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COMPARATIVE ANALYSIS OF *IN VITRO* ANTIOXIDANT CAPACITY OF FRESH AND DRY LEAF EXTRACTS OF *HUGONIA MYSTAX* LINN

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ABSTRACT

Hugonia Mystax is a valued medicinal plant in traditional folk medicine used in siddha and ayurveda for various ailments. It is a woody evergreen liana distributed throughout India in the dry tropical forest, mostly found in south Indian forest (Tamilnadu and Kerala). *Hugonia Mystax* locally known as modirakanni, mainly used for skin diseases, rheumatism, antidote for snake bite, anthelmintic, febrifuge, astringent, fever, verminosis, peptic ulcers and intestinal worms. Preliminary phytochemical analysis of *Hugonia Mystax* extracts showed the presence of carbohydrates, flavonoids, phenolic groups, saponins, steroids, tannins and terpenoids. *In vitro* antioxidant activity of n-hexane, chloroform and ethanol extracts of fresh and dry leaves of *Hugonia Mystax* showed significant activity by reducing power assay method using ascorbic acid as a standard drug. Absorbance was measured at 700 nm using UV-spectrophotometer showed significant activity. In conclusion, the results of this study clearly indicated that the extracts of *Hugonia Mystax* possess significant antioxidant activity and could be used as a potential source of natural antioxidant agent that may be due to the presence of phytochemicals.

KEYWORDS: *Hugonia Mystax*, Antioxidant, Preliminary analysis.

INTRODUCTION

In India there are about 7500 wild plants used for medicinal purposes by different tribal inhabitants. Recently WHO (World Health Organization) estimated that 80% of people worldwide rely on herbal medicines for some aspects of primary health care needs. Treatment with medicinal plants was considered very safe as there is minimal side effects.

Plants have been utilized as medicines for thousands of years. *Hugonia Mystax* "Fig. 1" is an important and unexplored medicinal plant in the Indian medicinal system, it belongs to the family Linaceae.

Figure No: 1 *Hugonia Mystax*.

Taxonomic name: *Hugonia Mystax* Linn
Family: *Linaceae*
Habitat: Konkan and Northkanara throughout dry forest of Tamilnadu
Folk: Kakibeeraa, Kansamara.

Common name
English: Climbing flax
Malayalam: Moderakanni, Motirakanni
Sinhalese: Bugatteya, Mahagetiya
Telugu: Gatrinta, kodivirai, modirakanni, pisangi, renangi, tivyaputiki, ungaralapidemu
Tulu: Mullankol
Tamil: Agori, kodivirai, modirakanni, agure, motirakodi, pisangi, kakiabira, pentapeeda, motirakanni, kotivi.

Plants are potential source of antioxidant. Antioxidants are substances that neutralize free radicals and their actions. Antioxidant plays an important role in inhibiting and scavenging free radicals and providing protection to human against infections and diseases. Plants are rich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, tannins, flavonoids, coumarins, alkaloids, amines and other metabolites which are rich in antioxidant activity. Many herbal plants contain antioxidant compounds which protect cells against degenerative effects of reactive oxygen species (ROS) which is a free radical such as singlet oxygen, superoxide, peroxyl radicals and hydroxy radicals. Flavonoids, tannins, saponins, terpenoids, phenol and amino acids are examples for antioxidants and many more having the capability to scavenge the free radicals.

Literature revealed no work was found on this plant on the aspect of comparative analysis of fresh and dry leaves. Hence in the present study, preliminary phytochemical screening and antioxidant capacity of various extracts of fresh and dry leaves of *Hugonia Mystax* was carried out by reducing power assay method to provide a scientific evidence to prove the ethnobotanical usage. A major part of the world population mainly in the developing countries still uses traditional, folk medicine.[1-3]

MATERIALS AND METHODS

Collection of plant material

The leaves of *Hugonia Mystax* were collected from Dindigul District, Tamilnadu, India. The collected plant material was authenticated by Botanical Survey of India (BSI/SRC/5/23/2018/Tech/149). The herbarium specimen was prepared and deposited at the Natural Product Research Laboratory, J.K.K. Nattraja College of Pharmacy, Namakkal, Tamilnadu for future reference.

