ANTIOXIDANT, ANTICANCER, ANTIBACTERIAL PROPERTIES AND PHYTOCHEMICAL ANALYSIS OF LEAVES OF THE MIRACLE FRUIT PLANT, *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell

A dissertation work submitted to University of Kerala in partial

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CONTENTS

CHAPTER – 1

INTRODUCTION

Synsepalum dulcificum, commonly known as **miracle fruit** or **miracle berry**, is an evergreen shrub which belongs to the Family Sapotaceae, native to tropical West Africa. The plant is well known and grown for its fruits which makes sour foods taste sweet. Therefore it is used to sweeten palm wine and other beverages locally in West Africa.

1.1 Plant Description:

a. Systematic position

Kingdom: Plantae

Division : Magnoliophyta (Angiosperms)

Order : Ericales Bercht. & J. Presl

Family : Sapotaceae Juss.

Genus : *Synsepalum*

Species : *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell

(World Flora Online Consortium, 2017; Sapotaceae, World Flora Online Data, 2022)

Fig 1: *Synsepalum dulcificum* plant

b. Habit : dense shrub or small tree, usually not more then 5.5 metres in height and will be smaller when there are cultivated.

c. Stem : erect, woody, quadrangular, contains milky latex, aromatic.

d. Leaves: simple, oval, tapering at base with smooth margins and with reticulate venation, aromatic, clustered spirelike at the end of small branches.

e. Flower : flowers creamy-white, very small, clustered in leaf axils.

f. Fruit: oval shaped berry, fleshy, becomes scarlet red when ripens which contains white pulp and a single seed. The fruit contains **miraculin**, a protein based molecule which is responsible for making sour or bitter foods sweet, hence the species epithet 'dulcificum' meaning 'very sweet' (Pharm. J. Trans., 1852).

A glycoprotein, miraculin, results in the flavouring altering mechanism. In 1968, a Japanese researcher Kenzo Kurihara, first isolated miraculin from the fruit. Miraculin itself is not sweet, but it binds to the receptors on the taste buds and causes the acidic foods to be regard as sweet and the effect lasts for a half hour to two hours.

Looking into the history, miracle fruit had a specfic role in the production of beverages which are produced by the fermentation of maize, millet and palm wine and are named as Koko and Kenkey, the traditional drinks of West Africans. It has been also used in sour foods to taste as sweet (Akinmoladun, *et al*., 2016, Afolabi et.al., 2020). The glycoprotein, miraculin which inhibits the signals of sour taste from the brain by binding to the sweet receptors of the tongue (Liqing *et al*., 2014).

Apart from the flavour and colour, miracle fruit is a good source for functional food applications as it possess antioxidant activity (Zuxing *et al*., 2016). The miracle fruit contains enoromous amout of fundamental vitamins which includes vitamin A which is good in vision,

formation of healthy bone and immune system, vitamin C helps in the prevention of infections, vitamin E enhaces fertility and maintenance of cellular integrity and vitamin K which a crucial element for blood clotting. Essential amino acids such as lysine, leucine, isoleucine, phenylalanine, threonine *etc* and non-essential amino acids such as glycine, proline, serine, tyrosine are exclusively found in the miracle fruit (Njoku *et al*., 2015).

The leaves of *S. dulcificum* contains eight chemical compounds which includes, β-sitosterol and stigmasterol mixture, lupeol, lupeol acetate, lupenone, pheophytin-a, pheophytin-b and α-tocopheryl quinone (Chen *et al*., 2010). In type 2 diabetic rats, the leaf extracts of *S. dulcificum* which is rich in methanol and flavonoids have exposed the anti-diabetic potential (Obafemi *et.al*., 2017).

1.2 Role of Plant Medicines:

Medicinal plants are the most widely used medicines in non-industrialized societies because apart from the modern medicines, they are more cheaper and can be readily available. It was estimated to be $2.2 US Billion dollars as the global export value of the thousands of types of plants with suspected medicinal properties in 2012. Since medicinal plants are considered as a reserve of ingredients and therefore can be used in drug development either pharmaceuticaly, non-pharmaceuticaly or synthetic drugs. Besides this, for the development of human cultures around the world, these plants play a major role. Due to the presence of high nutrients, some plants are considered as a repository of nutrition and they include aloe, turmeric, ginger, pepper, green tea, etc (Mahtab, *et al*., 2016).

Oxidative stress and an increase in reactive oxygen species are the major effects of diabetes and these can be controlled by many plants as they contain several natural antioxidants, specifically flavonoids, tannins, vitamin E and C which have the ability to maintain β-cells execution and reduction in the glucose level in the blood. In the case of

diabetes mellitus, these medicinal are more affordable and have less complexity and are more effective when compared to synthetic drugs (Wesam *et.al*., 2016).

Herbal medicines have been in use since long before modern medicine existed, around one forth of the drugs directed to patients in modern medicine are extracted from medicinal plants. Three chief kinds of advantages may provided by the medicinal plants, financially to the people who harvest, process and distribute them for sale, healthier to the people who consume them as medicines and wider societies such as taxation income, job oppurtunities and a healthier labour force. (Shadia *et al*., 2016).

1.3 Medicinal Plants in India:

The country has a deep history conventional healing techniques, many of which list the use of these plants. For example, Hortus Malabaricus, the oldest printed book on Indian medicinal plants, a 12-volume dissertation on the medicinal plants of the Malabar region along India's west coast- dates back to 1678.

