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Pharmacognostic, Phytochemical and Pharmacological Evaluation of Ipomoeapes-Capraelinn. R. Br

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ABSTRACT: The main objective is to pharmacognostic, phytochemical and pharmacological evaluation of ipomoeapes-capraelinn. R. BR. The pharmacognostical, phytochemical profile including preliminary phytochemical screening, analysis and quantification of kaempferol in the plant extract by HPLC and pharmacological studies were carried out for the in vitro anti-inflammatory activity and invivo antineuropathic pain activity in vincristine induced neuropathy model. Pharmacognostical studies establishes macroscopical, microscopical and analytical standard and characterization of leaves of this plant. These evaluation can be used further as identification and standardization parameters of the leaves. Preliminary phytochemical screening on the leaves of Ipomoea pes-caprae confirms the presence of tannins, flavonoids, sterols, carbohydrates and saponins. The quantification of total phenolic, flavonoid and tannin was determined for ethanolic extract of leaves of Ipomoea pes-caprae which were found to be 22.27 mg/g, 217.1 mg/g and 157.259 mg/g respectively. It proves the significant concentration of these phytoconstituents in the ethanolic extract of Ipomoea pes-caprae. HPLC studies of IPC extract and standard kaempferol revealed that the presence of kaempferol in this extract and the t_R of standard and sample were found to be 5.87 mins and 5.88 mins respectively. Kaempferol content present in this extract was quantified as0.034%. So for kaempferol has not beenreported fromthe leaves of this plant. This is the first time we have reported the presence of this compound from the leaves of this plant. IPC showed significant radical scavenging activity for Hydrogen peroxide, DPPH, Total antioxidant and reducing power assay. This may be due to the presence of flavonoid constituent like kaempferol. IPC extract exhibited significant anti inflammatory activity in the in vitro membrane stabilization model (IC_{50} 944.8 µg/mL) when compared with standarddrug Diclofenac sodium (IC_{50} 836.2 µg/mL).

KEYWORDS: Extraction, Phytochemical screening, Drug, Medicine, Phytconstituents

I. INTRODUCTION:

Ever since the birth of mankind there has been a relationship between disease and plants. There is no record that people in prehistoric times used synthetic medicines for their ailments. But they tried to make use of the things, which were easily available, i.e. the plants and animals. They started using plants and found that majority of plants were suitable as food, where as other were either poisons or medicinally useful. By their experience this knowledge of herbal remedies was transferred to generation as folk medicine. So, the history of herbal medicine as old as human history.

Plants have been used for medicinal purpose long before and recorded in the history. For example ancient Chinese and Egyptian papyrus writing describe medicinal plants and their uses. Indigenous culture (eg: African and Native American) used traditional medicinal systems eg: ayurvedic and traditional Chinese medicine in which herbal therapies were used systematically. Scientists found that people in different parts of the globe tended to use the same or similar plants for the same purpose.

In the early 19th century when method of chemical analysis first become available scientists started extracting and modifying the active ingredients fromplants. Later chemists began making their own version of plant compounds, beginning the transition from raw herbs to synthetic pharmaceuticals. Over time, the use of herbal medicines declined in favour of pharmaceuticals.

Recently the WHO estimated that 80% of people worldwide rely on herbal medicine for some aspect of their primary healthcare. In the last twenty years in the United States, increasing public dissatisfaction with the cost of prescription medications combined withaninterest inreturningto natural or organic remedies has led to an increase in theuse ofherbalmedicines.In Germany roughly 600 to700 plant based medicines are available and are prescribed by approximately 70% of

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Germanphysicians.

Neuropathy is a collection of disorders that occurs when nerves of the peripheral nervous system (the part of the nervous system outside of the brain and spinal cord) are damaged. The conditionis generally referred to as **peripheral neuropathy** and it is most commonly due to damage of nerve axons. Neuropathy usuallycauses pain and numbness in the hands and feet. It can result from traumatic injuries, infections, metabolic disorders and exposure to toxins.