Preparation of the extracts

Dry leaves

The plant material was collected and chopped into small pieces, dried under shade condition and coarsely powdered. The coarse powder was subjected to successive extraction with n-hexane, chloroform and ethanol (95%) solvent of increasing order of polarity by Soxhlet extraction method. The extracts were collected and distilled at atmospheric pressure and the trace of solvent was removed *in vacuo*.

Fresh leaves

The fresh leaves were chopped into small pieces. Container contained full of menstrum and maintained for 3-7 days, shaken frequently until the extraction of plant material was completed. n-hexane, chloroform and ethanol were used for extraction process. The extract was filtered and dried to get a solid mass.

The fresh and dry leaves extracts were used for phytochemical analysis.[4-7]

Preliminary phytochemical analysis

Phytochemical constituents of fresh and dry leaf extract of *Hugonia Mystax* Linn.

Table No:1

Sr.No.	Phytochemical Constituents	Fresh leaf extract of *Hugonia Mystax* Linn			Dry leaf extract of *Hugonia Mystax* Linn		
		n-Hexane	Chloroform	Ethanol (95%)	n-Hexane	Chloroform	Ethanol (95%)
1.	Alkaloids	-	-	-	-	-	-
2.	Flavonoids	+	+	+	+	+	+
3.	Phenolic Compounds	+	+	+	+	+	+
4.	Fats and oils	-	-	-	-	-	-
5.	Steroids	+	+	+	+	+	+
6.	Tannins	+	-	+	+	-	+
7.	Amino acids	-	-	-	-	-	-
8.	Saponins	+	-	+	+	-	+
9.	Terpenoids	+	+	+	+	+	+
10.	Carbohydrates	-	-	+	-	-	+
11.	Glycosides	-	-	-	-	-	-

+ ---- Present - ---- Absent

The result of preliminary phytochemical screening is given in the Table No:1. Which revealed the presence of flavonoids, phenolic compounds, steroids and terpenoids in all the three extracts of fresh and dry leaves. Saponins

and tannins were present in both n-hexane and ethanol extracts. Carbohydrates present in ethanol extract only. Alkaloids, fats and oils, amino acids and glycosides were absent in all the extracts.

***In vitro* antioxidant capacity**

The reducing power of n-hexane, chloroform and ethanol extract of *Hugonia Mystax* was determined by the method of Oyaizu (1986).[8-10]

Table No. 2: Reducing capacity of various extracts of *Hugonia Mystax* Linn.

Sr. No.	Extract	Concentration				
		10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
1.	Ascorbic acid	0.510	0.544	0.574	0.600	0.674
2.	Fresh leaf extract of n-hexane	0.025	0.059	0.080	0.099	0.103
3.	Fresh leaf extract of $CHCl_3$	0.126	0.161	0.154	0.176	0.194
4.	Fresh leaf extract of ethanol (95%)	0.084	0.111	0.136	0.153	0.202
5.	Dried leaf extract of n-hexane	0.088	0.154	0.175	0.182	0.184
6.	Dried leaf extract of $CHCl_3$	0.119	0.130	0.151	0.156	0.179
7.	Dried leaf extract of ethanol (95%)	0.243	0.263	0.311	0.312	0.362

Potassium ferricyanide + Ferric chloride ⟶ Potassium ferrocyanide + Ferrous chloride

Different concentration of the various extracts of fresh and dry leaf of *Hugonia Mystax* Linn and its fractions of (10, 20, 30, 40, 50 µg/ml) was added to 2.5ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% v/v potassium ferri cyanide solution. The reaction mixture was vortexed well and then incubated at 50˚C for 20 minutes. At the end of the incubation, 2.5 ml of 10% tri chloro acetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with 2.5 ml of deionised water and 0.5 ml of 0.1% ferric chloride. The colored solution was read at 700 nm with standard drug (Ascorbic acid) using UV- Spectrophotometer. The reducing power of the samples were compared with standard drug was showed in Table No.2

Antioxidant capacity of fresh and dry leaf extract of *Hugonia Mystax* Linn.

