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Phytochemical Screening And Evaluation Of Antioxidant, Anti-Inflammatory Activity Of Leaves And Flowers Of Law's Ceropegia

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Abstract: The present study was designed to evaluate the antioxidantof methanolic extract of Law's Ceropegia leaves and flowers using in vitro approaches and anti-inflammatory activity.Tannins, Saponins, Triterpenes, Alkaloids, Flavonoids, Steroids, Anthraquinones, Sterols phytochemicals present in the leaves extract and Tannins, Saponins, Triterpenes, Alkaloids, Flavonoids, Steroids, Anthraquinones, Sterols and Reducing Sugar present in flower extract of Law's Ceropegia plant.CCl_4 has shown better performance as the concentration increased 0.05 to 0.3 mg/ml as well as it shown better antioxidant activity in both leaves and flower extract. The ethanol extracts have better anti-inflammatory activity for both leaves and flower than petroleum ether, chloroform, ethyl acetate and n-butanol extract in both leaves and flowers of Law's Ceropegia plant.

Keywords: Law's Ceropegia, Phytochemicals, Anti-inflammatory, Antioxidant, Free radicals.

1. INTRODUCTION

A huge number of free radicals are produced as a result of pollution. Animals are exposed to these free radicals or oxidizing agents, which have a significant impact on their metabolism and the endogenous ones created by metabolism(Sen et al. 2010). Most chemical entities, such as superoxide anion (O_2^-), nitric oxide (NO•)oxygen species (ROS), hydroxyl (HO•), and peroxyl (ROO•) radicals, or nitric oxide (NO) reactive nitrogen species (RNS), peroxynitrite anion, contain reactive radicals.($ONOO^-$) which include agents like and hypochlorous acid (HClO) hydrogen peroxide (HOOH)(Sasane et al. 2021). The majority of these free radicals, such as ROS and RNS, induce lipid peroxidation, protein crosslinking, and DNA damage, resulting in cataracts, atherosclerosis, chronic inflammation, diabetes, cardiovascular disorders, malignancies, liver, and neurological illnesses(Phaniendra et al. 2015; Nayak et al. 2021). Antioxidants are employed in the food business to extend the shelf life of foods, particularly those that cause polyunsaturated fats to peroxide due to lipid peroxidation. As a result, food deterioration, discolouration, and nutritional losses, among

other things, rise(Farag et al. 2020). Various synthetic antioxidants are available, including butylated hydroxyanisole (BHA), tertiary butylhydroquinone, butylated hydroxytoluene (BHT), and propyl-gallate(Kalita et al. 2018). These are some examples of plants that have been thoroughly researched for antioxidants in recent years.

Inflammation is caused by tissue that has anti-inflammatory activity in response to injury. This is an example of the complicated process of inflammation, which is often linked to pain and includes events such as increased vascular permeability, increased protein denaturation, and membrane modification(Medini et al. 2015). It consists of a series of fluidic and cellular changes. The generation of different radicals such as O_2, OH, and non-free radical species (H_2O_2) leads to an over activation of phagocytes, which has anti-inflammatory properties. These are extremely potent oxidizing agents. As a result, medicines that can scavenge reactive oxygen species may be useful in the treatment of both inflammatory and oxidative illnesses(Kalita et al. 2018).

Plants have been the basis of traditional medicines from time immemorial throughout the world and continue to provide new targets for remedies for many afflictions of mankind(Antony et al. 2011). The plant of *Law's Ceropegia* is a woody herb from Apocynaceae family(Kambale and Yadav 2015). Its height about 30-60 cm with larger and fresh green leaves. Its flowering in month of august and flowers are white along lantern-shape, associated with Ceropegias. The flower tube has 5 lobes, curved into a closed bowl shape. *The Law's Ceropegia*plant was named of amateur botanist, John Sutherland Law. This species is in danger of extinction. It is now highly endangered as natives consume its tubers in large quantities.It is mostly found in the Western Ghats of Maharashtra, India(Singh and Karthikeyan 2000).

