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Pharmacognostic evaluation and *in-vitro* antioxidant potential of Bhovara (*Ipomoea cairica* (L.) crude extracts using various solvents

Research Article

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Abstract

In Ayurveda *Ipomoea cairica* (L.) Sweet has its own importance to treat various health problems. It is tropical plant and a wild relative of sweet potato (*Ipomoea batatas*), which is considered as one of China's most invasive alien species. *Ipomoea cairica* (L.) is a climber plant frequently used as a medicinal herb in India and Western Asia. In this study two different solvents like water and ethanol were used for the extraction of *Ipomoea cairica* (L.). The highest extraction yield was obtained by using Ethanol. The antioxidant activity, total phenolic content, and total flavonoid content of aqueous and Ethanolic extracts were investigated using various *in vitro* assays. Study revealed that Ethanolic extract showed the highest antioxidant potential by DPPH (2,2- diphenyl-1-picrylhydrazyl) radical scavenging activity. The same extract also exhibited the highest phenolic content (22.03±1.21 mg Gallic acid equivalent/g dried *Ipomoea cairica* L.) and the highest flavonoid content (8.54±0.42 mg Quercetin equivalent/g of *Ipomoea cairica*). These results indicate that ethanolic extract of *Ipomoea cairica* (L.) can be used to reduce oxidative stress.

Key Words: Extraction, *Ipomoea cairica (L.),* Antioxidants, Total phenolic, Total flavonoid, TLC.

Introduction

Ayurveda is an Indian traditional system of medicine being practiced from thousands of years. It became a vital part of the Indian culture. Use of herbal medicine for alleviation of diseases and betterment of mankind is well practiced from centuries. These are estimated to by around 25,000 plant based formulations, used in folk medicine and known to rural communities in India. Because of this current trend of increasing use of herbal medicine and their growing popularity, the search for drug and pharmacognostic evaluation of plant have accelerated in recent years. (1,2)

``*Ipomoea* is the largest genus in the flowering plant, with over 500 species. (3,4) The family is widespread in both tropical and subtropical areas. *Ipomoea cairica* (L.), commonly known as rail road wine or morning glory is an environmental weed, found in waste land areas (5) which is traditionally known to possess various therapeutic activity such as larvicidal, (6) antifungal, anti-inflammatory, hepatoprotective,(7) antitumor,(8) antimicrobial activity (9) due to presence of various phytochemicals such as flavonoids, alkaloids, tannins, saponins, phenols, amino acids, glycosides, steroids anthraquinones, and lignans. The phytonutrient like flavonoid content shows anti-inflammatory effect and protect the body cells from oxidative damage which leads to various disease. (10) Phenolic and flavonoid compounds are important plant constituent with redox properties, which are responsible for antioxidant activity. (11,12)

Antioxidants which are immensely present in *Ipomoea cairica* (L.) work by significantly slowing or preventing the oxidative damage from oxidative process caused by free radicals. In continuation of our effort in this study the pharmacognostic evaluation and *In vitro* antioxidant potential of *Ipomoea cairica* (L.) Using different solvents has been evaluated. The alcoholic and aqueous extracts of *Ipomoea cairica* (L.) is screened for their free radical scavenging properties using ascorbic acid as standard antioxidant by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay. (13, 14)

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Materials and Methods

Materials

All the chemicals and reagents were purchased from Sigma-Aldrich Ltd. and Quercetin, Folin-ciocalteu reagent. Gallic acid, Ascorbic acid, DDPH reagent, purchased from HIMEDIA.

Method

Collection of the plant

The plant was collected from Local area of Belagavi during the month of September–October. Authentication of collected plant was done by ICMR-NITM (National institute of traditional medicine), Belagavi and the herbarium specimen of the same has been deposited in ICMR herbaria with accession number RMRC-1601.

Processing of plant material

The fresh plant material was washed, shade dried, powdered and stored in air tight container until further use.