Medicinal plants compose an chief constituent of the plant resource spectrum of Kerala. Analysis upto the minute reveals that out of estimated 4600 flowering plants in Kerala, about 900 own medicinal values. Of the particulars, 540 species are appeared to occur in forest ecosystems. In Ayurveda and Sidha, the Indian system of medicine, indegenous or naturalized plants over 150 species are used. *Asparagus racemosus, Solanum anguivi, Desmodium gangeticum, Cissus quadrangularis, Pseudartheria viscida, Strobilanthes ciliatus, Dysoxylum malabaricum, Piper longum, Aristolochia indica, Ceasalpinia bonduc, Tribulus terrestris, Sarcostemma acidum, Baliospermum montanum, Aristolochia bracteolata* etc. are the major medicinal plants acquired from the forests of Kerala (ENVIS, Kerala).

1.4 Need for herbal drugs:

In India and Africa, 70% and 90% natives respectively depends on traditional medicine to aid to link up their health care needs. Over 90% of general hospitals in China have sub-divisions for herbal remedies and it accounts about 40% of all health care implemented (WHO, 2005).

The main ground for using herbal remedies are that it is economical, diminish the troubles about the adverse effects of synthetic drugs. The eminent use of herbal medicines is for health giving and treatment for incurable, as resistant to lethal conditions. Although, the consumption of folk medicines increases when allopathic medicines are worthless in the treatment of diseases, including in advanced cancer and to all appearances of new contagious diseases (Benzie *et.al.,* 2011).

1.4.1.Antimicrobial drugs

For the prevention and treatment of varied health disorders has been in practice from ages. Broadly, it is believed that the perils related with the herbal drugs is very less, but studies on consequential reactions are representing the need for the expansion of productive marker systems for isolation and identification of the solitary units. The utilization of plants for releiving purposes antedates the chronicles and forms the emergence of much of modern medicine. A great deal of standard drugs derive from the plant sources, in the ages ago, a mass of less potential drugs were plant based (Vadhana *et al.,* 2015) .

Antimicrobial drugs are chemical stuffs of natural or synthetic genesis that vanquish the growth of or demolish microorganisms which includes bacteria, fungi, protozoa, helminths and viruses. Antimicrobial drugs are extensively used in cosmetics, industrial biocides, topical medicaments and household products. Based on their concentrations, they can perform as disinfectants, preservatives or antiseptics (Dahanukar *et al.,* 2000).

1.4.2 Antioxidants

A free radical is any species capable of independent existence that contains one or more unpaired electrons. These radicals will be formed in the human body by the phagocytes and by the side effects of the chemicals and are unfasten by enzymic and non-enzymic anti-oxidant defence systems. When the anti-oxidant shielding is insufficient, the oxidative stress occurs and can destroy DNA, proteins, lipids and carbohydrates. To keep down the tissue injury in human diseases, may be one way forward is the usage of drugs with numerous mechanisms of protective action, includes anti-oxidant properties. Some naturally occuring anti-oxidants in the body are Ascorbic acid (vitamin C), Lactoferrin, Nicotinamide, Adenosine etc. Thiols, ebselen (anti-inflammatory), iron iron chelators (in thalassaemia, leukaemia, stroke, haemorrhagic shock, malaria, traumatic brain injury), etc. are synthetic drugs (Barry Halliwell, 1991).

1.4.3. Anticancer drugs

Cancer is observed as one of the overriding diseases that bring about morbidness and impermanence in millions of people intercontinentally and by virtue of its universality, there is doubtlessly extensive need to light on novel anti-cancer drugs. Even so, the traditional methods of drug disclosures and the process of development is long-lasting and extravagant. In order to minimize and save the time and expense, the implementation of *in silico* techniques and optimization algorithms in drug discovery projects can layout the solutions. 617 assorted anti-cancer drugs tagged as active chemicals and 2,892 natural products tagged as inactive phytochemicals and are noted that a few of the 2,892 natural products had the aptitude to be anti-cancer compounds, but the outcome of acceptance on the calibre of the forecast model was negligible (Anwar, *et al*., 2017).

Organizing experimental data and clinical evidences proposes that using the drug combination is a good way to tackle the tumour growth and metastasis concurrently.

Nevertheless, the deleteriousness of drug combination are collateral by the increase of drug numbers. Pharmacognenetics, drug sensitivity tests and cancer biomaker detecting and pharmacogenetics are drafted to sort out effectual and adequate numbers of anti-cancer drugs and abandon ineffectual drugs for commercial or medicinal reasons and medicament cogitations, includes nano-drugs or liposome-entrapped drugs (Lu DY, *et al*., 2015).

1.5 Need for Phytochemical analysis

Phytochemicals have a great potential as therapeutic agents. Due to the emergence of new infectious diseases, there should be a continuous and emergent need to discover new therapeutic compounds with various chemical structures and novel mechanisms of action (Mohan, *et al*., 2021). The phytochemical analysis helps to know the chemical constituents present in the plant through the extracts made using suitable solvents. This analysis may leads to a break through to the medical field and results in the production of natural drugs with less toxicity.

