Cleome aspera*	+++	+	+++	+	-	+	-
6.	*Clerodendrum viscosum*	+++	+	+	+++	+	+++	-
7.	*Coccinia grandis*	+	+	+	+++	+	+++	+
8.	*Eclipta prostrata*	+	+++	++	+++	-	+	+
9.	*Euphorbia hirta*	+++	+++	+++	+++	+	+++	++
10.	*Heliotropium indicum*	+++	+++	+	+++	+	+++	+
11.	*Ludwigia octovalvis*	+	+	+++	+++	-	++	+
12.	*Plumbago zeylanica*	+	+++	+	+++	+	++	++
13.	*Vitex negundo*	+++	+++	+	+++	-	+++	+

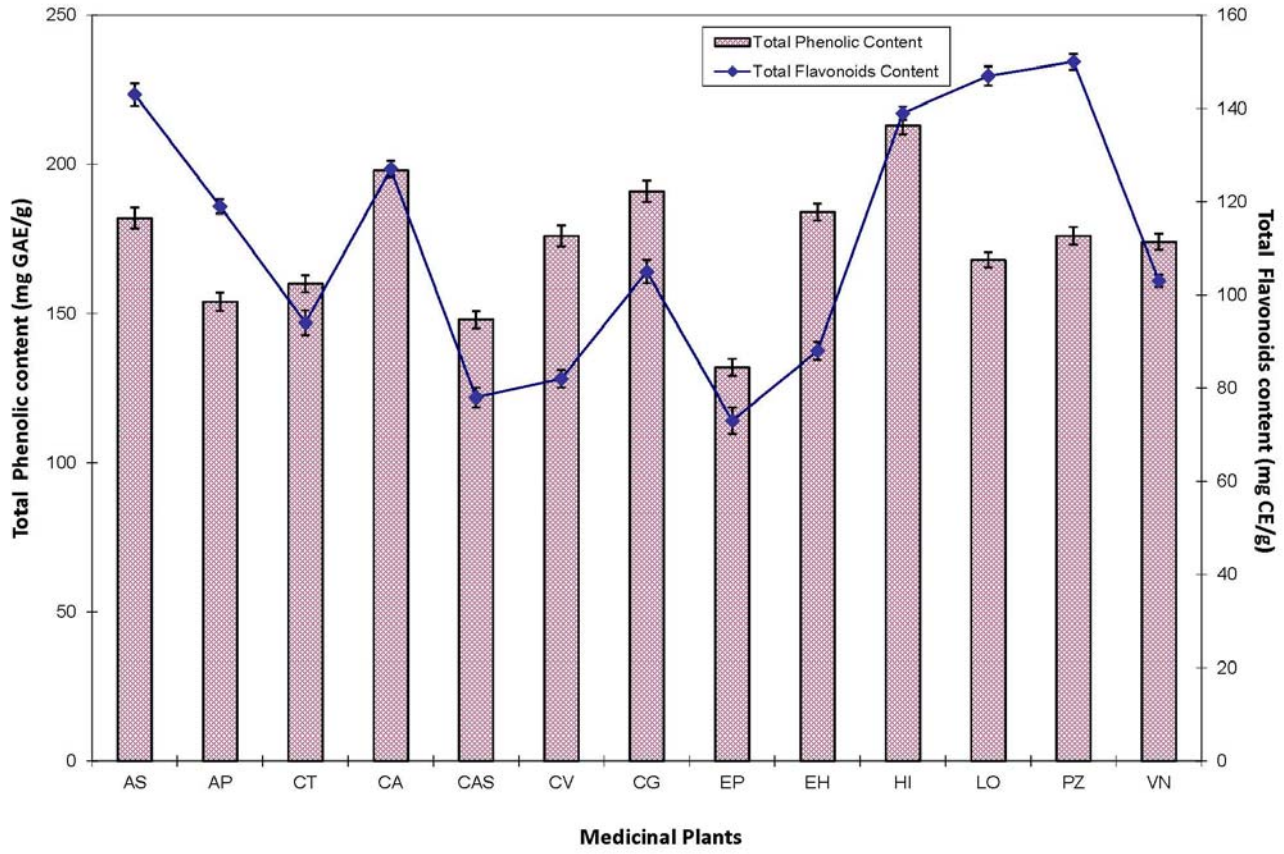

Fig 3: Total phenolic and flavonoid content of aqueous extract of studied medicinal plants use by fisher folks of Chilika lagoon. The codes of the plants depicted in Table -1.

