In Vitro Anti Arthritis Activity of Muntingia Calabura Fruit

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Abstract— The present study was carried out to identify the hidden nutraceutical properties of the Muntingia calabura fruit. As natural, the entire Muntingia calabura plant had the ability to act as anti-cancer, anti-diabetic activity, anti-hyperglycemic activity, antioxidant activity, cardio protective activity, antipyretic activity, anti-inflammatory activity, acute toxicity, anti-proliferative activity, cytotoxic activity, quinone reductase activity, antiplatelet aggregation activity, hepatoprotective activity, gastroprotective activity, mycardial infarction, anti-hypertensive activity, anti-anxiety activity, anti-aging activity, and anti-hyperuricemic activity. Arthritis is a chronic inflammatory disorder that can be treated with anti-inflammatory drugs. Several medicinal plants show the potential to be used in arthritis therapy, one of which is Muntingia calabura fruit. The objective of this research was to study the anti-arthritic activity of Muntingia calabura fruits by using ethanol extract. The free radical scavenging was analyzed by DPPH (2,2-diphenyl-1-picrylhydrazyl). The bioactive components of Muntingia calabura were screened by phytochemical and qualitative analyses like the Mayers test, ninhydrin test, amino acids, protein test, saponins, terpenoids, Borntragners test, and Molisch's test. We underwent the qualitative and quantitative analysis of Muntingia calabura in some solvents like water, methanol, ethanol, chloroform, and ether. Therefore, ethanol extraction shows a higher concentration. Antioxidants are supplements present in Muntingia calabura fruits that are used to prevent inflammation disorders. Finally, we have analyzed the activity of anti-arthritis drugs in Muntingia calabura to cure or prevent the arthritis disorder.

Index Terms- Phytochemical, antioxidant, arthritis, muntinga calabura fruits.

I. INTRODUCTION

Muntingia calabura is commonly known as Jamaican cherry, strawberry cherry tree, jam tree, Chinese cherry, Madras Pea Pumpkin, Jam tree and Cotton Candy Berry, Singapore Cherry, Bird‟s Cherry, Calabura, Poor Man"s Cherry, Panama Berry, Pazham (Fruit), Japanese cherry (India) and cherry Chettu, Nakkaraegu (Telugu), Ten Pazham (Tamil), Paanchara (Marathi), Gasagase Hannina Mara (Kannada)[1].The calabur tree (*Muntingia calabura L.)* a plant belonging to the *Muntingia* genus and the Tiliaceae family. Although the plant is mainly distributed in tropical America, it can also be found in gardens and roadsides in Indonesia. This plant is rich in flavonoids, with flavones, flavanones, flavans, and biflavans as the major compounds and also contains polyphenols and steroids, which are anti-inflammatory compounds that can be used for the treatment of rheumatoid arthritis. The pharmacological activities of *Muntingia calabura* support its traditional use for the treatments of pain, fever, and inflammatory illness [2], and antibacterial [3], anti diabetic, antimicrobial, antioxidant, anti proliferative, and anti nociceptive agents [4].

II. METHODOLOGY

2.1 Preparation of sample

2.1.1 Selection of sample

The length of *Muntingia calabura* fruits were measured using measuring tape around 1-1.25 cm wide with red or sometimes yellow, smooth, thin, tender, skin, soft, and juicy pulp, with very sweet with minute yellowish seeds, too time to be noticed while eating.

Fig 2.1 Selection of sample

2.1.2 Physical characteristics

Muntingia calabura fruit is known for its vast medical properties such as Antioxidant, Antidiabetic, Anti-inflammatory, Antiulcer and Antipyretic, activity. The selection of fresh and fully ripe fruits sample of muntingia calabura were taken [5].

2.1.3 Procurement of sample:

The fruits required for the analysis were collected form the local street at sempatti near Gandhigram. The fresh fruit were washed thoroughly clean stored in refrigerator until used for analysis.

