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Pharmacognostical, physico-chemical and phytochemical findings of different extracts of *Capparis zeylanica* Linn. (Capparidaceae)

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Abstract
The aim of the study is to investigate pharmacognostical, physico-chemical and phytochemical screening of the different extract of *Capparis zeylanica* It has total ash 3.1% w/w, water soluble ash 0.5% w/w, acid insoluble ash 0.5% w/w, water soluble extractives 3.8% W/W and alcohol soluble extractives 10.18% W/W. The plant showed loss on drying 9.2% W/W. The extractive values of Methanol, Pet ether, Chloroform and Ethyl acetate were 6.3%, 2.2%, 1.2% and 1.3% w/w respectively. In determination of leaf constant it revealed that stomata were present on upper epidermis of leaf having average stomatal number-9, stomatal number range- 8-10, average stomatal index-17.75 and stomatal index range-16-20. The leaf had average palisade ratio 1:7.95. And average vein-islet number is 58.4 having vein–islet range-50-63.The Fluorescence characteristic of powdered flower produce different colors with different reagents in visible and UV light. Qualitative phytochemical study of *C. zeylanica* showed different responses for alkaloid, glycosides, saponin, phenolic, carbohydrate etc.

Keywords: *Capparis zeylanica* Linn, traditional use, pharmacognostical, physicochemical, phytochemical screening, fluorescence analysis

1. Introduction
As we know very well that everything in this world change time by time, since thousands of year the era was of ayurveda or herbal origin drug. But last few decades it was replaced by allopathic system of medicine, which was firstly accepted worldwide, but later due to its lots of adverse effect again men step down on Ayurveda because of its better therapeutic result and safety profile and now the people are more believing in natural origin drug. Herbal drugs have played a vital role in curing so many aliments throughout the history of medicine as well as the existence of mankind. If we take a worldwide comparison of patronization of modern and alternative medicine, it is depicted that 75% of the population world over is per forced, compelled to use the alternative system of medicine especially the herbal medicine indigenous to that part of the world [1, 2].

1.1 Plant profile
Capparis zeylanica Linn. is commonly known as Indian caper; a climbing Scandent shrub and found throughout India *Capparis zeylanica* Linn is belonging to the family Capparidaceae plants are 2-3m in height, armed with 3-6mm long recurved thorns, branched, leaves are elliptic or broadly lanceolate, base rounded, apex mucronate; flower profuse, pinkish white, later turning pink, berries are globular or ellipsoid, 3-4 cm in diameter, and seeds are globase, embedded in white pulp. It is grows in moist habitat. Large climbing shrubs with hooked spines, stems woody, rough, young parts green, rusty tomentose with pungent smell, leaves ovate or elliptic,3.5-6.5x2.5-4 cm, rusty-tomentose when young, glabrosa at maturity, base cuneate,entire,tip mucronate, flowers yellowish-white or white in supra-axillary, solitary, 2-3 pedunculate, berries globose, scarlet red [3]. Fig- 1 to 4.

1.2 Taxonomical classification [4]
Kingdom	:	Plantae
Subkingdom	:	Viridaeplantae
Phylum	:	Tracheophyta
Subphylum	:	Euphyllophytina
Infraphylum	:	Radiatopses
Class	:	Magnoliopsida

Subclass	:	Dilleniidae
Super order	:	Violanae
Order	:	Capparales
Suborder	:	Capparineae
Family	:	Capparaceae.
Genus	:	Capparis
Specific epithet	:	zeylanica - L.
Botanical name	:	*Capparis zeylanica* L.

Vernacular name

Oriya	:	Asadhua
Sanskrit	:	Karambha
Bengali	:	Kalokera
Hindi	:	Ardanda
Kannad	:	Aantundikayee
Marathi	:	Govindi
Tamil	: -	Adondai
Telugu	: -	Adondai

1.3 Occurrence
The plant is a many branched thorny, sub-scandent climbing shrub. It is grows in moist habitat.

1.4 Distribution
The plant distributed throughout the major parts of India, Bangladesh and some parts of Pakistan.

1.5 Phytochemicals
Root bark of *Capparis zeylanica* contains an alkaloid, a phytosterol, a water soluble acid and mucilaginous substance. It's leaves contain-β-carotene. The whole plant contain- A saponin, p- hydroxybenzoic, syringic, anillic, ferulic and p-coumaric acid, l-stachydrine, rutin and β -sitosterol. It's seeds and leaves contain Thioglucoside, glucocapparin, n-tricontane, α-amyrin and fixed oil [5, 6].