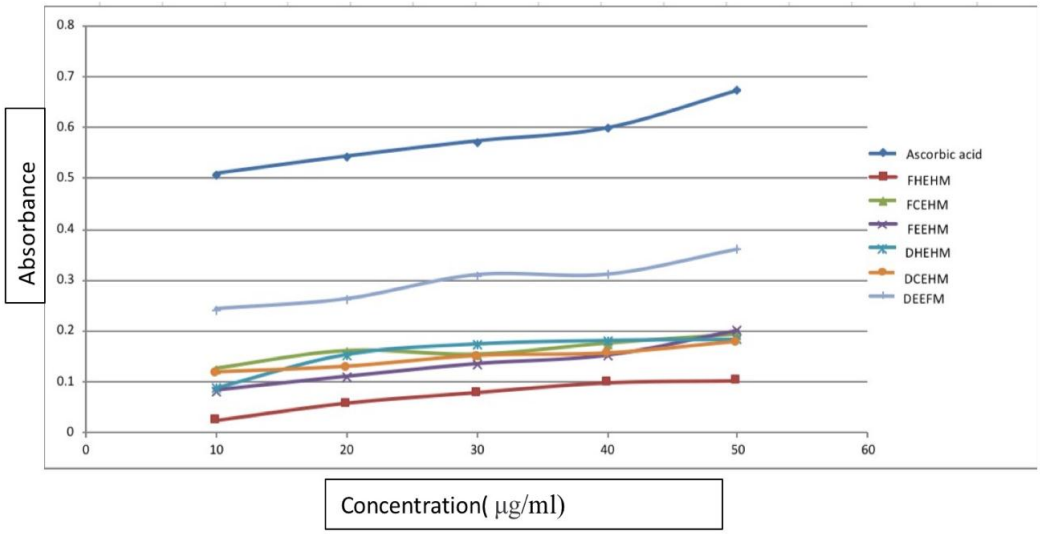

Figure No:2

FHEHM- Fresh hexane extract of *Hugonia Mystax*
FCEHM- Fresh chloroform extract of *Hugonia Mystax*
FEEHM- Fresh ethanol extract of *Hugonia Mystax*
DHEHM- Dried hexane extract of *Hugonia Mystax*
DCEHM- Dried chloroform extract of *Hugonia Mystax*
DEEHM- Dried ethanol extract of *Hugonia Mystax*

RESULT AND DISCUSSION

Plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity.[11] High levels of free radicals or active oxygen species create oxidative stress, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death.[12] There is continuing interest on the screening of medicinal plants with a view to determine new sources of natural antioxidants.[13,14]

Hugonia Mystax an important Indian medicinal plant, fresh and dry leaves was tested for the first time in the present study to their antioxidant activity. These results indicate that all the extracts have a noticeable effect on antioxidant activity, the dried ethanol extract possess

greater antioxidant activity which may be attributed to the presence of carbohydrates, flavonoids, phenolic group, saponins, steroids, tannins and terpenoids.

Both the extracts (dry and fresh leaf) at the concentration of 10, 20, 30, 40, 50 µg/ml produced significant antioxidant activity in a dose dependent manner. This activity also increases with increasing concentration. The antioxidant activity was determined by reducing power assay method and serve as a significant reflection of the antioxidant activity by forming a colored complex with potassium ferri cyanide, tri chloro acetic acid and ferric chloride which was measured at 700 nm using UV-Spectrophotometer. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.[15-21]

Antioxidant compounds convert the oxidation form of iron (Fe^{3+}) in ferric chloride to ferrous (Fe^{2+}). From "Fig. No 2", dried leaves of ethanol extract of *Hugonia Mystax* showed an increased absorbance when compared to the standard drug ascorbic acid. The extracts of these plants can be regarded as promising candidate for a plant derived antioxidant compound.

CONCLUSION

n-hexane, chloroform and ethanol extract of fresh and dry leaves of *Hugonia Mystax* possesses concentration dependent activity due to the presence of plant secondary metabolites such as carbohydrates, flavonoids, phenolic group, saponin, steroid, tannin and terpenoids.

From the above result, it can be concluded that dried ethanol extract of *Hugonia Mystax* showed increased antioxidant activity at the concentration of 50 µg/ml with increased absorbance.

Hugonia Mystax offers immense value, which can form the basis of drug supplementation and should be used for the promotion of public health. It may also be considered for the treatment of diseases as an alternative therapy.

Further, it is necessary to isolate the bioactive molecules responsible for the activities to develop novel leads of pharmaceutical interest.

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