Phytochemical analysis were done in the extracts made from the fresh leaves of lemongrass, oregano, rosemary and thyme, along with the leaves of tulsi, neem , *Aloe vera* and bryophyllum using hexane, chloroform, ethanol, methanol and water as the solvents. This revealed the presence of tanins and saponins, Terpenoids and reducing sugars in methanol and ethanol extracts. Flavonoids were detected in the methanol extracts of tulsi and *Aloe vera* and anthraquinone is found only in the extracts of *Aloe vera*. These plants have the capacity to be an antimicrobial agent against MDR clinical isolates and therefore the extracts of these plants are also active against MDR bacteria under low level concentrations consequently reduce the possible toxic effects. This leads to the evolution of some stable, biologically active compounds which can be hired in the synthesis of antimicrobial agents (Praveen and Sharmishtha, 2012).

1.6 Importance of *S. dulcificum* in Medicine

S. dulcificum is a remarkable and magnificient shrub because of its marvellous pharmacological and nutritional values. Various components of the plant are useful for industrial and therapeutic purposes. The plant is recorded as one of the capitalistically dominant African medicinal plants with higher capabilities as food and nutritional adjunct (Lykke and Padonou, 2019).

The miracle fruit plant has inherent anticancer, anticovulsant, antioxidant, antihyperuricemia and cholestrol-lowering possessions among others. Particularly, the glycoprotein, miraculin has an appreciable nutritional and therapeutic assistance as a low-calorie sweetner and an antidiabetic agent. The evolution of drugs from the plant for the governance of diabetes and diabetic issues are practically viable areas of focus (Afolabi Clement *et al*., 2020).

1.7. Significance of the study

The interpretation of antimicrobial activities and phytochemical analysis of conventionally supreme medicinal plants give a lot of knowledge about their medicinal properties. This knowledge put an end to the unconsiousness of people about the primordial system of medication using various medicinal plants. Antimicrobial prospective of different medicinal plants is being widely experimented all over the world (Ahmad *et al*., 1998).

In this study, the evaluation of antimicrobial activities and phytochemical analysis of plants drew on standard procedures give sufficient scientific proof of their medicinal properties. Based on the informations, people can make use of the medicinal plants for the therapies of contagious diseases rather of other drugs which betray several side effects and restricted potency. The leaves of the *S. dulcificum* was foregather and scrutinize the

antimicrobial properties. The scientific researchers based on its antimicrobial activities has not been studied much. Consequently, the current exploration is sighted towards the inquiry of the antimicrobial properties of the plant, *S. dulcificum* and a preceding examination was also made on its phytochemical resources.

CHAPTER – 2

AIM AND OBJECTIVES

This study aimed to investigate the biological activities like- anti-bacterial, anti-oxidant, anti-cancerous potentials and phytochemical analysis of the leaf extracts of *S. dulcificum* (Schumach. & Thonn.) Daniell. The objectives are as follows:

i. To investigate the anti-bacterial properties of the plant *S. dulcificum* against pathogenic microorganisms.
ii. To examine the anti-oxidant activities of leaves of *S. dulcificum* to use it as a good source of food.
iii. To study the anti-cancerous potential effect of the *S. dulcificum* plant leaves.
iv. To analyze the phytochemical compounds to evaluate the phenols, flavonoids, carbohydrate, protein and lipids.

CHAPTER – 3

REVIEW OF LITERATURE

To keep down the tissue injury in human diseases, may be one way forward is the usage of drugs with numerous mechanisms of protective action, includes anti-oxidant properties. Some naturally occuring anti-oxidants in the body are Ascorbic acid (vitamin C), Lactoferrin, Nicotinamide, Adenosine, etc. Thiols, ebselen (anti-inflammatory), iron iron chelators (in thalassaemia, leukaemia, stroke, haemorrhagic shock, malaria, traumatic brain injury), etc. are synthetic drugs (Barry Halliwell, 1991).

The latex produced in papaya (*Carica papaya*) is a complex mixture of chemicals. A well-kwown proteolytic enzyme papain is the most important among them. An alkaloid, carpaine, terpenoids are also present which have the anti-bacterial properties and are bacteriostatic to *Bacillus subtilis, Salmonella typhi, Enterobacter cloacae, Proteus vulgaris, Escherichia coli, Staphylococcus aureus*. Ashwagandha (*Withania somnifera* root), Cauvery 100 (a mixture) and Livo-vet are the preparations done in the field of Ayurvedha in India which are used to treat animals as well as humans. They exhibit antimicrobial activities and additionally, immunomodulatory, antidiarrheal, psychotropic and anti-cancer properties (Marjorie, 1999).

The methanol and chloroform extracts from the fruit and pulp of *S. dulcificum* has a key role in anti-tyrosinase and anti-oxidant effects and therefore it could be potentially bid to food add-ons and medical cosmetology products (Chen, *et al*., 2009).

Through solvent partitioning and chromatographic separation, the methanol extract from leaves of *S. dulcificum* runs to five pure substances and the chemical constituents of its were separated with the help of column chromatography which results in the isolation of eight

compounds, inclusive of, a mixture of β-sitosterol and stigmasterol, lupeol, lupeol acetate, lupenone, pheophytin-a, pheophytin-b and α-tocopheryl quinone (Chen *et al*., 2010).