1.6 Traditional uses
Its root bark is demulcent, sedative, stomachic, good appetizer& finds useful for internal application in colic. It is antihydrotic, bitter, cholague and used in cholera [7, 8]. It's aerial parts are used as sapasmolytic [9]. The whole plant useful in fever. It has also bactericidal properties. It is given in neuralgia, pleuracy, rheumatism & colitis [10]. It leaves are irritant ground in to paste and used for external application in glandular swelling, piles & boils. Decoction of leaves is given in syphilis [8, 11].

2. Materials and Methods
The following drugs and chemicals were used for the different experimental study. The Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. Methanol and Petroleum ether was purchased from Qualigens chemicals. Mumbai. And all others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.

2.1 Plants collection, identification and processing
The plant was collected from adjoining area of Barpali (Dist-Bargarh, Odissa) in the month of Oct-Nov on dated 25-10-2019. The plant was identified by Botanist Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D, P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust if any; initially it was dried in sunlight for an hour and shade dried completely. Also all the foreign matters like dead or destructed part were removed precautionary. The dried plant stem was cut into chips and powdered by means of wood grinder and was sieved through sieve no. 18 & 60 to get the coarse powder, fine powder was used for further detailed powder microscopy, and the coarse powder was extracted with different solvents for further studies.

2.2. Pharmacognostical studies
The plants parts were subjected for macroscopic study. The leaves, stem, flower, root Transverse Section were processed with glycerine and saffranine for better visualisation and it was observed under compound microscope (Olympus 100MB, Universal Pvt. Ltd., Mumbai) at magnification of 100 X under day light.

Macro and mcroscopical studies

2.2.1 Macroscopical investigation [12-16]
A. Morphological group of stem

Type of stem	:	Woody
Outer surface	:	Rough, spines or remantits of the spines are also found
Fracture	:	Irregular & fibrous
Odour	:	Characteristic
Taste	:	Bitter
Colour	:	Green

B. Morphological group of leaf Fig-5

Type of leaf	:	simple
Colour	:	Green
Odour	:	Characteristics
Taste	:	Bitter

C. Morphological group of root Fig-6

Type of root	:	Tap root
Outer surface	:	Fairly smooth transverse cracks
Fracture	:	Short
Odour	:	Indistinct
Taste	:	Slightly bitter
Colour	:	Yellowish grey

2.2.2 Microscopical investigation [17, 18]
2.2.2.1 Transverse section of stem
A transverse section of stem shows a single layer of epidermis, followed by 6-10 layer of parenchymatous cortex. The central region was occupied by wide pith, composed of thin-walled, circular to isodiametric parenchymatous cells, some of which are pitted. The secondary growth starts in the usual manner. The cork cambium arises in the outermost or the second layer of the cortex giving rise to the cork towards the outer and phlloderm towards the inner side Fig7, 8.

2.2.2.2 Transverse section of leaf
A transverse section of leaf shows distinct layer of upper and lower epidermis. Upper epidermis was covered with thick cuticle. Vascular bundles were distributed in the middle zone. Each vascular bundle is surrounded by bundle sheath Fig-9

2.2.2.3 Transverse section of root
A transverse section of root shows a single layered epidermis, some of which elongated to form unicellular hairs. The epidermis was followed by 2-3 layered parenchymatous cortexes. The endodermis was distinct with casparian dots on the anticlinal walls. The pericycle was single layered and

encloses a triarch stele. The phellogen arises in the epidermis Fig-10, 11.

Powder microscopy [17, 18, 19]

2.2.2.4 Powder microscopy of stem

From the above microscopy it shows that the stem part is containing xylem, unlignified vessel, and epidermis Fig-12 to 14.

2.2.2.5 Powder microscopy of leaf

Some important characters of the leaf of *Capparis zeylanica* contains Calcium oxalate- it occur as cluster in the cell of mesophyll and as prism in a sheath of cells around the fiber**,** glandular trichome Anomocytic stomata present on upper epidermis, simple parenchyma cells were found numerously and stone cells were also found Fig-15,16.

2.2.2.6 Powder microscopy of stem

It contains wood element as xylem vessel with numerous bordered pitted thickening. It xylem fibres- Large number of thick walled, elongated fibres mostly in groups. The walls of a few fibres show pitted thickening. it also contains calcium oxalate crystal-Large number of big elongated prism either entire or in fragments, some may also appear cubical in form, prisms are found scattered all over. Cork cells are thin walled, some colourless and other brown. It also contains sieve tubes Fig-17 to 20.

Powder microscopy of root

It contains Parenchyma which are thick walled cells containing oil globules and minute acicular raphides. And also contains wood element- Vessels with boarded piths, scalarriform and spiral thickening Fig-21, 22.