2.1.4 Preparation of Sample for fresh extract:

For the fresh extract the cleaned *Muntingia calabura* fruit selected and macerating in the mortar and pestle for the extraction 2g of fresh fruits were weighed and different solvent like Aqueous, Methanol, Ethanol, Acetone, Ether and chloroform are used in 20ml variation and incubate for 24 hours in a dark place, after incubation filter the extract and used for the further analysis.

Fig 2.1.3 Preparation of sample for fresh extract

2.2. Test for Phytochemicals

Preparation of Aqueous extract:

2g of fresh fruits are taken in a clean, dry test tube. To this add gradually 20 ml of water and heated at 60^0C using heating mantle few minutes for boiling. The samples are then cooled and incubated for 24hrs in a dark place. After incubation the samples are refrigerated at 4^0 C for further analysis.

Preparation of Ethanolic extract:

2g of fresh fruits are taken in a clean, dry test tube. To this add 20ml of ethanol and then incubated for 24 hrs in a dark place. After incubation, the samples are refrigerated at 4^0C for further analysis.

Preparation of Chloroform extract:

2g of fresh fruits are taken in a clean, dry test tube. To this add 20 ml of chloroform and then incubated for 24 hrs in a dark place. After incubation, the samples are refrigerated at 40 C for further analysis.

Preparation of Petroleum Ether extract:

2g of fresh fruits are taken in a clean, dry test tube. To this add 20ml of petroleum ether and then incubated for 24 hrs in a dark place. After incubation, the samples are refrigerated at 40 C for further analysis.

Preparation of Acetone extract:

2g of fresh fruits are taken in a clean, dry test tube. To this add 20ml of acetone and then incubated for 24 hrs. After incubation, the samples are refrigerated at 40 C for further analysis.

2.3.1. Qualitative Analysis of *Muntingia calabura* fresh fruits

The phytochemicals cure diseases without causing any harm to human beings these can also be considered as “manfriendly medicines”. The phytochemical analysis of Aqueous, Ethanol, Petroleum Ether, Acetone and Chloroform extract of fresh and dehydrated betel leaf are analyzed by qualitative chemical method are as follows[6].

Test for Alkaloids

Mayer’s Test

To a few ml of different extract of sample, two drops of Mayer’s reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

Test for Amino acids

The extract (100 mg) of sample is dissolved in 10 ml of distilled water and filtered through Whatmann No.

1 filter paper and the filtrate is subjected to test for Amino acids.

Ninhydrin Test
Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of filtered extract sample. Appearance of purple colour indicates the presence of amino acids.

Test for Carbohydrates
Molisch's Test
To 2 ml of different sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

Test for Glycosides
Borntragner's Test
To 2 ml of different filtered extract, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for Phenolic compounds
Ferric Chloride Test
The extract of different solvent was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

Test for Tannins
10 ml of bromine water was added to the few ml of different solvent extract. Decoloration of bromine water showed the presence of tannins.

Test for Terpenoids
2 ml of choloroform was added with 5ml of different solvent extract and evaporated on water bath and boiled with 3 ml of Con.H2SO4.

Test for Flavanoids:
Alkaline Reagent Test
The solution of different solvent extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavanoids.
Test for Proteins
The different solvent extract was dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for proteins.

Biuret Test
2 ml of different solvent filtrate extract was treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

Test for Saponins
The different solvent extract is diluted with distilled water and made up to 20 ml. The suspension is shaken for 15 minutes. A two cm layer of foam indicates the presence of saponins.