3.3. Antioxidant activity

We use *in vitro* models such as DPPH, super oxide radical (O_2^-), hydroxyl ion (OH^-) and nitric oxide (NO) to test antioxidant activities of aqueous leaf extract of the studied medicinal plants. The results show the extracts having very active antioxidant activity (Fig. 4). DPPH assay is one of the most widely used methods for screening of antioxidant activity of plant extracts [39]. DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicrylhydrazine, with the addition of the aqueous plant extract in a concentration dependent manner. Lower the IC_{50} value better is the scavenging ability of the sample. In the present study, we find highest DPPH scavenging activity in extract of *Heliotropium indicum* (IC_{50} = 42 ± 2.41 µg/ml) followed by in *Centella asiatica* (IC_{50} = 49 ± 2.54 µg/ml), *Coccinia grandis* (IC_{50} = 54 ± 1.67 µg/ml) and *Euphorbia hirta* (IC_{50} = 57 ± 1.46 µg/ml). These extracts significantly quenched DPPH radical, though, it showed lesser activity than the standard Butyl Hydroxyl Toluene (BHT).

The superoxide (O_2^-) radical is one of the most dangerous free radicals in humans and also the source of hydroxyl radicals (OH^-) [40]. Superoxides are produced from molecular oxygen due to oxidative enzymes of the body [41] as well as via non-enzymatic reaction such as autoxidation by catecholamines [42]. In the present study, superoxide radical reduces NBT to a blue colored formazan that is measured at 560 nm [43]. The results of superoxide radical scavenging activities of the extracts show *Heliotropium indicum* is very promising having IC_{50} 94 ± 1.34 µg/ml followed by in *Plumbago zeylanica* (IC_{50} = 102 ± 1.24 µg/ml), *Vitex negundo* (IC_{50} = 105 ± 1.05 µg/ml) and *Cleome aspera* (IC_{50} = 112 ± 1.29 µg/ml).

Hydroxyl radical (OH^-), which is the most reactive free radical, has the capacity to conjugate with nucleotides, causes breaking of DNA strands and lead to carcinogenesis, mutagenesis and cytotoxicity [44]. Among the plant extracts *Plumbago zeylanica* is having very good hydroxyl radical scavenging activity with IC_{50} = 54 ± 1.67 µg/ml. The other potential OH^- scavengers are from *Ludwigia octovalvis* (IC_{50} = 61 ± 2.07 µg/ml), *Heliotropium indicum* (IC_{50} = 63 ± 1.45 µg/ml) and *Centella asiatica* (IC_{50} = 71 ± 1.76 µg/ml).

In addition to reactive oxygen species, nitric oxide (NO) is also implicated in inflammation, cancer and other pathological conditions. The extract effectively reduced the generation of nitric oxide from sodium nitroprusside. *In vitro* inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [45]. The results show *Vitex negundo* is having potential nitric oxide scavenging activity (IC_{50} = 47 ± 2.76 µg/ml). Besides the plant extracts of *Andrographis Paniculata* (IC_{50} = 49 ± 3.1 µg/ml), *Alstonia scholaris* (IC_{50} = 61 ± 3.5 µg/ml) and *Coccinia grandis* (IC_{50} = 63 ± 3.62 µg/ml) also very active nitric oxide scavenging activity.