2.2.2.7 Determination of leaf constants [19, 20]

The average stomatal number is 9, stomatal number range is 8-10, average stomatal index is 17.75 and stomatal index range of *C. zeylanica* is 16-20 Table-1, Fig-23.

The average palisade ratio of *C. zeylanica* linn: 1: 7.95 Table-2:

The average vein-islet number of *C. zeylanica* is -58.4 and vein-islet range is -50-63 Table-3 and Fig-24

2.3 Physio-chemical evaluation [21-24]

2.3.1 Ash Values

The ash value of *C. zeylanica* is in Table 4

2.3.2 Determination of extractive values [25-27]

Extractive values Table-5 of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug. The ethanol soluble extractive and chloroform water-soluble extractive Table-5.

2.3.3 Determination of moisture content (Loss on drying)

The Percentage of Moisture content was found to be 9.2%

2.4 Preliminary phytochemical screening [24, 28-31]

The powder plant material was extracted with methanol in soxhlet extractor for 72 hours at 60^0C. The extract was kept solvent free using vacuum filtrations. Then methanolic extract was extracted with pet ether (60^0-80^0), chloroform, methanol, ethanol, ethyl acetate and water successively in separating funnels for 24 hours. The solvent remove from all the extract using vacuum filtration and concentrated. Then the extracts were stored at 4^0C until use. All extract were studies to their colour, consistency and odour for further reference and phytochemical work. The value was recorded in Table -6 to 7.

2.4.1 Determination of fluorescence characteristics of powder drug & extract [32-34]

Fluorescence characteristics of the powder and extracts of *Capparis zeylanica* was observed with different chemical reagents under Day light and UV light (at 254 & 366 nm.) shown in Table 8 -9.

2.4.2 Thin layer chromatography [35-37]

Thin layer chromatography is a separation technique used for qualitative screening of different plant extracts which serve as a very important tool in the overall phytochemical research studies. By trial and error method, methanolic extract of *C. zeylanica* showed isolation and resolution of spots with following solvent systems: Chloroform:Methanol (3:2) and Methanol: Ethyl acetate (3:2) having Solvent Front–5.5cm and Solvent Front–6.3cm. The different spots developed in each solvent system were identified under day light by using Anisaldehyde–sulphuric acid as spraying reagent and the calculation of R_f value made by following the below formula and tabulated in Table 10 and Fig-25, 26

3. Results and Discussion

It has total ash 3.1% w/w, water soluble ash 0.5% w/w, acid insoluble ash 0.5% w/w, water soluble extractives 3.8% W/W and alcohol soluble extractives 10.18% W/W. The plant showed loss on drying 9.2% W/W. Table 4 to 5.The extractive values of Methanol, Pet ether, Chloroform and Ethyl acetate were 6.3%, 2.2%, 1.2% and 1.3% w/w respectively. Table-6.The study showed that water extract shows positive response for alkaloid, glycosides, saponin, and shows negative response for phenolic compound and carbohydrate. The ethanol extract shows positive response for alkaloid, saponin, and carbohydrate and negative response for phenolic compound and glycosides. The chloroform extract shows positive response for alkaloids and negative response for all other constituents. The methanol extract shows positive response for alkaloid and negative response for all other constituents. The ethyl acetate extract shows positive response for alkaloid and saponin and shows negative response for all other phytoconstituents. The pet ether extract shows positive response for alkaloid and saponin and negative response for all other constituents Table- 7.

Methanolic extract was subjected to thin layer chromatography on silica gel for the identification of chemical constituents. It showed 6 spots with Rf values 0.27, 0.3, 0.38, 0.6, 0.83 & 0.94 respectively in solvent system chloroform: methanol (3:2) and 7 spots with Rf values 0.25, 0.33, 0.5, 0.62, 0.69, 0.8, 0.93 respectively in solvent system methanol: ethyl acetate(3:2). The Rf values found by TLC give the information about the chemical constituents present in the plant Table-10.

Transverse section of stem of *C.zeylanica* showed a single layer of epidermis covered externally by a thick cuticle, followed by 6-10 layer of parenchymatous cortex, which was differentiated in to two zones, the outer zone consisting of smaller cells while the inner zone consisting of larger cells. The endodermis was inconspicuous. The pericycle was represented by unlignified cells in the early stages, but as the growth proceeds, they get lignified and were converted in to fibers. On further growth, the parenchymatous cells between