Neuropathy can affect nerves that control muscle movement (motor nerves) and those that detect sensations such as coldness or pain (sensory nerves).In some cases of autonomic neuropathy-it can affect internal organs, such as the heart, blood vessels, bladder or intestines. Pain fromperipheral neuropathyis oftendescribed as a tingling or burningsensation. There is no specific lengthof time that the pain exists, but symptoms often improve with time ─especially if the neuropathy has an underlying condition that can be cured. The condition is often associated with poor nutrition. A number of diseases and pressure or trauma but many cases have noknown reason and other wise called as idiopathic neuropathy.

Neuropathy may also be categorized based on functional classification (motor, sensory, autonomic or mixed)or the type of onset (acute ─ hours or days, sub- acute ─weeks or months or chronic ─months or years). The most common form of neuropathy is (symmetrical) peripheral polyneuropathy, which mainly affect the feet and legs on both sides of the body.

II. MATERIALS AND METHODS:

MACROSCOPY OF THE LEAVES

Organoleptic characters such as colour, odour, texture, shape, size, apex, venation and arrangement of the leaves were studied for this plant.

Collection of plant material: Plants were collected from Ramnadand identified by taxonomist.

Specimen collection: Petiole and leaf were fixed in FAA solution (70% ethyl alcohol, formalin and acetic acid in the ratio of 90 mL:5 mL: 5 mL). The materials were kept in the fluid for three days, after which they were washed in water and dehydrated with tertiary butyl alcohol. Paraffin waxwas filtered and the specimens were embedded in wax for sectioning.

Sectioning: Transverse sections of petiole and leaf were taken using microtome andstained with toluidine blue. All sides,after stainingintoluidine blue were dehydrated by employing graded series of ethyl alcohol (70 %, 90%, 100% alcohol) and xylol- alcohol (50-50) and passed through xylol and mounted inDPX mountant.

Clearing of leaves: Clearing of leaves for studying stomatal number and stomatal index was done by using 5% sodium hydroxide with chlorinated soda solution supplemented with gentle heat. Quantitative microscopy of leaves was carried out was studied as per the procedure given standard procedure. Photomicrographs were taken with the help of Nikon Eclipse E200 Microscope. Powder microscopy of the leaf powder was also studied.

PRELIMINARYPHYTOCHEMICALSCREENING

All the extracts were subjected to qualitative chemical analysis. The various chemical tests were performed on the extracts for the identification such as sterols, terpenoids, flavones, anthraquinones, sugars glycosides, alkaloids, quinones, phenols and tannins.

Preparation of ethanolic extract of Ipomoeapes-caprae

The shade dried and coarsely powdered leaves of Ipomoeapes-caprae was defatted with petroleum ether for three days by defatted marc was extracted with 70% ethanol by triple maceration and filtered. The filtrate was concentrated under reduced pressure to obtain a solid residue which was dark green in colour.

III. RESULT AND DISCUSSION:

Macroscopic studies of leaves
Colour – Dark green colour
Odour- Characteristic odour
Texture- Coarse
Venation-Pinnate Leaf arrangement -Alternate Leaf type- Simple
Margin- Lobed
Shape- Eliptic(oval) **Microscopic studies of the leaves Leaves**

The leaves has broad and thick midrib and thin lamina. The midrib consist of wide adaxial part, bowel shaped and thickabaxial part. It is 1.1 mm, adaxial cone is 150 x250 µm in size and the abaxial part is 1.8 mm wide.

The epidermal layer around the midrib is intact and includes small, thick walled- squanish cells. The ground tissue is parenchymatous; the cells are angular or circular and compact.The cells

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in the adaxial cone are collenchymatous. The palisade zone extends up to the lateral shoulders of the adaxial cone.

The vascular strand is shallowly saucer shaped. It is 300 µm thick and 1 mm broad.The vascular strand consists of three or four separate units of bi-collateral bundles. In each bundle occur short, radial lines of three or four xylem elements. The elements are wide, angular and thick walled. The Metaxylem elements are 40µm.