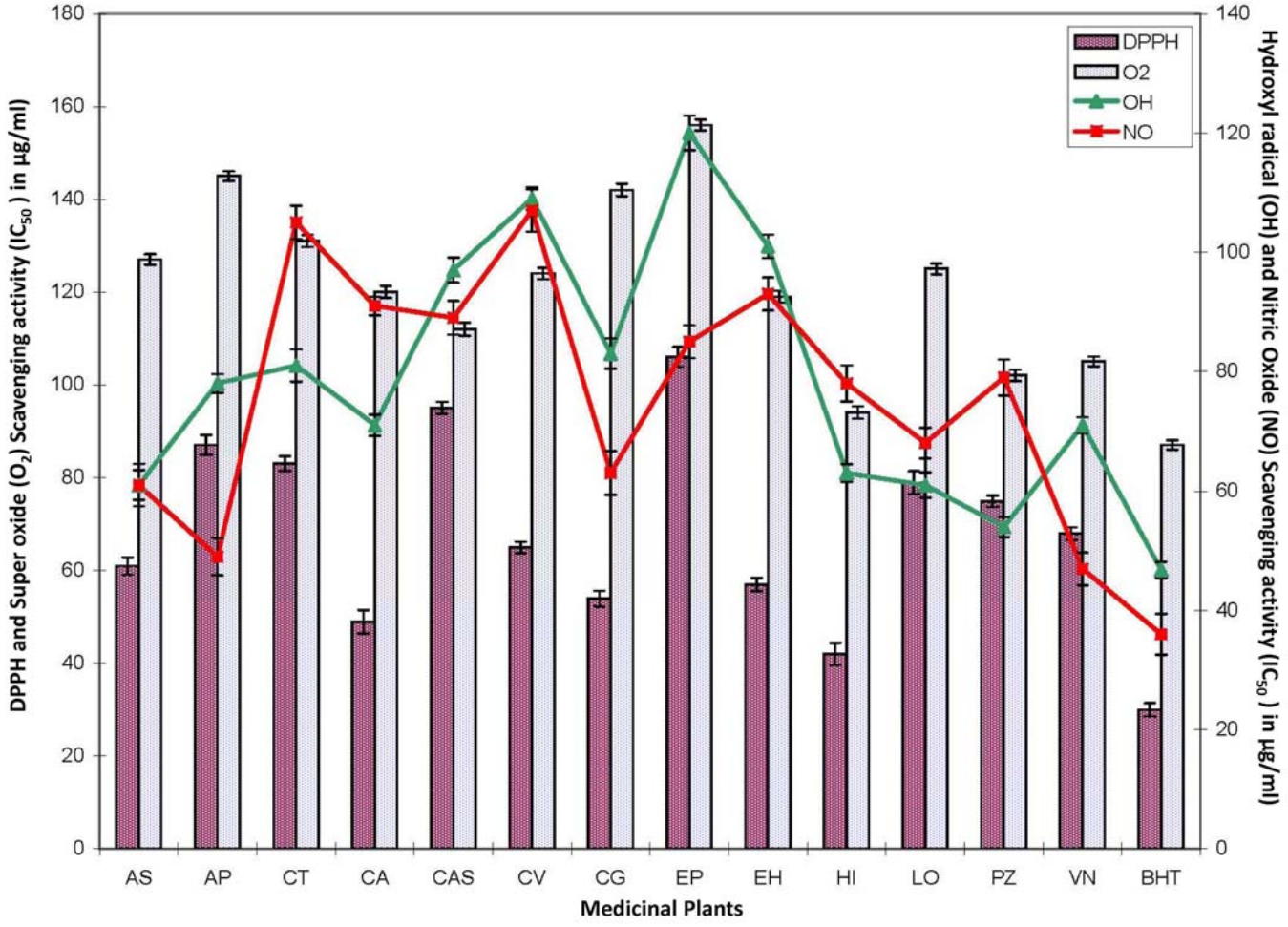

Fig 4: Antioxidant activity of aqueous extract of leaves of medicinal plants of Chilika. The codes of the plants depicted in Table -1.

3.4. Canonical correspondence analysis

We used Canonical correspondence analysis (CCA), to visualize and correlate between the medicinal plant species and ailments for which it was used by the fisher folks with response to phytochemical variables (total phenolic content, total flavonoids, DPPH, O_2^-, OH^-, NO scavenging activities) (Fig. 5). The length of an explanatory variable arrow in the ordination plot corresponds to the variable's importance in explaining its influence on the species and ailments. The proximity of points representing similarity in influence on the species and explanatory variable ailments. The two species those are close together, having a similar phytochemical composition than two species that are further apart on the ordination diagram. Considering the length of

arrows, the EC_{50} value of superoxide scavenging activity is the most important phytochemical variable. However, the lower IC_{50} value indicates higher radical scavenging activity. Therefore, DPPH radical scavenging activity (having a short length of arrow) is the most important antioxidant activity in the studied medicinal plants (Fig. 5). The important phytochemical variables in the studied medicinal plants can be ranked as DPPH, NO, OH^-, O_2^-, total phenolic content and total flavonoid content. The ordination diagram also shows, with increase in total phenolic and flavonoids content, the antioxidant activity, particularly DPPH, O_2^- and OH^- scavenging activity increases in the studied plants. In the ordination diagram *Heliotropium indicum* (HI), *Plumbago zeylanica* (PZ), antidote (ANT), total phenolic (TP) and total flavonoids (TF) forms one cluster. (Fig. 5). Interestingly, in *Heliotropium indicum* phenolic content is highest with maximum DPPH and O_2^- scavenging activity and in *Plumbago zeylanica* highest flavonoid content was recorded with maximum OH^- scavenging activity. Therefore, it might be due to its high antioxidant properties, *Heliotropium indicum* and *Plumbago Zeylanica* act as antidote for snake bite or for poison consumed cases. The position of *Vitex negundo* (VN), in the ordination diagram, is opposite to the arrow representing nitric Oxide (NO) scavenging activity which shows low NO EC_{50} and high NO scavenging activity. *Centella Asiatica* (CA) and *Ludwigia octovalvis* (LO) lies in one cluster and occur more or less equidistant to the arrows representing nitric Oxide (NO) scavenging activity; total phenolic (TP) and total flavonoid (TF) content therefore have moderate nitric Oxide (NO) scavenging activity. *Andrographis paniculata* (AP), *Coccinia grandis* (CG) and *Euphorbia hirta* (EH), *Cassia tora* (CT) and CAS (*Cleome aspera*) are closely represented in the ordination diagram so, their photochemical composition seems to be similar.