Phytochemical analysis were done in the extracts made from the fresh leaves of lemongrass, oregano, rosemary and thyme, along with the leaves of tulsi, neem , aloe vera and bryophyllum using hexane, chloroform, ethanol, methanol and water as the solvents. This revealed the presence of tanins and saponins, Terpenoids and reducing sugars in methanol and ethanol extracts. Flavonoids were detected in the methanol extracts of tulsi and aloe vera and anthraquinone is found only in the extracts of aloe vera. These plants have the capacity to be an antimicrobial agent against MDR clinical isolates and therefore the extracts of these plants are also active against MDR bacteria under low level concentrations consequently reduce the possible toxic effects. This leads to the evolution of some stable, biologically active compounds which can be hired in the synthesis of antimicrobial agents (Praveen and Sharmishtha, 2012).

The imminent constitution shows that *Synsepalum dulcificum* holds 59.55% moisture content, 3.26% fat, 7.75% protein, 4.36% ash, 18.84% carbohydrate and 6.24% crude fiber and the mineral inspection reveals that pulp of *Synsepalum dulcificum* carries 24.20ppm iron, 6.22ppm copper, 9.49ppm zinc, 100ppm calcium, 0.01ppm cobalt and 0.01ppm chromium while the vitamin estimation shows that it accommodates 0.04% vitamin A, 22.69% vitamin C, 0.01% vitamin D and 0.02% vitamin K (Chinelo Nkwocha, *et al*., 2014).

The biosynthesis of silver nanoparticles (AgNPs) using the leaf and seed extracts of *Synsepalum dulcificum*, heads to the fabrication of equitably spherical molecules of 4-26nm in size which expresses an utmost absorption at wavelengths of 438.5 and 440nm for the leaf and seed AgNPs, respectively. The FTIR spectra proves that the phytosynthesis of particles have rich phytochemicals, peculiarly phenolics and proteins in the extract. The two AgNPs expresses anti-bacterial and anti-fungal properties against *Aspergillus niger, A. fumigatus and A. flavus*

and also, they bring off approximately 80% deterioration of malachite green under ambient conditions. Simultaneously, AgNPs showed potent anticoagulant and thrombolytic activities. Hence, they could be used as nano catalysts to deteriorate dyes in effluents, as antibacterial agents and in nano medicine for the treatment of blood coagulation disorders (Agbaje, *et al*., 2016).

Imminent investigation, constituents' inquiry and phytochemical analysis were conducted on leaves and roots of *Synsepalum dulcificum* (miracle fruit). The imminent investigation done in the leaves sample results: crude lipid (12±2.00)%, crude fibre (17.5±0.50)%, ash (6.70±2.00)%, protein (6.62±0.02)%, carbohydrate (57.60±0.01)% and moisture(40.30±1.53)% while that of root samples showed crude lipid (7.5±1.4)%, crude fibre (20.30±1.53)%, ash (8.00±1.56)%, protein (5.6±0.07)%, carbohydrate (58.60±0.01)% and moisture (29.2±1.06)%. Meantime, the phytochemical analysis of the leaves revealed the results: flavonoids (2.8±0.2)%, polyphenols (3.52±0.10)%, alkaloids (0.6±0.20)%, cardiac glycosides (3.44±0.20)% and saponins (2.80±0.20)%. These concentrations thus make *S. dulcificum* an important source of phytomedicine (Osabor, *et al*., 2016)

In the type 2 diabetic rats, the methanolic and flavonoid rich leaf extracts of *S. dulcificum* have anti-diabetic potential (Obafemi, *et al*., 2017). The methanol extract of *S. dulcificum* leaves has noticeable *in-vitro* anti-oxidant activity and mineral content which points to the healthy potentiality especially against the diseases caused by oxidants. The IC50 of the extract for DPPH radical scavenging were 139.45 µg/ml (Olabisi, *et al*., 2017).

In *in-vitro* condition, α-glucosidase inhibitory and anti-oxidant activities and physiochemical properties (monosaccharide composition and molecular weight) shows sizeable differences in MFP-S and MFP-L in *S. dulcificum* (Huajun, *et al*., 2017).

The comparative study done in the leaves of *S. dulcificum* and *Morus alba* reveals that the crude extract of *S. dulcificum* develops an anti-bacterial component against *Listeria monocytogenes* and *Morus alba* from mature leaves of methanolic extract showed better performance (Wasoh, *et al*., 2017).

In order to minimize and save the time and expense, the implementation of in silico techniques and optimization algorithms in drug discovery projects can layout the solutions. 617 assorted anti-cancer drugs tagged as active chemicals and 2,892 natural products tagged as inactive phytochemicals and are noted that a few of the 2,892 natural products had the aptitude to be anti-cancer compounds, but the outcome of acceptance on the calibre of the forecast model was negligible (Anwar, *et al*., 2017).

Flavoring tablets were prepared through the effects formulated by the optimum ratio of *Synsepalum dulcificum: Siraitia grosvenorii* (1:3), which productively intervene the taste of children's medicine, thus decipher the practical issues regarding taking medications in children (Chen Liu, *et al*., 2018).

The antioxidant property of the extract showed up an appreciable protective effect in both the MSD and FSD on the kidney and liver against the toxicity induced by lead-acetate in Wistar albino rats (Obafemi *et al*., 2019).

S. dulcificum pulp extract carries high α-amylase and 2-glucosidase inhibitory activities and it exposes stronger inhibitory activities compared to standard drug, acarbose and hence it was integrated with yogurt results the highest α-glucosidase and α-amylase inhibitory activities and therefore the pulp can be expanded as utilitarian component with anti-diabetic activities in food application (Fazilah *et al*., 2020).