Pharmacognostic evaluation

Plant material was evaluated for various pharmacognostic characters like morphology, microscopy, physicochemical and phytochemical evaluation. (15, 16)

Morphology characteristics

Plant was subjected for organoleptic examination and studied for various morphological characters; Fig.1. (2)

Powder microscopy

Various microscopical characters of the plant material were studied using trinocular microscope as per standard procedure. (3) The characteristic features of cell walls, cell content trichomes, fibers, vessels etc. have been studied in detail and shown in Fig.2. (13)

Physicochemical studies

Ash value

Total ash, Acid insoluble ash**,** Water soluble ash and Sulphated ash of *Ipomoea cairica* (L.) was evaluated by standard procedure. Results are tabulated in Table.1. (15, 16)

Total Ash value

About 2g of powdered drug was weighed accurately into a tarred silica crucible. Incinerated at 450°C. The crucible was cooled and weighed. Percentage of total ash was calculated with reference to air dried substance.

Acid insoluble ash

Ash obtained from the total ash was boiled with 25ml of dil. HCl for a few minutes than filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible and the insoluble matter obtained was washed with hot water and ignited. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated with reference to air dried substance.

Water soluble ash

Ash obtained from the total ash was boiled with 25 ml of distilled water for five minutes and filtered through an ash less filter paper then filter paper was transferred into a tarred silica crucible and Incinerated at 450°C.The crucible was cooled and weighed the difference in weight was the water-soluble ash and the percentage of water-soluble ash was then calculated.

$$\%Ash\ value = \frac{(Z - X)}{Y} \times 100$$

X= weight of empty dish
Y= weight of the drug taken
Z= weight of the dish + ash (after complete incineration)

Moisture content

The presence of moisture was determined by loss on drying method as per standard procedure. Method includes weighing out 2gm of powdered drug and placed in thin porcelain dish and heated at 105°C in hot air oven, up to two repetitive weights do not differ by more than 0.5mg. Cool in desiccator and weigh. Table.1 The loss in weight is usually considered as moisture level in powdered drug. (5)

$$Loss\ on\ drying = \frac{Loss\ in\ weight\ of\ the\ sample}{Weight\ of\ the\ sample} \times 100$$

Extractive value

Extractive value of plant material was performed using water, ethanol and ether as solvent as per standard procedure. (1, 4) The extractive value is calculated by the percentage of weight of the extract with reference to the air-dried drug; Table.1.

$$Extractive\ value = \frac{Weight\ of\ extract}{Weight\ of\ dried\ plant} \times 100$$

Preparation of plant extract

The coarse powdered plant material (40g) was extracted by using two different solvents i.e. water and ethanol (Table.2) initially plant material was subjected for solvent extraction by Cold maceration for 7 days. Then both the extracts were filtered and concentrated under reduced pressure using a rotary evaporator; and stored in an air tight container at cold temperature for future usage.(17)

Phytochemical evaluation

The plant possesses various bioactive compounds, (2) both aqueous and alcoholic extracts of *Ipomoea cairica* (L.) were tested for various components by their specific tests and the results were tabulated in Table.3. (3,4)

Thin layer chromatography (TLC)

Thin-layer chromatography is a simple and efficient method used to identify and quantify secondary metabolites in herbal extract, TLC analysis for both the extracts was carried out using silica gel 60 F254 (merck) plates as stationary phase. Both the extract samples and standard quercetin was prepared by dissolving in methanol then spotted on TLC plates. The solvent system taken was Toluene: Ethyl acetate: Formic acid (7:3:0.5) the chromatograph was developed in presence of aluminum chloride reagent and Rf value was calculated; Fig 4. (12)

Total flavonoid content (TFC)

The total flavonoid contents in crude extract was estimated by using Aluminum chloride colorimetric method with slight modification. Quercetin was used as standard. 10mg of Quercetin was dissolved in 10ml of ethanol further serial dilutions ranging from 20-100 µg/ml were prepared by using ethanol. 0.5 ml of resulting solution was pipetted out to this 1.5 ml of ethanol (95%) 0.1 ml of aluminum chloride (10%) 0.1ml of potassium acetate (1M) and 2.8ml of distilled water was added and

incubated at room temperature for half an hour and absorbance was measured at 415nm using UV-spectrophotometer sample solutions were prepared in same way as that of standard and content of total flavonoids was estimated by utilizing Quercetin standard calibration curve Fig 5. and TFC was expressed as mg QCE/gm dried extract of *Ipomoea cairica* (L.)*;* Table 4. (18, 19)