2.4 Total Anti oxidant activity of *Muntingia calabura* fresh fruits
DPPH radical scavenging activity
The free radical scavenging activity of samples was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) The scavenging activity for DPPH free radicals was measured according to the procedure described by [7]. An aliquot of 3 ml of 0.004% DPPH solution in methanol and 10 to 100 µl of plant extract/ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolourization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)=045}}{\text{A518 (control)}} \times 100$$

2.5 Anti Arthritis activity of *Muntingia calabura* fresh fruits

Inhibition of protein denaturation
Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi 1968 and Sakat

et al. 2010 with slight modification. 500 µL of 1% bovine serum albumin was added to 10, 20, 30, 40 and 50 µL of plant extract. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using

$$\text{Percentage inhibition \%} = \frac{\text{100-(O.D. of test – O.D. of product control) x100}}{\text{O.D. of Control}}$$

III. RESULT AND DISCUSSION

3.1 Physical characteristics of *Muntingia calabura* fruit

Table 3.1.1 Physical characteristics of *Muntingia calabura* fruit

S. No	Attributes	Physical character
1.	Size	Round or spherical.
2.	Shape	1-1.5 cm diameter.
3.	Colour	Green, crimson when mature.
4.	Peel Texture	Smooth, slender, succulent.
5.	Fruit Texture	Lightish-brown, velvety, pulp with sweet, musky flavour.
6.	Seed Texture	Extremely light yellow.

The physical characteristics of *Muntingiacalabura* fruits are rounded or spherical in shape, about 1-1.5 cm in diameter. It is green in colour and becomes crimson when fully mature. The fruit has a smooth, slender, succulent peel and a lightish-brown, velvety, tender pulp with a sweet, musky, fig-like flavor and visible extremely little yellowish seedy [8].

3.2 Qualitative analysis of *Muntingia calabura* fruit.

The qualitative analysis of *Mutiniga calabura* result obtained after the manual analysis .The sample of *Mutiniga calabura* are evaluated for qualitative analysis by using different solvent. The listed qualitative analysis namely protein, ninhydrin, amino acid, mayers, molisch's, saponin, terpenoid, borntragners have shown various result positive (+) and negative (-). The result shows higher solubility in the ethanol and aqueous solvent. The ethanol extract is used for the further analysis purpose.

3.3 Anti-oxidant activity of *Muntingia calabura* fruit

Table-3.3.1 Anti-oxidant activity of *Muntingia calabura* fruit

S.No	Concentration	% of Inhibition Ascorbic acid	Sample
1.	20 µl	34.91	30.10 %
2.	40 µl	45.61	46.27 %
3.	60 µl	68.82	70.00 %
4.	80 µl	74.34	75.50 %
5.	100 µl	89.23	91.28 %

The table 3.3.1 shows that the Anti-oxidant activity of *Muntingia calabura* fresh fruit sample 20 µl - 30.10 % , sample 40 µl - 46.27 %, sample 60 µl - 70.00 %, sample 80 µl -75.50 %, sample 100 µl-91.28 %.*Muntingia calabura* fruit shows higher amount of Antioxidant activity compare with Standard Ascorbic acid.

3.4 Anti-arthritis activity of *Muntingia calabura* fruit

Table 3.4.1 Anti-arthritis activity of *Muntingia calabura* fruit

S.No	CONCENTRATION	% INHIBITIONOFSAMPLE
1.	10 µl	08.09 %
2.	20 µl	40.60 %
3.	30 µl	48.90 %
4.	40 µl	57.20 %
5.	50 µl	70.82 %

Table 3.4.1 shows that the Anti-arthritis activity of *Muntingia calabura* fresh fruit sample10 µl-08.09 %,sample 20 µl-40.60 %,sample 30 µl-48.90 %,sample 40 µl-57.20 %,sample 50 µl-70.82 %.. *Muntingia calabura*shows Anti-arthritic properties. The higher concentration of 50 µl of the extract shows 70.82 % of Arthritic activity.

CONCLUSION

From the above study the *Muntingia calabura* fruit herbal products are either consumed in traditional medical setting or taken as food supplements. Several medicinal plants have been shown to offer an alternative to synthetic drugs in preventing and treating some chronic and mild diseases. So it's recommended for gaining health benefits. The higher concentration of anti oxidant properties in *Muntingia calabura*, it is often used to develop Pharmacological agents like drugs and tablets. We confirmed that the *Muntingia calabura* fruit has 70.82% of anti arthritis activity. We suggest that the *Muntingia calabura* fruit can be used for the prevention or curing of arthritis disorder.

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