The miracle fruit plant has inherent anticancer, anticovulsant, antioxidant, antihyperuricemia and cholestrol-lowering possessions among others. Particularly, the glycoprotein, miraculin has an appreciable nutritional and therapeutic assistance as a low-calorie sweetner and an antidiabetic agent. The evolution of drugs from the plant for the governance of diabetes and diabetic issues are practically viable areas of focus (Afolabi Clement *et al*., 2020).

The investigation conducted by DPPH and FRAP assays in the extracts of seed and fruit pulp of *Synsepalum dulcificum* reveals that the anti-oxidant properties were higher in seed extract when compared to the fruit pulp extract (Gabriela, *et al*., 2020).

In the berries of *S. dulcificum*, a hemagglutinating activity analysis has been done. In this study, they conclude that due to its low hemagglutinating activity, *S. dulcificum* is harmless and is guaranteed by the comparison of hemagglutination levels with those of raspberry and blueberry (Adrián, Raquel *et al*., 2021).

The petroleum ether extract of *S. dulcificum* fruits employs higher antimicrobial activity than the ethanol extract. Expresses significant anticancer activity by the ethanol extract ($p<0.05$). The calculated half-maximal concentration (IC50) of the extract on HCT-116 cells at 24, 48 and 72h are 14.99, 8.97 and 8.54 µg/ml respectively, while the IC50 of the extract on PCE cell lines at 24, 48 and 72h are 236.25, 206.09 and 196.72 µg/ml respectively. Therefore, they can use an anticancer agent against colorectal cancer (Sheryar Afzal, *et al*., 2021).

CHAPTER-4

MATERIALS AND METHODS

The present investigation entitled "Anti-oxidant, Anti-cancer, Anti-bacterial Properties and Phytochemical Analysis of the Miracle Fruit Plant, *S. dulcificum*", was carried out, the following methodology and chemical analysis were applied to arrive at the results and to makeup lucidity of research design. This section therefore elucidate in detail, the chronological steps used for the research, which are evidently marked out under the following headings.

4.1 Collection of plant leaves

Selected species *S. dulcificum* (miracle fruit plant) samples were collected from the area of Puthiyakavu, Mavelikara, Alappuazha district (Figs. 2 &3).

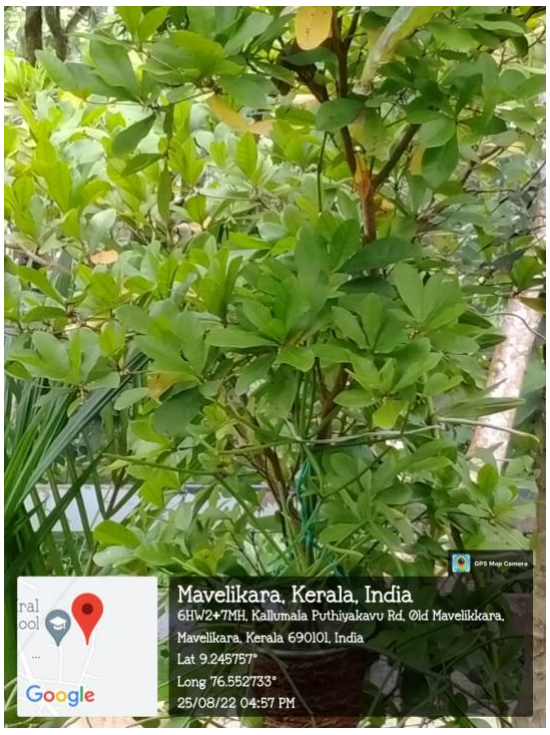


Fig. 2: *Synsepalum dulcificum* plant along with GPS location

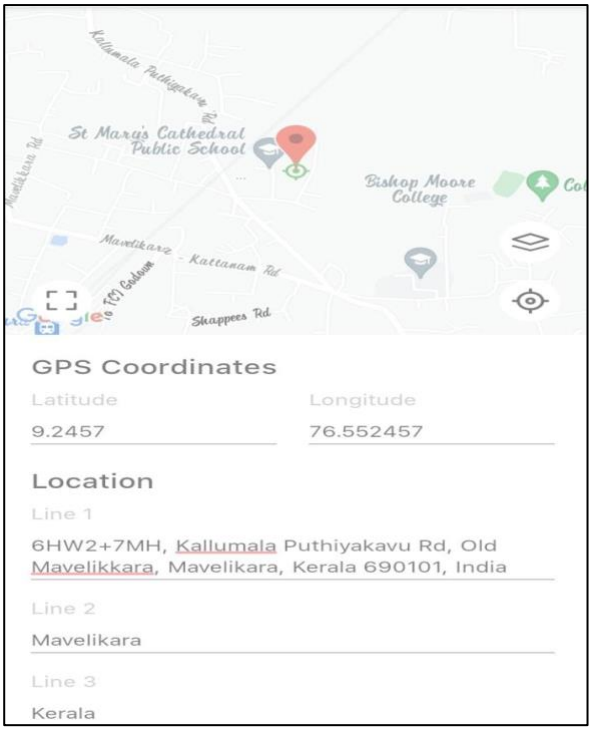


Fig. 3: Map of sample collection

4.2. Extraction of metabolites from *S. dulcificum* leaves

The samples collected were thoroughly washed several times using normal water and then followed by distilled water to remove the impurities. For the study, both fresh and dry samples of *S. dulcificum* leaves were prepared. For dried samples, the cleaned samples were subsequently dried under sunshade to remove the moisture content completely and powdered by using mechanical grinder. It was kept in dry place till use. The fresh samples were prepared by cleaning the leaves and immersing it in the different solvents such as ethanol, chloroform and distilled water in the ratio 1:4 for about 2 weeks.