Total phenolic content (TPC)

The amount of total phenolics in both the extracts was determined with Folin-ciocalteu reagent method. Gallic acid was used as a standard and the TPC content was expressed as milligram of gallic acid equivalent per gram of dried extract of *Ipomoea cairica* (L.) (mg GAE/ gm). For this purpose, the calibration curve of gallic acid was plotted by mixing 1ml aliquots of 20, 40, 60, 80 and 100µg/ml of gallic acid solutions with 5.0 ml of Folin-ciocalteu reagent and 4.0 ml of sodium carbonate solution (75 g/l). Then samples (1mg/100ml) were prepared using methanol. 1ml of resulting sample solutions were mixed separately with same reagents. Tubes were covered with parafilm and allowed to stand for 30 min at room temperature for incubation and absorbance was measured at 765nm using UV-spectrophotometer; Table 4. (18)

Antioxidant activity

Antioxidant assay was performed as per standard procedure by utilizing DPPH Assay method. 39.4mg DPPH was dissolved in 1000ml of methanol. Free radical scavenging properties of the extract was estimated using ascorbic acid as a standard. Which is strong anti-oxidizing agent. It was prepared by dissolving 10mg of ascorbic acid in 10ml of methanol then the serial dilutions were made by taking 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml from above solution and made up the volume to 10ml using same solvent to get 2%, 4%, 6%, 8% and 10% of Ascorbic acid respectively.

Sample of aqueous and alcoholic extracts were also prepared in different concentration by fallowing sample procedure and 3ml of each solution was mixed with 1ml DPPH to produce 2%, 4%, 6%, 8% and 10% test sample solution. The prepared solutions of ascorbic acid and test sample were incubated in dark for half an hour then absorbance was measured at 517nm using UV spectrophotometer the percentage inhibition; Fig 7, and IC_{50} was calculated; Table 4. (20, 21)

Results

Pharmacognostic evaluation

Morphological characteristic

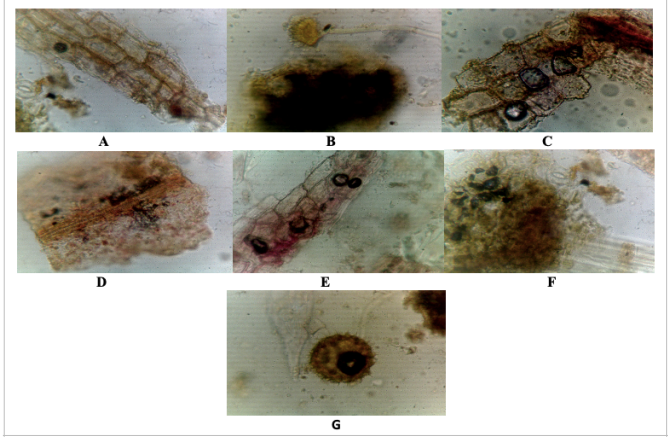
Ipomoea cairica (L.) is a vining, herbaceous, perennial plant with palmate leaves and large, showy white to lavender flowers. A species of morning glory, Leaves palmately 5 to 7-partite; segments elliptic - obovate or lanceolate, narrowed at both ends, retuse, mucronate at apex, glabrous. Flowers in 1 to 3-flowered cymes. Calyx- segments unequal, ovate, mucronulate, tuberculate on the back of outer ones. Corolla 6 -7 cm long, white or purple. Capsules 2 -celled, 4 -valved. Seeds pubescent.

Fig 1: *Ipomoea cairica* in its natural habitat

Powder microscopy

Various microscopical characters were studied and represented in Figure 2.

