International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 05-10-2021, Accepted: 17-10-2021, Published: 06-11-2021
Volume 6, Issue 6, 2021, Page No. 30-34

In vitro anti-oxidant activity of *Muntingia calabura*

J Banurekha[1*], P P John[2]

[1] Department of Pharmaceutical Chemistry, Vinayaka Missions College of Pharmacy, Salem, Tamil Nadu, India
[2] Nirmala College of Health Science, Chalakudy, Thrissur, Kerala, India

Abstract

Excessive physiological burden of free radicals leads to oxidative stress which is root cause of various human diseases. Enriching of body systems with antioxidants prevents the onset as well as treats the disease caused due to free radicals and related oxidative stress. *Muntingiacalabura Linn*. is a folklore medicinal plant used against disease produced due to oxidative stress. Continuous hot percolation process is used with various solvents to prepare the extract from the stem of the plant. The extracts obtained especially petroleum ether & ethanols were subjected to the preliminary phytochemical studies. The phytochemical evaluation shows the presence of flavonoids, phenolic compounds, glycosides, saponins and carbohydrates in ethanolic extracts of the plant. Hence, the ethanolic extract which has the polarity in between was selected for further pharmacological evaluation. The ethanolic extract of stem of *Muntingiacalabura Linn*. was used for the evaluation of antioxidant activity. Various *in-vitro* models are used to generate the free radicals and the ability of anti-oxidant to reduce or disproportionate the radical is measured. Among those we have undergone with DPPH free radical scavenging activity. Total DPPH scavenging potential of the extracts at concentration 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ ml were measured and results were depicted in Table No: 3 and Fig.1. Significant DPPH radical scavenging activity was evident at all the tested concentrations of the extract. Thus, from the results of the present study, it was concluded that the ethanolic extract of stem of *Muntingiacalabura* has very good in-vitro anti-oxidant activity against DPPH method and can be used as a supplementary plant drug for various diseases.

Keywords: *Muntingiacalabura,* antioxidant, DPPH, percolation

Introduction

As one of the aspects of the body's natural ecosystem, it is increasingly being realized now that a majority of the disease/disorders are mainly due to the imbalance between pro-oxidant and anti-oxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to increased generation of free radicals and/or their poor quenching/scavenging into the body [1].

Hyper physiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress that is being suggested as the root cause of ageing and various human disease like atherosclerosis, stroke, diabetes, cancer, and neurodegenerative disease such as Alzheimer's disease and Parkinsonism. Therefore, in modern Western medicine, the balance between anti oxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system. Researches in the recent past have accumulated enormous evidence advocating enrichment of body systems with antioxidants to correct vitiated homeostasis and prevent the onset as well as treat the disease caused/fostered due to free radicals and related oxidative stress. Current understanding of the role of free radicals and oxidative stress in pathogenesis of various disease and advancements made in developing anti-oxidant-based therapeutics and also discussed the opportunities to develop therapeutics from traditional medicinal practice [1].

Based upon ethno pharmacological survey, the stem of *Muntingia calabura* has been selected to prove scientifically having antioxidant activity.

Materials and Methods

Selection of the plant

The isolation of secondary plant metabolites begins with the selection of a plant, the most critical aspect of the project, in order to locate a plant previously in folklore practices, one should turn to the discipline of ethno botany. Ethno botany literally means "peoples botany'' and is defined as the study of plants important to primitive people. Ethno botany can include present day people and involves and involves inter disciplinary study surrounding a core of botany with chemistry, pharmacology and anthropology among others [2].

As the medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. The library search undertakes on that will yield detail of folk medicinal plants utilize in particular area. This will ensure the availability of plants for collection purposes [2].

So, for the selection of a medicinal plant for an active antioxidant activity, undergone discussion with a tribal medical practitioner for the traditional and tribal uses of this plant which have not been evaluated for antioxidant activity. A search of the genus and species will yield past studies that have been done. The complied list of plants must therefore be subjected to a literature survey to confirm that the plant has not been previously investigated for antioxidant activity. In addition to the verification that the plant constituents have yet to be identified, note must be taken of which plant were utilized and how they were culturally prepared [3].