- **Solvents used** : Organic solvents such as ethanol (w/v) and chloroform (w/v) and aqueous extracts (w/v) were used for the extraction procedure.

- **Extraction** : The coarse powdered leaves successively extracted with solvents of increasing polarity such as ethanol, chloroform and distilled water to obtain the extracts of *S.dulcificum* leaves.
- **Cold extraction** (Solvent extraction) : The plant material is extracted in ethanol, chloroform and distilled water. The organic solvents were added in a ratio of 1:4 (w/v) and kept for an overnight at room temperature. The extract were filtered and stored. Re-extracted is done with the same solvents respectively, obtain crude extracts.

4.3 Antioxidant Assay DPPH Radical scavenging Assay

- **Principle:**

Radical scavenging activity of the leaf extracts of *S. dulcificum* against stable 2,2-diphenyl 2-picryl hydrazyl hydrate (DPPH) was determined according to the method of Brand-William *et al*., (1995) with slight modification. DPPH reacts with an anti-oxidant compound, which can donate hydrogen and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at the optical density 515nm on a UV visible spectrophotometer.

- **Procedure:**

For DPPH assay the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1mg/ml., w/v). a 60µM solution of DPPH in methanol was freshly prepared and a 200µl of this solution was mixed with 50µl of the leaf extract at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800 µg/ml). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank.

Radical scavenging activity was calculated by the following formula

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

4.4 Anti Cancer Assay by MTT

- **Principle:**

The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NSD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan (Mosmann *et al.*, 1983). The insoluable formazan crystals are dissolved using a solubilizing solution (100% DMSO) and the resulting purple colored solution is quantified by measuring absorbance at 750nm using an ELISA plate reader.

- **Procedure:**

Human melanoma cell lines (SK-MEL) (2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37º C and 5% CO_2 environment in the incubator for 24 hours. The test samples were prepared in DMEM media (100 mg/ml) and filter sterilized using 0.2µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, 100 µg/ml respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in oreder to minimize errors. After treatment with the test

samples the plates were further incubated for 24 hours. After incubation period, the media from the wells were aspirated and discarded. 100µL of 0.5mg/ml MTT solution in PBS was added to the wells. The plates were further incubated for 2h for the development of formazan crystals. The supernatant was removed and 100µL DMSO (100%) were added per well. The absorbance at 570nm was measured with micro plate reader. Two wells per plate without cells served as blank. All experiments were done in triplicates. The cell viability was expressed using the following formula:

$$\textbf{Percentage of cell viability} = \frac{\textbf{Average absorbance of treated}}{\textbf{Average absorbance of control}} \times 100$$

- **IC 50 value:**

The IC 50 value is the half maximal inhibitory concentration of the sample. The IC 50 values were calculated using the equation for slope (y=mx+C) obtained by plotting the average absorbance of the different concentrations of the test sample (6.25-100 µg/mL) in Microsoft Excel.

4.5 Assessment of Antibacterial properties in the leaves of *S. dulcificum* using Agar well Diffusion method

- **Procedure:**

Agar well diffusion is widely used to evaluate the antimicrobial activity of the tset sample. Mueller-Hinton agar (15-20mL) was poured on glass petriplates of same size and allowed to solidify. Standardized inoculum of the test organism was equally spread on the surface of the plates using sterile cotton swab. Four wells with a diameter of 8mm (20mm apart from one another) were punched aspetically with a sterile cork borer in each plate. The test samples (leaf extracts of *S. dulcificum*, 100µL) were added into

the wells directly from the samples. Gentamycin (40µl from 4mg/ml stock) and the solvent used for sample dilution were added as positive and negative control respectively. The plates were incubated for 24 h at 36ºC ± 1ºC, under aerobic conditions. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

- **Culture Media Details:**

Muller Hinton Agar medium (HI Media, Mumbai-M173) is used for determination of susceptibility of microorganisms to antimicrobial agents. Suspend 38 grams in 1000ml distilled water. Heat until it boils to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121º C) for 15 minutes. Cool to 45-50ºC. mix well and pour into sterile petri plates.

- **Test Organisms:**

Inoculums were procured from The Microbial Type Culture Collection (MTCC) Chandigarh. The microorganisms used for the test are *Escherichia coli* (MTCC443), *Staphylococcus aureus* (MTCC87), *Vibrio cholerae* (MTCC3906) *and Serratia marcescens* (MTCC86). They are incubated at 37℃ for 24 hours. *Escherichia coli, Vibrio cholerae* and *Serratia marcescens* belongs to Gram-negative bacteria whereas *Staphylococcus aureus* belongs to Gram-positive bacteria.

4.6 Qualitative phychemical analysis of leaf extracts of *S. dulcificum*

The extracts using different organic solvents were screened for the qualitative analysis of different category of natural compounds using the procedure of Sofowora (1982). The first-

rate phytochemical compounds which are appreciable medicaments were examined in the present study and are:

1. Carboxylic acids
2. Coumarins
3. Flavonoids
4. Phenols
5. Proteins
6. Quinones
7. Resins
8. Steroids and phytosterols
9. Tanins
10. Phlobatannins
11. Xanthoproteins
12. Sugars
13. Glycosides
14. Alkaloids
15. Terpenoids
16. Carotenoids

4.6.1. Test for carboxylic acids

One ml of different extracts was individually served with few ml of saturated solution of Sodium bicarbonate ($NaHCO_3$). Detection of effervescence (due to the deliverance of CO_2) pointed out the the presence of carboxylic acids.