Fig 2: A. Epidermal parenchyma **B**. Glandular trichomes. **C**. Polygonal Epidermis. **D**. Reticulate xylem vessels. **E**. Stone cells **F.** stomata **G.** pollen grain



Physicochemical studies

Several Physicochemical parameters were studied for plant material namely; Ash content, Extractive value, and moisture content.

Table 1: Physicochemical parameters of plant *Ipomoea cairica* (L.)

Parameters	Values in % w/w
Total Ash	10%
Acid insoluble ash	0.5%
Sulphated ash	2.3%
Water soluble ash	4.5%
Moisture content	11.3%
Aqueous extractive matter	42.8%
Alcoholic extractive matter	36.27%
Ether extractive matter	31.6%

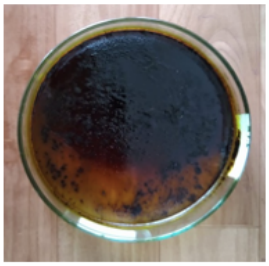
Extraction

The Plant *Ipomoea cairica* (L.) (40g) was subjected for extraction using two different solvents i.e. water and ethanol.

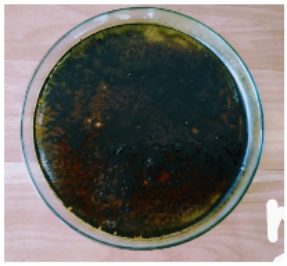
Table 2: Extraction Yield

Extract	Yield% W/W	Consistency	Color
Ethanolic extract	17.12	Non sticky	Light greenish
Aqueous extract	14.50	Sticky	Light brown

Fig 3. Different extracts of *Ipomoea cairica* (L.)



A. Aqueous extract



B. Ethanolic extract

Phytochemical evaluation

Table 3: Phytochemical evaluation of different extractions of plant *Ipomoea cairica* (L.)

Sl.No	Test	Plant constituents	*Ipomoea cairica* plant Extract			
			Methanol extract	Pet. Ether extract	Aqueous extract	Alcohol extract
1	Mayer's Test	Alkaloid	+	-	+	+
2	Dragendorff's		+	-	+	+
3	Wagner's test		-	+	+	+
4	Hager's test		+	+	+	+
5	$Fecl_3$ test	Tannins	+	-	+	+
6	Lead acetate		+	-	+	-
7	Bromine water		+	-	+	-
8	Foam test	Saponins	+	-	+	-
9	Test for tyrosine	Amino acid	-	-	-	-
10	Cysteine test		-	-	-	-
11	Fehling's test	Reducing sugar	-	-	+	-
12	Benedict's test		-	-	+	-
13	$Fecl_3$ test	Flavonoids	+	-	+	+
14	Lead acetate		+	-	+	+
15	NaOH solution		+	-	+	+

Thin layer chromatography

Rf value of both the extracts of *Ipomoea cairica* (L.) was calculated and compared with the standard quercetin. TLC observation indicates the presence of quercetin in both the extracts with the Rf value of 5.2; Fig 4A -4B

Fig 4A: Aqueous extract (sample A) and quercetin (std).
Fig 4B: Alcoholic extract (sample B) and quercetin (std)

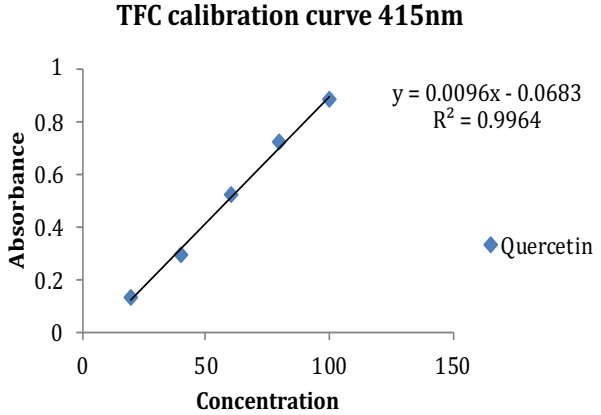
Fig 4A

Fig 4B

Total flavonoid content (TFC)

The TFC of the extract measured using standard quercetin. Total flavonoid values were obtained from the calibration curve y = 0.0096x - 0.0683 $R^2 = 0.9964$ where y is the absorbance and c is the concentration of Quercetin solution (mg/mL) expressed as mg QCE/g dried extract.In the same way both the extracts of *Ipomoea cairica* (L.) Shown the total flavonoid content, which is further compared with the standard Quercetin Table 4.