Muntingia calabura is a folklore medicinal plant used against disease such as antidiuretic, antidiabetic, antioxidant activity and anti-inflammation of the pharynx membranes. Eczema, flow of blood or secretion, skin disorder or irritation genital disorder Leucorrhoea, relief of vagina, conjunctivitis, Epiphora, relieving erectile dysfunction, night fall, affect the male genital organs. Hence, the present study is an attempt to investigate antioxidant activity of the plant *Muntingia calabura* [4].

Collection and authentification of plant material

The stems of *Muntingia calabura* were collected from the foothills of Shevaroys hills, Salem district in the month of November 2018. The plant was taxonomically identified and authorized by the botanist *Balasubramanian.*

Preparation of the extract

The stem of *Muntingia calabura* was collected and air dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve no.40 and stored in an airtight container for the extraction. The collected, cleaned and powdered aerial part of *Muntingia calabura* was used for the extraction purpose. 1000 gms of powdered material was evenly packed in the Soxhelt apparatus. It was then extracted with various solvents from nonpolar to polar such as petroleum ether, chloroform, acetone, ethanol and aqueous successively. The solvents used were purified before use. The extraction method used was continues hot percolation and carried out with various solvents, for 72 hrs. The aqueous extraction was carried out by cold-maceration process [5].

Methods of extraction

Continuous hot percolation process

Requirements

Shade dried coarse powder of stem of *Muntingia calabura*, Soxhlet apparatus.

Solvents used

Petroleum ether, alcohol & distilled water.

Preparation of Extracts

Petroleum ether extract of stem of *Muntingia calabura*

The shade dried coarsely powdered stem of *Muntingia calabura* (1 kg) were extracted with petroleum ether (60^0C-80^0C), for 72 hrs. After completion of extraction, the defatted extracts were filtered while hot through Whatman filter paper (no.10) to remove any impurities if present. The extract was concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extract was transferred to 100ml beaker and the remaining solvent was evaporated on a water bath. Dark greenish yellow coloured extract was obtained. The concentrated extract was then kept in a desiccator to remove the excessive moisture. The dried extract was packed in air tight glass container for further studies [6].

Ethanol extract of stem of *Muntingia calabura*

The marc left after Acetone extraction was dried and then extracted with ethanol 99% v/v (75^0C-78^0C), for 72 hrs. After completion of extraction, the solvent was removed by distillation. Dark greenish coloured extract was obtained. The extract was then stored in a desiccator to remove the excessive moisture. The dried extract was packed in air tight glass container for further studies [7].

Identification of Phytochemical Active Constituents Preliminary phytochemical studies

The extracts obtained (Pet. ether, Ethanol) was subjected to the following preliminary phytochemical studies.

Test for alkaloids

a. **Dragendorff's test:** To 2 mg of the extracts, 5 ml of distilled water was added, 2mlHydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragenderff's reagent was added. Formation of orange or orange red precipitates indicates the presence of alkaloids.
b. **Hager's test:** To2 mg of the extracts were taken in a test tube, a few drops of Hager's reagent was added. Formation of yellow precipitates confirms the presence of alkaloids.
c. **Wagner's test:** 2 mg of extracts were acidified with 1.5% v/v of hydrochloric acid and afew drops of Wagner's reagent were added. A brown precipitate indicates the presence of alkaloids.
d. **Mayer's test:** To a few drops of the Mayer's reagent, 2mg of extracts were added.Formation of white or pale-yellow brown precipitate indicates the presence of alkaloids [8].