The phloem elements are small, thick walled and darkly stained. They are present in bothadaxial and abaxial sides of the vascular bundle. The abaxial phloem unitsare thickand circular masses.The abaxialunitsaresmall clusters.

Lamina

The lamina is bifacial (heterofacial), amphistomatic and mesomorphic. The lamina is 340 µm thick. The adaxial epidermis is thin and the cells are narrow and cylindrical. The abaxial epidermis is thickand it consists ofrectangular-oblongcells. The stomata are slightly raised above the epidermal surface. Themesophyll tissuesare differentiated into adaxial band of vertical filaments; the filament is two or three cells and looselyarranged. The spongyparenchyma cells are shortand cylindrical and forms a reticulate system of aerenchyma**.**

Epidermalcellsandstomata

Thestomataandepidermal cells areobservedintheparadermal (parallel tothe epidermal surface) sections.Theepidermal cellsarepolygonal withstraight,thickanti clinal walls. Stomata of both adaxial and abaxial sides are paracytic type. The stoma has two subsidiary cells, one or either side and parallel guard cells. The guard cells are 20 x 40 µmin size.

Glandulartrichomes

Peltate type of glandular trichomes are common on the epidermal layer. The gland has a short one-celled stalkand circular 8- celled flat head. The gland is 30 µm in diameter.

Venation

The venation is densely reticulate. The secondary and tertiary veins are uniformly thin. The veinislets are wide and polyhedralin outline.

Table 1: Quantitative microscopical parameters of the leaf of Ipomoeapes-caprae

S. No.	Parameters	Values* Obtained
1	Stomatal number	52.43
2	Stomatal index	14.65
3	Veinislet number	8.92
4	Vein termination number	11.28

Fluorescence analysis

The organic molecules absorb light usually over a specific range of wave length and many of them emit such radiations. So if the powder is treated with different chemical reagents and seen in the UV chamber, different colours will be produced. The results of fluorescence analysis revealed the purity of this plant material

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Table: 2 Fluorescence Analysis of extracts of Ipomoeapes-caprae

Extracts	Consistency	Colouring Day Light	Colour under UV Lamp	
			366 nm	254nm
Petroleum Extract	Semisolid	Yellow	Orange	Yellow
Ether Extract	Semisolid	Greenishbrown	Green	Greenish brown
Chloroform extract	Semisolid	Brownishgreen	Orange	Orange
Ethanol Extract	Semisolid	Lightgreen	Green	Orange
Methanol Extract	Semisolid	Green	Darkgreen	Orange
Aqueous Extract	Semisolid	Brown	Brownish green	Darkgreen

Physical parameters

The result of the ash value, acid insoluble ash value and water soluble ash value presented. Total ash of the drug is inclusive as well as physiological ash. Physiological ash and non-physiological ash. Physiological ash is derived from the plant tissues. While non-physiological ash consists of residue of the extraneous matter(such as sand, soli etc),adhering totheherb itself.Many a timethe crude drug are admixed with various mineral substance like sand, soli, calcium oxalate, chalk powder or other drugs with different inorganic contents. For determining ash, the powdered drug is incinerate as to burnout all organic matter. Ash value is criterion to jude the identity or purity of crude drugs. Total ash usually consists of carbontes, oxides, phosphates, silicatesand silica. Adhering dirt and sand maybe determined by acid insoluble ash.

Table: 3 Analytical parameters of Ipomoeapes-caprae

S. No	Parameters	Values*expressedas%
1.	**Foreign organic matter**	0.01± 0.12
2.	**Moisture content**	11.85 ± 0.46
3.	**Ash values**	
	Total ash	8.01 ± 0.35
	Acid insoluble ash	4.32 ± 0.47
	Watersolubleash	4.2±0.90
	Water insoluble ash	7.8 ± 0.91

Extractive values

The result of the extractive values are presented**.** The extracts obtained by exhausting crude drug are indicative of approximate measure of their chemical constituents. Takinginto considerationthe diversityinchemical nature and properties of contents of drugs, various solvents are used for determinations of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substance desired. Highest extractive value was obtained for the hydro-ethanolic extractive of this plant (15.76%). It indicates that therapeutically viable active principles are predominantly present in hydro-ethanolic extract. Hence this
Extract was chosen for phytochemical and pharmacological studies.