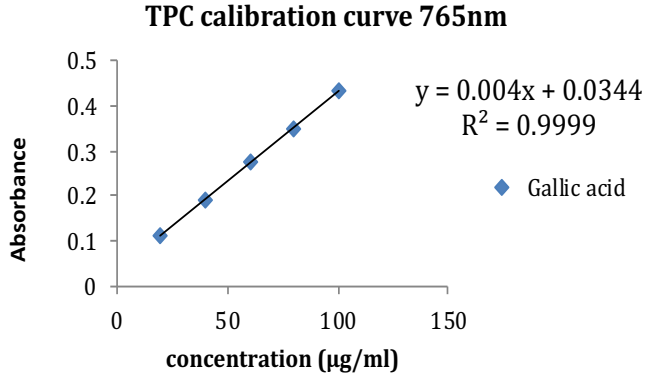
Fig 5: Standard calibration curve for Quercetin



Total phenolic content (TPC)

Total Phenolic Content of the extract measured using the FC method. TPC values were obtained from the calibration curve y = 0.004x + 0.0344 with R^2 = 0.9999, where y is the absorbance and c is the concentration of Gallic acid solution (mg/mL) expressed as mg GAE/g. In same way both the extracts of *Ipomoea cairica* (L.) shown the total phenolic content, which is further compared with the standard Gallic acid Table 4.

Fig.6: Standard calibration curve for Gallic acid



Estimation of Antioxidant

2,2-diphenyl-1-picrylhydrazyl is very stable free radical having an absorption band at 517nm. DPPH scavenging activity of the standard ascorbic acid and extracts of *Ipomoea cairica* (L.) in a concentration-dependent manner, decrease in the absorbance is due to increase in the antioxidant property. And % inhibition increases with increase in concentration of the extract.

IC_{50} Value

The IC_{50} of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the DPPH concentration by 50%, which is obtained by interpolation from a linear regression analysis.[18] (y=0.6393x+0.3606 R^2= 0.999) A lower IC_{50} indicates a highest antioxidant activity of a compound. Ethanolic extracts possesses 138.6µg/ml IC_{50} value while aqueous extract showed 154µg/ml; Table 4.

Fig 7: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity *of Ipomoea cairica* (L.) extracts in different solvents

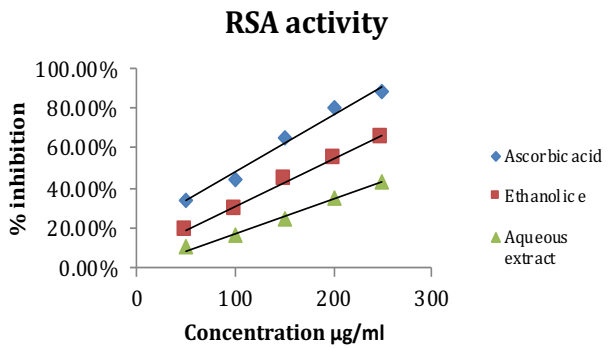


Table 4: TFC, TPC, and IC_{50} values of *Ipomoea cairica* extracts

Extracts of *Ipomoea cairica* (L.)	TFC (mg QCE/g)	TPC (mg GAE/g)	IC_{50} Value (µg/ml)
Aqueous extract	6.45±0.31	14.6±0.54	154±µg/ml
Ethanolic extract	8.54±0.42	22.03±1.21	138.6µg/ml