Test for carbohydrates

a. **Anthrone test:** 2 mg of extracts were shaken with 10 ml of water, filtered and the filtratewas concentrated. To this 2 ml of anthrone reagent solution was added. Formation of green or blue colour indicates the presence of carbohydrates.
b. **Benedict's test:** 2 mg of extracts were shaken with 10 ml of water, filtered and the filtratewas concentrated. To this 5 ml of Benedict's solution was added and boiled for 5 minutes.Formation of brick red coloured precipitate indicates the presence of carbohydrates.
c. **Fehling's test:** 2 mg of extracts were shaken with 10 ml of water, filtered and the filtratewas concentrated. To this 1 ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of red coloured precipitate indicates reducing sugar.
d. **Molisch's test:** 2 mg of extracts were shaken with 10 ml of water, filtered and the filtrate was concentrated. 2 drops of freshly prepared 20% alcoholic solution of α - naphthol was added. 2 ml of conc. Sulphuric acid was added so as to form a layer below the mixture. Red violet ring appears, indicating the presence of carbohydrates which disappear on the addition of excess of alkali [9].

Test for flavonoids

a. **Shinoda's test:** 2 mg of extracts were dissolved in 5 ml of ethanol and to this 10 drops ofdilute hydrochloric acid followed by a small piece of magnesium were added.
   Formation of pink, reddish or brown colour indicates the presence of flavonoids.
b. **With con. Sulphuric acid test:** Yellow orange colour (anthocyanins), yellow to orangecolour (flavones) and orange to crimson (flavanones)

Test for glycosides

Molisch's test: 2 mg of extracts were shaken with 10 ml of water, filtered and the filtrate wasconcentrated. To this 2-3 drops of Molisch's reagent was added, mixed and 2 ml of concentrated sulfuric acid was added carefully through the side of the test tube. Reddish violet ring appears, indicating the presence of glycosides [10].

Test for protein and free amino acids

Small quantities of the extracts were dissolved in few ml of water and treated with following reagents.

a. **Million's reagent:** Appearance of red colour shows the Presence of protein freeamino acids.
b. **Ninhydrin reagent:** Appearance of purple colour shows the presence of protein andfree amino acid.

Test for gums and mucilage

Precipitation with 95% alcohol: Small quantities of the extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

Test for saponins

Foam test: In a test tube containing about 5 ml of extracts, a drop of sodiumbicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honey comb like froth indicates the presence of saponins [11].

Test for sterols

a. **Liebermann-Burchard's test:** 2 mg of dry extracts were dissolved in aceticanhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicates the presence of steroids.
b. **Salkowski reaction:** 2 mg of dry extracts were shaken with chloroform, to thechloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

Test for fixed oils

Spot test: Small quantities of various extracts were separately pressed between two filterpapers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of various extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats [12].

Test for phenolic compounds and tannins

Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagent.

a. Dil. Ferric chloride solution (5%)-Violet colour.
b. 1% solution of gelatin containing 10% sodium chloride-White precipitate.
c. 10% lead acetate solution-White precipitate.

In the above stated extracts, chloroform, methanol and aqueous extracts showed the presence of same type of constituents. Hence, the ethanolic extract which has the polarity in between was selected for further pharmacological evaluation [13].

Pharmacological Screening

Antioxidant studies

The ethanolic extract of stem of *Muntingia calabura Linn*. was used for the evaluation of antioxidant activity.

***In-vitro* antioxidant studies**

Various in-vitro models are used to generate the free radicals and the ability of anti-oxidant to reduce or disproportionate the radical is measured. Among those we have undergone with DPPH free radical scavenging activity [14].

DPPH scavenging activity (1, 1 Diphenyl 2, picryl-hydrazyl)

DPPH scavenging activity or the Hydrogen donating capacity was quantified in presence of stable DPPH radical on the basis of Blois method. Briefly, to a methanolic solution of DPPH (100 µM, 2.95 ml), 0.05ml of test compounds dissolved in methanol was added at different concentration (20-100µg/ml). Reaction mixture was shaken and absorbance was measured spectrophotometrically (Spectra-Max-Plus, Molecular Devices, USA) at 517 nm at regular intervals of 30 seconds for 5 minutes, and the reading was taken till 20 min. Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated by using the following equation,

$$\text{Scavenging effect (\%)} = (1 - B/A) \times 100$$

Where,
A= Absorbance of DPPH control with solvent(517nm)
B= Absorbance of decolorized DPPH in presence of Test sample (517nm) [15, 16].