Table: 4 Analytical parameters–Extractive values of Ipomoeapes-caprae

S. No	Parameters*	Values*expressedas%
1.	**Extractive Values**	
	Petroleum extract	1.59± 0.48
	Chloroform extract	5.59± 0.04
	Ethanol extract	3.19 ±0.81

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	Hydro ethanolic extract (Ethanol: Water,70;30)	15.76 ±0.71
	Methanol extract	13.57 ± 0.01
	Aqueous extract	9.67± 0.74
2.	**Foaming index**	Less than100
3.	**Swelling index**	**Expressed as ml**
	Initial volume	3.6 ± 0.20
	Finalvolume	10.3 ± 0.97

Preliminary phytochemical studies

Preliminary phytochemical study was performed for various extracts and powdered leaf material of Ipomoea pes-caprae.

Tabe: 5 Preliminary phytochemical screening for the leaf powder of Ipomoeapes-caprae

S.NO	TEST	RESULTS
1.	**TESTFORSTEROLS**	
	a. Salkowski's test	+
	b. Libermann- burchard's test	+
2.	**TESTFORCARBOHYDRATES**	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
3.	**TESTFORPROTEINS**	
	a. Millon's test	+
	b.Biurettest	+
4.	**TESTFORALKALOIDS**	
	a. Mayer's reagent	+
	b. Dragendroff's reagent	+
	c. Hager's reagent	+
	d. Wagner's reagent	+
	e.Test for Purine group (Murexide test)	-
5.	**TESTFORGLYCOSIDES**	
	a. Anthraquinone glycosides	+
	i) Borntrager's test	+
	ii) Modified Borntrager's test	+

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	b. Cardiac glycosides	
	i) Keller Killiani test	-
	c. Cyanogenetic glycosides	-
6.	**TEST FOR SAPONINS**	+
7.	**TEST FOR TANNINS**	
	$Fecl_3$test	+
8.	**TEST FOR FLAVONOIDS**	
	a. Shinoda test	+
	b. Alkali test	+
	c. Acid test	+
9.	**TEST FOR TERPENOIDS**	+
10.	**TEST FOR VOLATILE OILS**	-
11.	**TEST FOR MUCILAGE**	-

Table: 6 Preliminary phytochemical screening for the various extracts of leaf Powder of Ipomoeapes-caprae

S. No.	Chemical Test	Hexane extract	Petroleum ether extract	Chloroform	Methanolic extract	Ethanolic extract	Aqueous extract
1.	Terpenoids	+	+	+	+	+	-
2.	Flavonoids	-	-	-	+	+	+
3.	Phytosterols	+	+	+	+	+	-
4.	Anthraquinone Glycosides	-	-	-	+	+	+
5.	Cardiac Glycosides	-	-	-	+	+	-
6.	Sugars	-	-	-	+	+	+
7.	Alkaloids	-	-	-	+	+	-
8.	Quinones	-	-	-	-	-	-
9.	Phenols	-	-	-	+	+	+
10.	Tannins	-	-	-	+	+	+
11.	Saponins	-	-	-	+	+	+
12.	Proteins&free aminoacids	-	-	-	+	+	+

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The rsults of the preliminary phytochemical studies showed the presence of terpenoids, tannins, phyto sterols, alkaloids, glycosides, flavonoids and saponins.