Fig.1. Law's Ceropegia Plant

The past couple of decades have seen considerable change in opinion regarding ethnopharmacological therapeutic applications of phytochemical. A great deal of effort therefore still focuses on identifying and using these Phytochemical as source of novel therapeutic molecules(Velavan 2015).

2. MATERIALS AND METHODS

Plant materials and Preparation of Extract

The flower and leaves of *Law's Ceropegia* collected Lonavala region of Maharashtra, India.these flower and leaves were are collected, washed with deionized water then dried at room temperature for two weeks.After completion of drying process. The dried samples were powdered using pestle and mortar.powdered plant samples were separately dissolved in 300 ml methanol. The solution was left to stand at room temperature for 30 hours with infrequent shaking and was filtered with Whatman No. 1 filter paper. The filtrate was used for the phytochemical analysis.

Qualitative Phytochemical Analysis
Phytochemical testing was carried out according tostandard protocol (Le BaoDuy et al. 2015; Dias et al. 2019). Phytochemistry of leaves and flowers Powder was Carried out as for Tannins, Saponins, Triterpenes, Alkaloids, Flavonoids, Steroids, Anthraquinones, Sterols, Reducing Sugar, Proteins, and Anthocyanin,

In Vitro Antioxidant Activity Determination

DPPH Scavenging Test
The reaction mixture contained 50 μL of test samples (or 80% MeOH as blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol.

Determination of Anti-inflammatory Activity
Study of anti-inflammatory activity (In-vitro models): *Law's Ceropegia* leaves and flowers extract was screened for anti–inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Mizushima and Kobayashi with slight modification at the doses of 200 mg/kg(Mizushima and Kobayashi 1968).

3. RESULTS AND DISCUSSIONS

Qualitative analysis of phytochemical constituents was tabulated in Table 1 for LC-L and LC-F. Tannins, Saponins, Triterpenes, Alkaloids, Flavonoids, Steroids, Anthraquinones,Sterols phytochemicals present in the leaves extract and Tannins, Saponins, Triterpenes, Alkaloids, Flavonoids, Steroids, Anthraquinones, Sterols and Reducing Sugar present in flower extract of *Law's Ceropegia* plant.

Tab. 1. Qualitative analysis of phytochemicals of LC-L, LC-F

Phytochemical Tested	TestPerformed	LC-L	LC -F
Tannins	Ferric chloride test (Mirgane et al. 2021)	+	+
Saponins	Frothing test (Auwal et al. 2014)	+	+
Triterpenes	Liebermann- Burchard test (Nath et al. 1946)	+	+
Alkaloids	Wagner's test (Ukoha et al. 2011)	+	+
Flavonoids	Alkaline reagent test (Ukoha et al. 2011)	+	+
Steroids	Salkowski's test (Zahran et al. 2007)	+	+

Anthraquinones	Borntrager's test (Le BaoDuy et al. 2015)	+	+
Sterols	Liebermann- Burcherd test(Mirgane et al. 2021)	+	+
Reducing Sugar	Fehling reagent test(Mirgane et al. 2021)	-	+
Proteins	Biuret reaction(Le BaoDuy et al. 2015)	-	-
Anthocyanin	NaOH Test(Ukoha et al. 2011)	-	-