Discussion

The Purpose of this study was to evaluate the pharmacognostic characteristics and *in-vitro* antioxidant activity of different extracts of plant *Ipomoea cairica* (L.). Initially areal part of the plant was collected, dried and powdered then subjected to preliminary pharmacognostic evaluations such as morphology and powder microscopy. In microscopical study different cellular and non-cellular components like epidermal parenchyma, Glandular trichomes, Polygonal epidermis, reticulate xylem vessels, Stone cells, stomata, pollen grain were identified. Then powdered material is examined for its physicochemical parameters like loss on drying (11.3%) and ash values like Total ash(10%w/w), acid insoluble ash (0.5%w/w), sulphated ash (2.3%w/w) and water soluble ash (4.5%w/w). The extractive value of the plant using different solvents is carried out it confirms that aqueous solvent has more extractive value (42.8%) than Alcoholic (36.27%) and ether solvent (31.6%). The plant material was subjected for extraction using alcohol and water as solvent and yield obtained 17.12% and 14.50% w/w respectively. Both the Aqueous and alcoholic extract possesses various bioactive compounds and these extracts were tested for various components by their specific tests and they show presence of different Phyto-constituents like Alkaloids, tannins, saponins and Flavonoids. In continuation of study thin layer chromatography confirms the presence of quercetin in the aqueous and ethanolic extract by comparing the Rf value (5.2) of quercetin in both the extracts.

The TPC of both ethanolic and aqueous extracts were evaluated by utilizing Folin-ciocalteu reagent method and values ranges from 22.03±1.21 mg GAE/g and 14.6±0.54 mg GAE/g of dried extract respectively and it shows that TPC of the ethanol extract is significantly higher than that of the aqueous extract. These phenolic compounds may possess more phenol groups or have higher molecular weights than the phenolics in the aqueous extract and the effect of solvents on TFC is similar to that on TPC. Ethanolic extract showed highest total flavonoid content i.e. 8.54±0.42 mg QCE/g dried extract as compared to aqueous extract (6.45±0.31 mg QCE/g). Radical scavenging activity of ethanolic and aqueous extracts shown promising results. IC_{50} values are inversely proportional to their antioxidant activity Ethanolic extracts possesses 138.6µg/ml IC_{50} value and aqueous extract shown 154µg/ml so it reveals that ethanolic extract has highest antioxidant capacity as compared to aqueous extract.

Conclusion

This study reveals the different pharmacognostic parameters and phytochemical constituents of *Ipomoea cairica* (L.) which are responsible for various pharmacological medicinal properties. And the Antioxidant activity of different extracts of *Ipomoea cairica* (L.) was evaluated using DPPH assay. Ethanolic extract showed remarkable radical scavenging activity than aqueous extract. The Total phenolic contents of extracts were consistent with the total flavonoid content and antioxidant activity. By considering both yield and antioxidant activity, Ethanolic extract provided significantly better results than Aqueous extract. The results of TPC, TFC and radical scavenging *in vitro* activity confirms ethanol can be used as preferable solvent as compare to aqueous solvent. These results indicate that ethanolic extract of plant *Ipomoea cairica* (L.) could serve as better medicine against oxidative stress.