Results and Discussion

Antioxidant activities

***In-vitro* antioxidant activities**

DPPH method

An antioxidant property of the ethanol extracts of *Muntingia calabura* was examined by using the stable free radical DPPH. Total DPPH scavenging potential of the extracts at varying concentration was measured and results were depicted in Table No: 3 and Fig.1. Significant DPPH radical scavenging activity was evident at all the tested concentrations of the extract. The scavenging effect increased with increasing extract concentration up to a certain extent and then leveled off with further increase.

DPPH is as table free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the ethanolic solution of DPPH shows a strong absorption band at 517nm. DPPH radicals react with suitable reducing agents and the electrons become paired and the solution loses color stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compounds/ plant extracts to acts free radical scavengers. Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517nm. The tested extract showed potent DPPH scavenging activity

Determination of extractive values of various extracts of the dried stem of *Muntingia calabura*

The shade dried coarsely powdered stem of *Muntingia calabura* was extract by using different solvents of increasing polarity *viz*. petroleum ether and ethanol by continuous hot percolation process using Soxhlet apparatus.
Extraction values were as follows:

1. Petroleum ether extract-5.8 %w/w
2. Ethanol extract-11 %w/w

The values were presented in the table no.1

Table 1: Extractive values of stem of *Muntingia calabura* wild.

Plant Name	Part Used	Method of extraction	Yield in %	
			Petroleum Ether	Ethanol
Muntingia calaburawild	Stem	Continuous hot percolation	5.8	11

Phytochemical evaluation

The phytoconstituents present in the various extracts were identified by chemical test and the results were showed in Table No: 2.

Petroleum Ether: Chlorophyll, Starch, Fat, Fixed oil.

Ethanol: Carbohydrates, Glycosides, Tannins, Saponins, Flavonoids and Phenolic Compounds.

The phytochemical evaluation shows the presence of Flavonoids, phenolic compounds, glycosides, saponins and carbohydrates an ethanolic extracts of the plant.

From the above stated extracts, ethanol extracts showed the presence of same type of constituents. Hence, the ethanolic extract which has the polarity in between was selected for further pharmacological evaluation.

Table 2: Preliminary phytochemical studies of various extracts of stem of *muntingia calabura* wild.

S. No.	Constituents	Test	Petroleum Ether	Ethanol
1.	Alkaloids	Mayer's test	-	+
		Dragondraff's test	-	+
		Hager's test	-	+
		Wagner's test	-	+
2.	Sterols	LibermannBurchard's	-	+
		Salkowski's	-	+
3.	Carbohydrates	Molisch's test	-	+
		Fehling's test	-	+
		Benedict's test	-	+
		Anthrone test	-	+
4.	Fixed oils and fats	Spot test	-	-
		FeCl3	+	+
6.	Protein and aminoacids	Biuret test	-	+
		Ninhydrin test	-	+
		Millon's test	-	+
7.	Saponins	Foam test	-	-
8.	Tannins	Gelatin test	-	-
		FeCl3 tests	-	+
9.	Gum and mucilage	Alcohol	+	-
10.	Flavonoids	Shinoda's test	-	+
		Conc.H2SO4	-	+
11.	Glycosides	Molisch's test	-	+

Table 3: Free radical scavenging activity of ethanolic extracts of *Muntingia calabura* (EMC) by DPPH method

S. No.	Treatment	Concentration %Inhibition				
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
1	EMC	67.01 ± 0.35	70.36 ± 0.37	77.13 ± 0.15	89.40± 0.23	94.05 ± 0.29
2	Ascorbic acid	71.50 ± 0.50	76.33 ± 0.33	81.50 ± 0.43	91.17± 0.40	95.34 ± 0.20

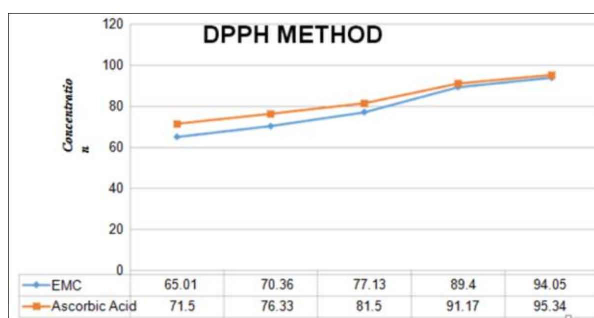

Fig 1: Free radical scavenging activity ethanolic extracts of *Muntingia calaburawilld* by DPPH