these fibres strands become lignified and were converted in to stone cells, encircling the vascular cylinder was formed. Some of the outermost cortical cells were also converted in to stone cells. The central region is occupied by wide pith, composed of thin-walled, circular to isodiametric parenchymatous cells, some of which were pitted. The secondary growth started in the usual manner. The cork cambium raised in the outermost or the second layer of the cortex giving raised to the cork towards the outer and phlloderm towards the inner side Fig-7-8.-Powder microscopy of leaf of *C.zeylanica* showed Calcium oxalate crystal- it occur as cluster in the cell of mesophyll and as prism in a sheath of cells around the fiber. Fig-13 Glandular trichome Fig-14, Anomocytic stomata present on upper epidermis, Simple parenchyma cells were found numerously and Stone cells were also found Fig-15-20. Powder microscopy of stem of *C. zeylanica* showed Wood element-xylem vessel with numerous bordered pitted thickening, Xylem fibers- large number of thick walled, elongated fibers mostly in groups. The walls of a few fibers show pitted thickening, Calcium oxalate crystal-large number of big elongated prism either entire or in fragments, some may also appear cubical in form, prisms are found scattered all over Cork cells- thin walled, some colourless and other brown and Sieve tube Fig 12-14.Powder microscopy of root of *C. zeylanica* showed Parenchyma-Thick walled cells containing oil globules and minute acicular raphides Fig-20, Wood element- Vessels with boarded piths, scalarriform and spiral thickening Fig -21-22.

Transverse section of root of *C. zeylanica* showed a single layered epidermis, some of which elongated to form unicellular hairs. The epidermis was followed by 2-3 layered parenchymatous cortexes. The endodermis was distinct with casparian dots on the anticlinal walls. The pericycle was single layered and encloses a triarch stele. The phellogen arises in the epidermis Fig-9-11. In determination of leaf constant it revealed that stomata were present on upper epidermis of leaf having average stomatal number-9, stomatal number range- 8-10, average stomatal index-17.75 and stomatal index range-16-20 Table-1. The leaf had average palisade ratio 1:7.95 Table-2. And average vein-islet number is 58.4 having vein –islet range-50-63 Table -3.


Fig- 1


Fig- 2


Fig- 3


Fig-4


Fig-5


Fig-6

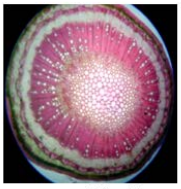


Fig-7

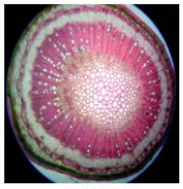


Fig-8

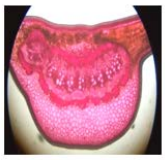


Fig-9

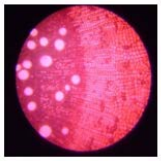
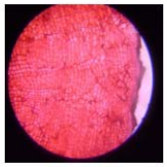


Fig-10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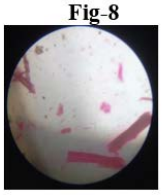
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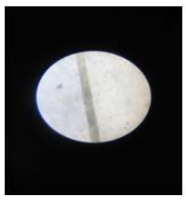
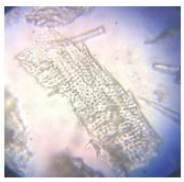


Fig 13


Fig-14


Fig-15


Fig-16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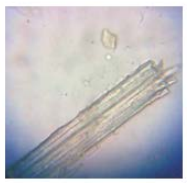
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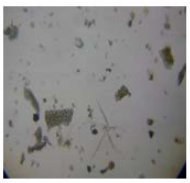
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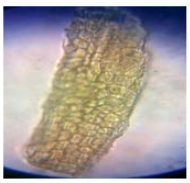
Fig- 20

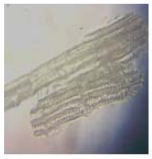
Fig-21


Fig-22:

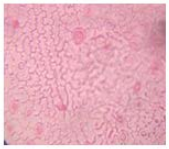
Fig-23

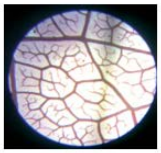
Fig-24

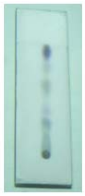

Fig-24

Fig-25

Table 1: Det[n] of stomatal number and stomatal index on upper epidermis of *C. zeylanica*

Field number	Number of stomata Per 1 sq. mm	Number of epidermal cells per 1 sq. mm	Stomatal index
1	10	45	18
2	8	41	16
3	8	32	20
4	10	47	17

Table 2: Determination of palisade ratio of *C. zeylanica*

Sl. No	Epidermal cells	Palisade cells	Palisade ratio
1	4	35	1: 8.75
2	4	41	1: 10.25
3	4	37	1: 9.25
4	4	21	1: 5.25
5	4	34	1: 8.5
6	4	22	1: 5.5