This naturally occurring antioxidant showed greater advantages over currently available synthetic source, namely butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters. As the synthetic source has low solubility, may prompt or cause negative effects and produced only moderate effect [40].

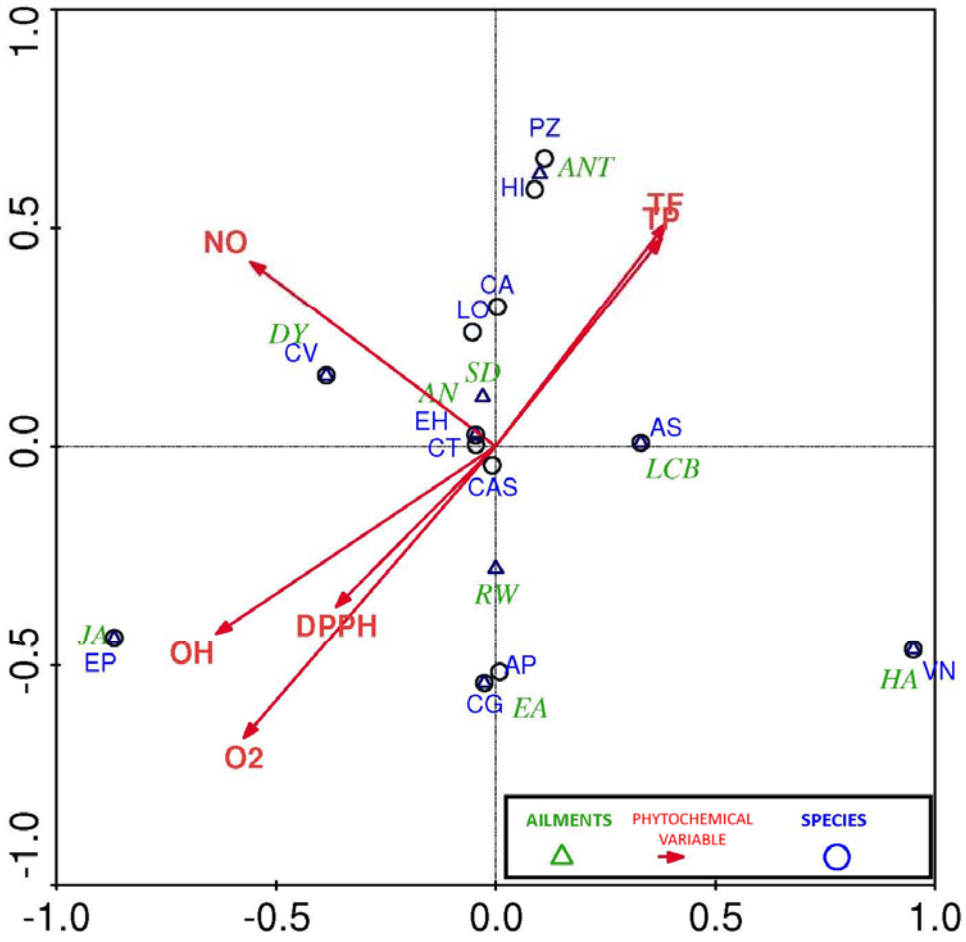

Fig 5: Canonical correspondence analysis (triplot) of medicinal plant species and ailments for which it was used by the fisher folks with response to phytochemical variables. The Response variables (Phytochemical parameters) represented by arrows and labeled as DPPH - DPPH radical scavenging activity, O_2^-superoxide radical scavenging activity, OH^- - hydroxyl ion radicals scavenging activity, NO Nitric Oxide scavenging activity, TP- Total phenolic content, TF- Total flavonoid content. The codes of the plants and their ailments are depicted in Table -1.

4. Conclusion

The result obtained from this study shows the medicinal plants used by fisher folks of Chilika lagoon having potent antioxidant activity. Most of the ethno medicinal information provided in this study is new, as they are not recorded earlier. However, due to increase attention in view of the resurgence

of interest in herbal medicines for healthcare all across the globe, their conservation is of prime importance. The data obtained in this study will lead to further detailed investigation and characterization of pharmaceutical lead compounds from these plants.

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