4.6.2. Test for Coumarins

One ml of ethanolic extract was treated with 10% NaOH solution and formation of dark yellow colour indicates the presence of coumarins.

4.6.3. Test for Flavonoids

- **Alkaline reagent test:** Crude extract was mixed with 2ml of 2% ammonium hydroxide (NH_4OH) solution. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid indicates the presence of flavanoids.
- **Concentrated H_2SO_4 test:** 1ml of conc. H_2SO_4 was added to the test solution. Formation of red colour indicates the presence of flavonoids.

4.6.4. Test for Phenols

- **Ferric chloride test:** Extracts were treated with 3-4 drops of aqueous 5% ferric chloride solution. Formation of bluish black indicates the presence of phenols.
- **Lead acetate test:** Extracts dissolved in distilled water is taken and to this 3ml of 10% lead acetate solution is added. A bulky precipitate indicates the presence of phenolic compunds.

4.6.5. Test for Proteins

- **Biuret test:** 1ml each of various extracts was warmed gently with 10% NaOH solution and a drop of diluted $CuSO_4$ solution. Formation of reddish-violet colour indicated the presence of proteins.
- **Ninhydrin test:** 1ml each of the various extracts was separately treated with few drops of Ninhydrin solution. Change in colour showed the presence of proteins.

4.6.6. Test for Quinones

A small amount of various extracts was treated with concentrated HCl and observed for the formation of yellow precipitate.

4.6.7. Test for Resins

1ml of various extracts was diluted with distilled water. Formation of bulk black precipitate indicates the presence of resins.

4.6.8. Test for Steroids and Phytosteroides

- **Salkowski's test:** To 2ml of the aqueous extract add 2ml of chloroform and 2ml of concentrated sulphuric acid (H_2SO_4). Shake well. If steroid present, the chloroform layer appear red and acid layer appear greenish yellow fluorescence.
- **Libermann's test:** The crude extract was mixed each with 2ml of chloroform and 2ml acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. Colour changes from violet to blue green indicated the presence of steroidal nucleus.

4.6.9. Test for Tannins

- **Gelatin test:** To 3ml of aqueous extract of plant sample add 2ml of 2% gelatin. Presence of curdy precipitate indicated the presence of tannin.
- **Ferric chloride test:** To the aqueous extracts of the plant sample, a few drops of ferric chloride solution were added. The colour changes indicates the presence of tannins.

4.6.10. Test for Phlobatannins

Plant extract was mixed with distilled water in a test tube, then shake well and filter. To each of the extract 1% aqueous HCl was added and then boiled using a hot plate stirrer. Formation of red coloured precipitate indicate the presence of phlobatannins.

4.6.11. Test for Xanthoproteins

1ml of each of various extracts was separately treated with a few drops of concentrated nitric acid and ammonia solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins.

4.6.12. Test for Sugars

- **Molisch's test:** To the extracts a few drops of alcoholic a-naphthol and 2ml of concentrated H_2SO_4 were added slowly through sides of test tubes. Formation of reddish brown precipitae indicated the presence of sugars.
- **Fehling's test:** A small portion of various filtrates were treated with 1ml of Fehling's solution 1 and 2 heated gently. Change in colour indicated the presence of sugars.
- **Anthrone test:** 1ml each of the various extracts in a watch glass were separately taken and mixed thoroughly using a glass rod with an equal quantity of anthrone reagent and a few drops of concentrated H_2SO_4 and heated on water bath. Formation of daerk green colour indicated the presence of sugars.

4.6.13. Test for Glycosides

- **Foam test (test for saponin glycosides):** Shake the plant extract vigorously with water. Persistent foam indicates the presence of saponin.

- **Keller-Kiliani test:** Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 rops of 2% solution of ferric chloride. The mixture was then poured into another test tube contaning 2ml of concentrated H_2SO_4. A brown ring at the interphase indicates the presence of cardiac glycosides.
- **Bromine water test:** The test solution was dissolved in bromine water and formation of yellow coloured precipitate indicates the presence of glycosides.

4.6.14. Test for Alkaloides

Extracts were dissolved in dil.HCl and then subjected to the following tests:

- **Dragendroff's test:** filtrates were treated with 1ml of Dragendroff's reagent. Formation of reddish-orange precipitate indicates the presence of alkaloid.
- **Wagner's test:** few drops of Wagners reagent are added to few ml of plant extract along the sides of test tube. A reddish-brown precipitate confirms the test as positive.

4.6.15. Test for Terpenoides

About 0.8g of the plant sample was taken in a test tube, 10ml of methanol was poured into it, shaken well and filtered to take 5ml extract. Then 2ml of chloroform were mixed in extract. Formation of reddish-brown colour indicates the presence of terpenoids

4.6.16. Test for Caroteinoides

1g of sample was extracted wih 10ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% H_2SO_4 was added. A blue colour at the interphase shows the presence of carotenoids.