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References

1. Srivastava D, Shukla K. *Ipomoea cairica*: a medicinal weed with promising health benefits. Int. j. inf. res. rev. 2015;2(5):687-94.
2. Dowlath MJ, Karuppannan SK, GI DR, SB MK, Subramanian S, Arunachalam KD. Effect of solvents on phytochemical composition and antioxidant activity of *Cardiospermum halicacabum (L.)* extracts. Pharmacogn. J.2020;12(6). DOI:10.5530/pj.2020.12.173
3. Shefali arora1, deepak kumar and shiba. Phytochemical, antimicrobial and antioxidant activities of methanol extract of leaves and flowers of *Ipomoea cairica*. Int. J. Pharm. Pharm. Sci.2013;Vol 5, Issue 1:198-202
4. Mandal S, Rahman CH, Sutapa C. Studies on Ipomoea cairica (L.) Sweet-A promising ethnomedicinally important plant J. innov. pharm. biol. sci. 2015;2(4):378-95.
5. Srivastava D, Shukla K. Morphological and physichochemical Analysis of *Ipomoea cairica* leaves. Asian J. of Adv. Basic Sci.; 3(1), 2014, 222-227.
6. Thomas TG, Rao S, Lal S. Mosquito larvicidal properties of essential oil of an indigenous plant, *Ipomoea cairica* Linn. Japanese journal of infectious diseases. 2004 Aug1;57(4):176-7.
7. Ilesanmi OB, Inala ER. Hepatoprotective effect of *Ipomoea cairica* (Convolvulaceae) leaf extract against cadmium chloride induced liver damage. Toxicologie Analytique et Clinique. 2022 Mar 1;34(1):30-7. https://doi.org/10.1016/j.toxac.2021.12.001.
8. Ramalingam S, Adithiya RA, Joseph A, Saravanan R. Anticancer activity of *Ipomoea carnea* on Ehrlich ascites carcinoma bearing mice. Indian J. Pharm. Educ. Res. 2019 Oct 1;53(4):703-9. DOI: 10.5530/ijper.53.4.134
9. Ferreira AA, Amaral FA, Duarte ID, Oliveira PD, Alves RB, Silveira D, Azevedo AO, Raslan DS, Castro MS. Antinociceptive effect from *Ipomoea cairica* extract. J. Ethnopharmacol. 2006 21;105(1-2):148-53. https://doi.org/10.1016/j.jep.2005.10.012
10. Sahu RK, Kar M, Routray R. DPPH free radical scavenging activity of some leafy vegetables used by tribals of Odisha, India. J Med Plants. 2013;1(4):21-7.
11. Steward JL, Keeler KH. Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae)? Oikos. 1988 Jul 1:79-86. https://doi.org/10.2307/3565666
12. Srivastava D. Detection of Arctigenin in. Ames BN. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Science. 1983 Sep 23;221 (4617):1256-64.Stadtman ER. Protein oxidation and aging. Science 1992; 257: 1220-1224. DOI: 10.1126/science.6351251
13. Velioglu Y, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem.1998 19;46(10):4113-7. https://doi.org/10.1021/jf9801973
14. Desai PV, Wadekar RR, Kedar GH, Patil KS. Free radical scavenging activity of roots of *Baliospermum muell-Arg*. Int. J. Green Pharm. 2008;2(1)
15. Khandelwal KR. Practical Pharmacognosy, Technique and Experiments. Nirali Prakashan, Ninth Edition. 2002; 23.10-23.11 & 25.1-25.6.
16. AhbiRami R, Zuharah WF, Thiagaletchumi M, Subramaniam S, Sundarasekar J. Larvicidal efficacy of different plant parts of railway creeper, *Ipomoea cairica* extract against dengue vector mosquitoes, Aedes albopictus (Diptera: Culicidae) and. J. Insect Sci. 2014 Jan 1;14(1).
17. Mahitha B, Archana P, Ebrahimzadeh MH, Srikanth K, Rajinikanth M, Ramaswamy N. In vitro antioxidant and pharmacognostic studies of leaf extracts of *Cajanus cajan* (l.) millsp. Indian J. Pharm. Sci. 2015;77(2):170
18. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal. 2014 Sep 1;22(3):296-302. https://doi.org/10.1016/j.jfda.2013.11.001
19. Pomilio AB, Sproviero JF. Acylated anthocyanins from *Ipomoea cairica*. Phytochemistry. 1972 1;11(3):1125-8. https://doi.org/10.1016/S0031-9422(00)88464-2
20. Maurya S, Singh D. Quantitative analysis of total phenolic content in *Adhatoda vasica* nees extracts. Int. J. Pharmtech Res. 2010 Oct;2(4):2403-6.
21. Bagali RS, Jalalpure SS, Patil SS. In-vitro Antioxidant and In-Vivo Hepatoprotective Activity of Ethenolic Extract of *Tectona grandis* bark Against CCl4 Induced Liver Injury in Rats. Pharmacogn J. 2020;12(3).

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