Quantitative estimation of phytoconstituents

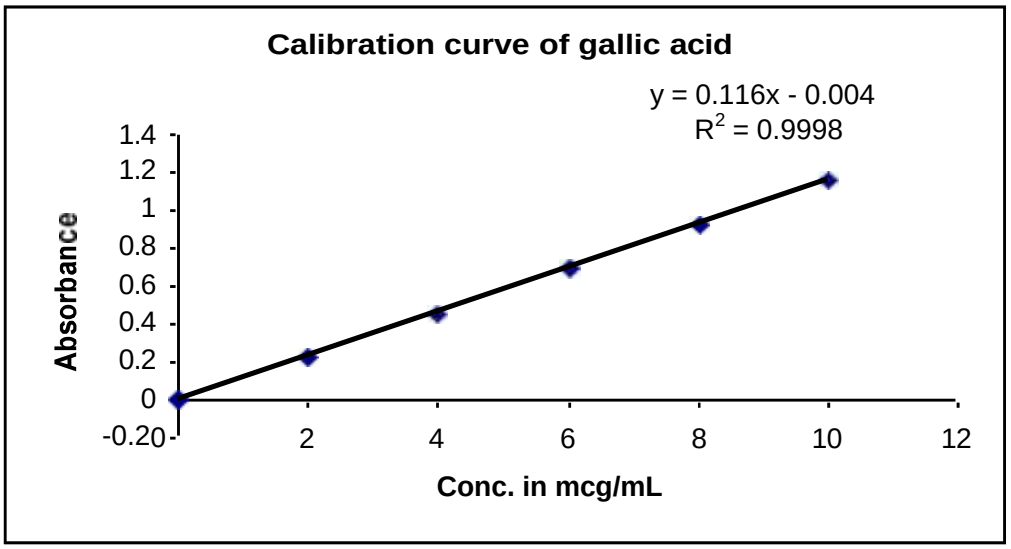
Total Phenolic Content

In the present study, total phenolic content present in the extract was estimated using modified Folin- ciocalteau method. Values are expressed as gallic acid equivalents. Total phenolic content for ethanolic extractof Ipomoea pes-capraewas found to be **22.27±0.19 mg/g.** The linear regression equation was found to be y=0.116x-0.004 while the correlationwas found to be 0.9998.

Table: 7 Total phenolic content in ethanolic extract of Ipomoeapes-caprae

S.NO	Conc. of gallic acid in µg/ml	Absorbance	Conc. of ethanolic extract in µg/ml	Absorance	Amount of Total phenolic content in terms mg GAE/g of extract*
1	2	0.229 ± 0.010	50	0.138±0.001	23.97±0.25
2	4	0.452 ± 0.006	100	0.243±0.001	20.98±0.13
3	6	0.695 ± 0.005			
4	8	0.918 ± 0.031			
5	10	1.162 ± 0.028		**Average**	22.27±0.19

Fig. 1 : Calibration graph of gallic acid



Phenolics are the most widely distributed secondary metabolite in plant kingdom. These groups of compounds have much attention as potential natural antioxidants in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenolic compounds are mainly due to their redox, hydrogen donating properties and singlet oxygenquenchers.

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Estimation of Flavonoid:

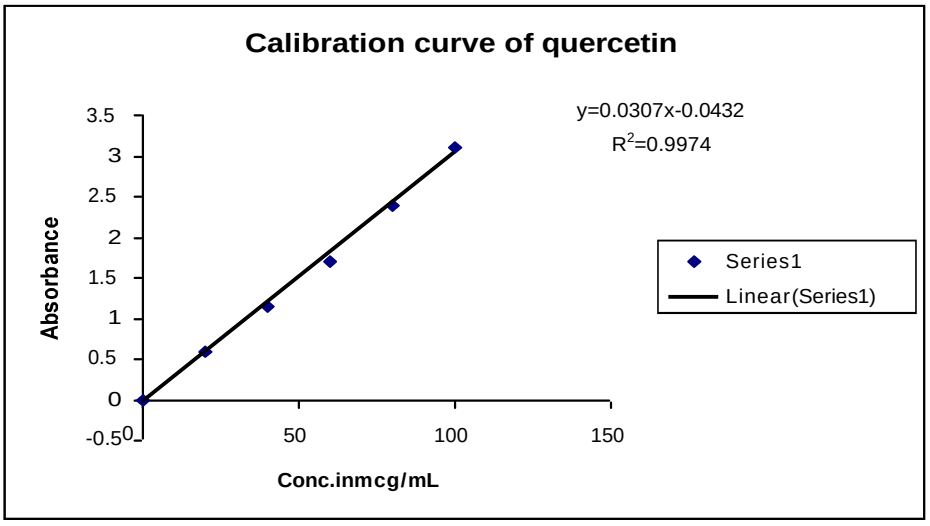
Flavonoidcontents were determined bycolorimetric method using $AlCl_3$. The flavones and flavonols react and form more stable complexes with Aluminium chloride. The amount of flavonoid was considered as the important index for evaluating the biological activity of drugs.