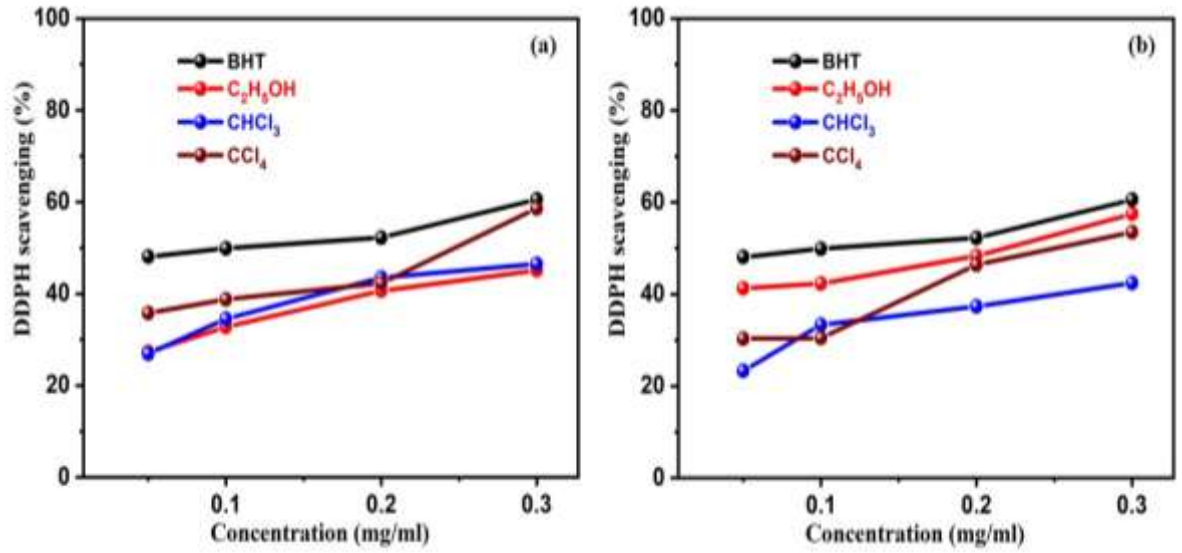
***+ = Positive test, - = Negative test**

Antioxidant Activity Determination

DPPHis anextremely stable free radical method and it has been extensively used to evaluate the antioxidant potential of several natural products(Khan et al. 2020).Antioxidant activity of LC-L and LC-F of *Law's Ceropegia*plant shown in Fig. 2 (a) and 2 (b) respectively. Fig. 2 (a) displayed that, In LC-L, CCl_4 has shown better performance as the concentration increased 0.05 to 0.3 mg/ml as well as it shown better antioxidant activity than C_2H_5OH and $CHCl_3$. The Fig 2 (b) shown that CCl_4 and C_2H_5OH have better antioxidant activities than $CHCl_3$.

Fig. 2 Antioxidant activity of LC-L and LC-F a) DPPH radical activity of LC-L b) DPPH radical activity of LC-F

Tab. 2. Antioxidant activity of LC-L



Extract Conc. mg/ml	BHT	C_2H_5OH	$CHCl_3$	CCl_4
0.05	48.1	41.50	23.83	25.60
0.1	49.91	38.84	34.23	35.80
0.2	52.24	47.34	23.50	44.33
0.3	60.57	55.22	43.00	48..60

Tab.3. Antioxidant activity of LC-F

Extract Conc. mg/ml	BHT	C_2H_5OH	$CHCl_3$	CCl_4
0.05	48.1	41.30	23.30	30.33
0.1	49.91	42.30	33.34	30.45
0.2	52.24	48.33	37.33	46.40
0.3	60.57	57.5	42.45	53.52