Summary and Conclusion

The last two decades has witnesseda revival of interest in natural drugs and herbal products primarily due to the widespread belief that "green'' medicine is healthier than synthetic products. This has led to a rapid spurt in demand for health products like herbal tea, ginseng and such products of traditional medicine. The health promotive and disease preventive strategy in treatment is widely prevalent in the oriental system, especially the Indian (Ayurveda, Siddha, Unani and Amchi) and the Chinese. System of medicines is finding increasing popularity and accept ance world over. Because of this sweeping "green wave", a large number of herbal drugs and the plant derived herbal products are available in world markets.

Several studies continue on through the world to identify antioxidant compound that are pharmacologically potent with low profile of side effects. Ayurveda, the oldest medical system in the world, provides lots of lead to find active and therapeutically useful compounds from plants. Rasayana formulations in Indian traditional medicine may have antioxidant activity arising from individual plants, or may acts synergistically to prevent ageing and related degenerative diseases. Ethno pharmacological approach for

the selection of the candidates for screening has been preferred as the chances of hitting the target is more with this approach rather than with the rather than with the random screening.

It is increasingly being realized that a majority of the disease of today are due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the current life or due to the poor scavenging/quenching in the body to the depletion of the dietary anti-oxidants. Thus, anti-oxidant has become the most common prescription drug of today.

Thus, from the results of the present study, it was concluded that the ethanolic extract of stem of *Muntingi acalabura* has very good in-vitro anti-oxidant activity against DPPH method.

Reference

1. Satoskar RS, Bhandarkar SD, Ainapure SS. Pharmacology and Pharmacotherapeutics. Popular Prakashan, Mumbai, India.1999.
2. Fransworth NR, Morris RW. Higherplants-The sleeping giant of drug development. American Journalof Pharmaceutical Education,1976:148:46-52.
3. Cragg GM, Newman DJ, Snader KM. Natural Productsin Drug Discovery and Development. Journal of Natural Products,1997:60:52-60.
4. Zakaria ZA, Fatimah CA, Mat Jais AM, Zaiton H, Henie EFP, Sulaiman MR *et al*. The *in vitro* antibacterial activity of Muntingiacalabura extracts. Int. J. Pharmacol,2006:2(4):439-442.
5. Perez-Arbealaez E. Plants medicinales y venenosas de Colombia, Hernando Salazar, Medellin, Colombia 192, 1975.
6. Morton JF. Jamaica cherry. In fruits of warm Climates Miam, 1987, 65-69.
7. Chin WY. A guide to the wayside trees of Singapore. BP Singapore Science Centre 145, 1989.
8. Akerale O. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram,1993:28:13-19.
9. Kalia AN. A text book of Industrial Pharmacognosy. CBS Publishers & Distributors, New Delhi, India, 2005.
10. Kokate CK. Practical Pharmacognosy, New Delhi: Vallabhprakashan, 2005.
11. World Health Organization, WHO Quality control methods for medicinal plantmaterial, Genava, 1998.
12. Ansari SH, Essential of Pharmacognosy, Delhi: Birla Publication Pvt. Ltd, 2009.
13. Kokate CK, Purohit AP, Gokhale. Pharmacognosy, Pune: Nirali Prakashan, 2003.
14. Madhu CG, Devi DB. Protective antioxidant effect of vitamins C and E in streptozotocin induced diabeticrats. Indian Journal of Experimental Biology,2002:38:101-104, 2000.
15. Mensor LL, Meneze FS, Leitao *et al*. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res,2001:15:127-130.
16. Mistuda H, Yuasumoto K, Iwami K. Anti oxidation action of in dole compounds during the auto oxidation of linoleic acid, Eiyo To Shokuryo,1996:19:210-214.