Table 3: Determination of vein-islet number of *C. zeylanica*

Sl. No.	No of vein-islet per square mm
1	57
2	62
3	50
4	60
5	63

Table 4: Determination of ash values of *C. zeylanica.*

Expt. No.	Weight of drug (g)	Weight of total ash(g)	% Weight of total ash	Mean (%)
1	2gm	0.06g	3.0%	3.1%
2	2gm	0.065g	3.2%	
Expt. No.	Weight of drug(g)	Weight of water soluble ash(g)	%Weight of water soluble ash	Mean (%)
1	2g	0.01g	0.5%	0.5%
2	2g	0.015g	0.5%	
Expt. No	Weight of drug (g)	Weight of acid insoluble ash(g)	% Weight of acid insoluble ash	Mean (%)
1	2g	0.01g	0.5%	0.5%
2	2g	0.01g	0.5%	

Table 5: Determination of extractive values

Types of extractive	Percentage (w\w)
Water soluble extractive	3.8%
Ethanol soluble extractive	10.18%

Table 6: Physical characters of *C. zeylanica* extracts

Sl. No.	Type of Extract	Colour	Odour	Consistency	Extractive Value
1	Pet. ether Extract(60^0 – 80^0C)	Light yellow	Characteristics	Greasy	2.2%
2	Chloroform Extract	Light Brown	Characteristics	Sticky	1.2%
3	Ethylacetate Extract	Light Brown	Characteristics	Sticky	1.3%
4	Methanolic Extract	Light Green	Characteristics	Greasy	6.3%
5	Ethanol	Light Brown	Characteristic	Greasy	
6	Water	Light Yellow	Characteristic	Greasy	

Table 7: Qualitative phytochemical screening of various extract of *C. zeylanica*

Sl. No.	Phytochemical Test	Pet. Ether (60^0 -80^0 C)	Chloroform	Ethyl acetate	Methanol	Ethanol	water
I	Test For Alkaloids -						
a.	Mayer's Test	Absent	Present	Present	Present	Present	Present
b.	Wagner's Test	Present	Present	Present	Present	Present	Present

c.	Hager's Test	Present	Present	Absent	Absent	Absent	Present
II	**Test for Carbohydrates and Glycosides –**						
a.	Molish's Test	Absent	Absent	Absent	Absent	Absent	Absent
b.	Fehling's Test	Absent	Absent	Absent	Absent	Present	Absent
c.	Barfoed's Test	Absent	Absent	Absent	Absent	Absent	Absent
III	**Test for Saponin -**						
-	Foam Test	Present	Absent	Present	Absent	Present	Present
IV	**Tests for Phenolic Compounds and Flavanoides -**						
a.	Ferric chloride Test	Absent	Absent	Absent	Absent	Absent	Absent
b.	Lead acetate Test	Absent	Absent	Absent	Absent	Absent	Absent

Table 8: Fluorescence analysis of powder drug with different reagents

Sl. No.	Reagent + Drug	Day light	UV Light Short	UV Light Long
1	Untreated Powder	Light green	green	Dark green
2	Powder + Sat. Picric Acid	Yellowish green	Dark Green	Deep Brown
3	Powder + Nitric Acid	Brown	Deep Green	Black
4	Powder + 1N HCL	Brownish Green	Green	Black
5	Powder + Conc. H_2SO_4	Dark Brown	Deep Green	Black
6	Powder + Glacial acetic Acid	Yellowish Brown	Green	Black
7	Powder + 1N NaOH	Brownish Green	Green	Black
8	Powder + Iodine	Pale Green	Deep Green	Black
9	Powder + Ferric Chloride	Light Green	Deep Green	Black

Table 9: Fluorescence characteristics of different Extract

Sl. No.	Types of Extract	Day Light	UV Light Short	UV Light long
1	Petroleum Ether ($60^0 – 80^0$ C) Extract	Yellow	Light Yellow	Black
2	Chloroform Extract	Green	Deep Green	Black
3	Ethyl acetate Extract	Light Green	Green	Deep Green
4	Methanolic Extract	Light Green	Green	Deep Brown

Table 10: TLC results of methanolic extract of *C. zeylanica*

Sr. No	Extracts	Solvent System	No of Spots	Colour of spots	Rf Values
1	Methanolic extract	Chloroform: Methanol (3:2)	6	pink	0.27
				pink	0.3
				Pink	0.38
				Grey	0.6
				Grey	0.83
				pink	0.94
2	Methanolic extract	Methanol:Ethyl acetate(3:2)	7	Grey	0.25
				Grey	0.33
				Pink	0.5
				pink	0.62
				Grey	0.69
				Grey	0.8
				pink	0.93

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