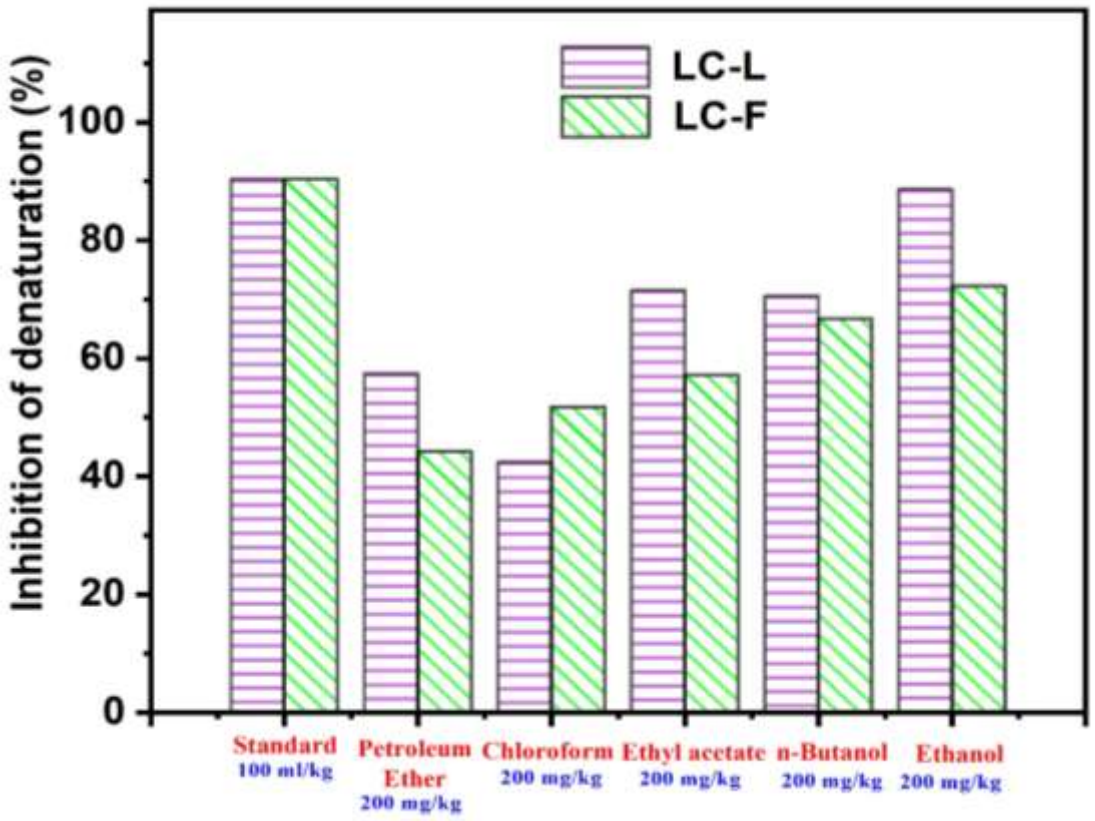
Anti-inflammatory Activity Determination

In vitro anti-inflammatory activityof leaves and flowers extract of *Law's Ceropegia*plant tabulated in Table 3. The Fig. 3 shown comparative analysis of LC-L and LC-F inhibition of denaturation of standard,petroleum ether, chloroform, ethyl acetate, n-butanol and ethanol extract. The ethanol extracts has better anti-inflammatory activity for both CL-L and LC-F than petroleum ether, chloroform, ethyl acetate and n-butanol extract.

Tab.4. Anti-inflammatory activity of CL-L and CL-F

Anti-inflammatory activity (Ethanol extract)	Dose (mg / kg)	LC-L		LC-F	
		Absorbance value (Mean + SE)	Inhibition of denaturation (%)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	5ml / kg	0.098	----	0.098	----
Standard (Ibuprofen)	100mg/kg	0.18	90.32	0.18	90.32
Petroleum ether extract	200mg/kg	0.15	57.44	0.12	44.18
Chloroform extract	200mg/kg	0.14	42.45	0.13	51.78
Ethyl acetate extract	200mg/kg	0.12	71.56	0.12	57.18
n-Butanol	200mg/kg	0.16	70.60	0.14	66.71
Ethanol	200mg/kg	0.17	88.60	0.12	72.18

Fig. 3 % inhibition of LC-L and LC-F for standard, Petroleum Ether, Chloroform, Ethyl acetate, n-Butanol and Ethanol



4. CONCLUSION

On the basis of present study, it can be inferred that the ethanolic extract from flower and leaves *Law's Ceropegia* exhibited the existence of various phytochemicals such as Tannins, Saponins, Triterpenes, Alkaloids, Flavonoids, Steroids, Anthraquinones, and Sterols. Mostly Ethanolic extract of the flower and leaves *Law's Ceropegia*possess*in-vitro* anti-inflammatory activity which might be attributed to the presence of various phytochemicals in the extract. Overall, flower and leaves of*Law's Ceropegia* is a source of natural antioxidant that can be significant in disease preventions as well as health preservation. Therefore, it's ethnomedical claims was rightaccording to the above experimental outcomes. This gives support to the claim for the traditional usage of the plant in the treatment of inflammation. The outcome of the study has seen to offer support for the use of flower and leaves of*Law's Ceropegia*. To encourage proper conservation sustainable usage of such plant resources, consciousness of local societies should be improved incorporating the traditional knowledge with scientific findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial orotherwise.

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