Total flavonoid content for ethanolic extract of leaves of Ipomoea pes-capare was found to be217.1±0.37 mg/g respectively. The linear regression equation was found to be y = 0.0307x-0.0432 while the correlation was found to be 0.9974.

Table 8: **Total flavonoid content of ethanolic extracts of Ipomoea pescaprae**

S.No.	**Conc. of quercetin in µg/Ml**	**Absorbance**	**Conc. of ethanolic extr act in µg/Ml**	**Absorbance**	**Amt of total flavonoid content in terms of mg quercetin equivalent/ g of extract**
1	20	0.589±0.01	100	0.26±0.001	202.2±0.96
2	40	1.151±0.04	200	0.66±0.002	232.1±10.79
3	60	1.710±0.09			
4	80	2.390±0.03			
5	100	3.112±0.03		**Average**	217.1±0.37

Fig. 2**: Calibration curve of Quercetin**



Flavonoids are the most diverse group of polyphenols and are consist of a basic C_6-C_3-C_6 flavone skeleton. Six classes of flavonoids are widespread in higher plant, and include the chalcones, flavanones, flavandiols, flavones, anthocyanins, catechins, and condensed tannins. Its antioxidative activity is dependent on the chemical structure, such as the number of hydroxyl groups substituted onthe B ring.

Total Tannin Content

Total tannin determination is carried out by spectrophotometry after oxidation of the analyte with the Folin–Denis reagent in alkaline medium.

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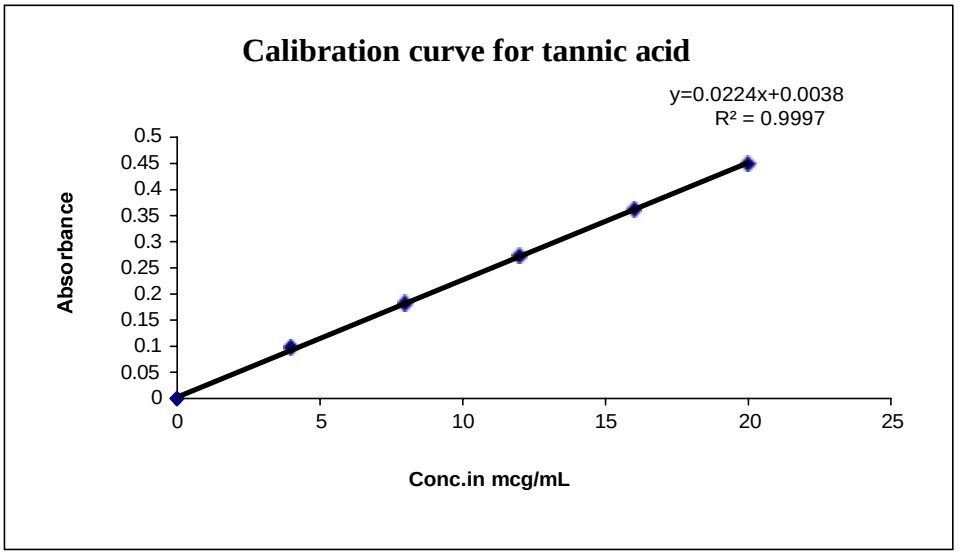
This method is based on a redox reactionand other reducing agents in the samples. Total tannin content for ethanolic extract of leaves of Ipomoeapes-caprae was found to be **157.25±3.99 mg/g** respectively.

Table: 9 Total tannin content in ethanolic extracts of Ipomoeapes-caprae

S. No.	Conc. of Tannic acid in µg/Ml	Absorbance	Conc. of ethanolic extract in µg/ml	*Absorbance	*Amt of total Tannin content in terms mg tannic acid equivalent/g of extract
1	20	0.589 ± 0.01	10	0.03±0.000	124.3±0.46
2	40	1.151 ± 0.04	20	0.05±0.0005	114.2±0.28
3	60	1.710 ± 0.09	40	0.07±0.0005	75.81±0.07
4	80	2.390 ± 0.03			
5	100	3.112 ± 0.03			
				Average	157.25±0.99

The linear regression equation was found to be y = 0.022x + 0.003. The amount of tannin content present in the ethanolic extract of Ipomoea pes-caprae was found to be **157.25±0.99mg/g** respectively.

Fig 3: Calibration curve for Tannic acid



It is known that plant tannin can be precipitated by many chemical reagents and these precipitation techniques have become the tool for the estimation. Tannins are antioxidants phytoconstituents.

PHARMACOLOGICALEVALUATION

Invitro antoxidant activity

Antioxidants obtained from plant are of greater benefit in comparison to synthetic one. It

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protects against free radicals and they are therefore essential in obtaining and preserving good health.

Hydrogen peroxide scavenging activity assay:

The ethanolic extracts of Ipomoeapes-caprae was also evaluated for hydrogen peroxide scavenging activity assay. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with $Fe^{2+,}$ and possibly Cu^{2+} ions toform hydroxyl radical and this may be the origin of many of its toxic effects.

$H_2O_2+Cu+orFe^{2+} \rightarrow Cu^{2+}orFe^{3+}+OH+\cdot OH-$

Table: 10 Percentage inhibition of EEIPC by hydrogen peroxide method

S. No.	Conc. In µg/mL	Percentage inhibition by Standard ascorbic acid	Percentage inhibition By EEIPC
1	40	42.72±0.68	37.2±0.34
2	80	71.08± 0.89	53.83±1.14
3	120	82.7± 0.96	66.9±0.95
4	160	85.45± 0.86	71.0±0.83
5	200	92.42± 0.65	77.2±0.82
	IC_{50}	**71.32µg/mL**	**97.28µg/Ml**

DPPH radical scavenging activity: The scavenging of the DPPH radical by hydrogen donating antioxidant is characterized by a rapid decline in the absorbance at 517 nm. The rapid reaction between antioxidants and DPPH occurs with the transfer of the most labile H atoms to the radical, while the subsequent slow step depends on the residual H-donating capacity of antioxidant degradation products. The antioxidants react with DPPH and convert it to 1, 1-diphenyl-2-picryl hydrazine with decolouration (from deep violet to light yellow).

Table 11: DPPH radical scavenging assay of IPC extract

S. No.	Conc. in µg/mL	Percentage inhibition by ascorbic acid	Percentage inhibition by Extract
1	40	76.44±1.68	65.86±0.55
2	80	81.22±1.22	72.77±0.69
3	120	90.33±2.38	82.75±0.32
4	160	93.45±1.47	85.92±0.87
5	200	95.68±2.02	89.24±0.63
	IC_{50}	**40.67µg/mL**	**56.52µg/mL**

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IV. SUMMARY AND CONCLUSION:

Many unknown and lesser know plants are used in folk and tribal medical practice as a source of medicine. The medicinal values of these plants are not brought in to the lime light of scientific world. One such plant is Ipomoeapes-caprae. Keeping this in view an attempt was made to bring the lime light of the commonly occurring plant Ipomoea pes-caprae. This dissertation work covers an extensive study on the leaves of Ipomoea pes-caprae also known as beach morning glory belongs to the family convolvulaceae.

Pharmacognostical studies establishes macroscopical, microscopical and analytical standard and characterization of leaves of this plant. These evaluation can be used further as identification and standardization parameters of the leaves.

Preliminary phytochemical screening on the leaves of Ipomoea pes-caprae confirms the presence of tannins, flavonoids, sterols, carbohydrates and saponins.

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