4.7. Quantitative Phytochemical estimation of *S. dulcificum* extracts

4.7.1. Determination of Total Phenolic content

The total phenolic content was estimated by standard methods (Malik and Singh, 1980). The plant sample of 0.5g was taken and ground with 5ml of 80% ethanol in a mortar and pestle. Homogenates was centrifuged at 10,000 rpm for 20 min. Supernatant was collected and the pellet was re-centrifuged. Then both the supernatants were collected and dried. Residues were dissolved in 5ml of distilled water. 0.5ml of the aliquot was taken in a test tube, the volume was made up to 3ml with distilled water and 0.5ml of Folin-ciocalteau reagent was added in to it. After 3 minutes, 2ml of 20% Na_2CO_3 was added to the test tube and mixed it thoroughly. Test tubes were placed in the boiling water bath for 1min and cooled it in room temperature. Then absorbance was measured at 650nm against the blank.

Concentration of phenol present in the given sample,

$$= \frac{\textbf{Concentration of standard} \times \textbf{OD of sample} \times 100}{\textbf{OD of standard} \times \textbf{Concentration of sample} \times \textbf{Weight of sample}}$$

4.7.2. Determination of Flavonoid content

About 4g of sample was suspended in 4mlof methanol, the crude extract is filtered out 0.25ml of each suspension was transferred into a test tube followed by the addition of 1.25ml distilled water and 5% $NaNO_3$. After 6 minutes, 15µl 10% aluminium chloride was added and the mixture left for 5 minutes in the dark. Adding 0.5ml 5% NaOH and 0.275ml of distilled water followed by drying in the oven, the dry weight was calculated.

Concentration of flavonoid present in the given sample=$\frac{\textbf{Final weight-initial weight}}{\textbf{2.5}}$

4.7.3. Estimation of Protein by Bradford's method

5g of the plant sample is weighed. Homogenize it in 5-10ml of phosphate buffer. Filter the extract through a double layered cheese cloth. Centrifuge the extract at 10,000rpm for 15 min. collect the supernatant, take an aliquot of the sample and make up to 1ml with extraction buffer. Add 1.5ml of Bradford reagent to all the test tubes and incubate at room temperature for 5minutes and read the absorbance at 595nm against the blank.

Concentration of protein present in given sample,

$$= \frac{\text{Concentration of standard} \times \text{OD of sample} \times 100}{\text{OD of standard} \times \text{Concentration of sample} \times \text{Weight of sample}}$$

4.7.4. Estimation of Carbohydrate content

The carbohydrate content was detected by Anthrone method. Take 1g of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature. Neutralized it with solid Sodium carbonate until the effervesence ceases. Made up the volume to 100ml and centrifuged, collected the supernatant and take 0.5ml for analysis. Prepared the standard by taking 0.2-1ml of the working standards, 1ml of water serves as blank and made up to the volume to 1ml in all the test tubes with distilled water, then added 4ml of Anthrone reagent, heated for 8 min in a boiling water bath, cooled rapidly and read the green to dark green colour at 630nm.

Concentration of phenol present in the given sample,

$$= \frac{\text{Concentration of standard} \times \text{OD of sample} \times 100}{\text{OD of standard} \times \text{Concentration of sample} \times \text{Weight of sample}}$$

4.7.5. Estimation of Total Lipid content

The amount of lipid in the sample was identified by Folchin method. 3g of powdered leaf sample was suspended in 50ml of chloroform (2):methanol (1) mixture. The mixture was mixed thoroughly and kept for 3 days. The solution was filtered. The chloroform content was evaporated by heating and the remaining consist of the lipid. Then measure the dry weight.

Concentration of lipid present in the given sample=Final weight-initial weight.

CHAPTER-5

RESULT AND DISCUSSION

5.1. ANTI-OXIDANT ASSAY

Radical scavenging activity of the leaf extracts of *S. dulcificum* against stable 2,2-diphenyl 2-picryl hydrazyl hydrate (DPPH), with Ascorbic acid as standard. It can be compared with test sample and ascorbic acid. Ascorbic acid showing IC 50 value value of 26.56mg/ml (Table 1) and test sample IC 50 at 806 µg/ml (Table 2; Fig. 4, Plate 1). This results shows that the leaf extracts of *S. dulcificum* contain moderate antioxidant activity.

Table 1: Anti-oxidant Assay (standard values using Ascorbic acid as standar)

Standard	Concentration (µg/ml)	OD at 515nm	% Of Inhibition
Control	-	0.9	-
ASCORBIC ACID (STANDARD)	1.56	0.82	8.89
	3.12	0.81	10.00
	6.25	0.77	14.44
	12.5	0.66	26.67
	25	0.49	45.56
	50	0.06	93.33
	100	0.06	93.33
	200	0.06	93.33

	400	0.06	93.33
	800	0.06	93.33
	1000	0.06	93.33
IC50	26.56		

Table 2. Table 1: Anti-oxidant Assay of *S. dulcificum* Leaves (test sample)

Sample	**Concentration (µg/ml)**	**OD at 515nm**	**% Of Inhibition**
Control	-	0.8610	
***S. dulcificum* leaf**	1.56	0.8544	0.76
	3.12	0.8416	2.24
	6.25	0.8368	2.81
	12.5	0.8331	3.23
	25	0.8245	4.23
	50	0.8213	4.61
	100	0.8173	5.07
	200	0.6307	26.74
	400	0.6133	28.76
	800	0.4302	50.03
	1000	0.4223	50.94
IC 50	806		

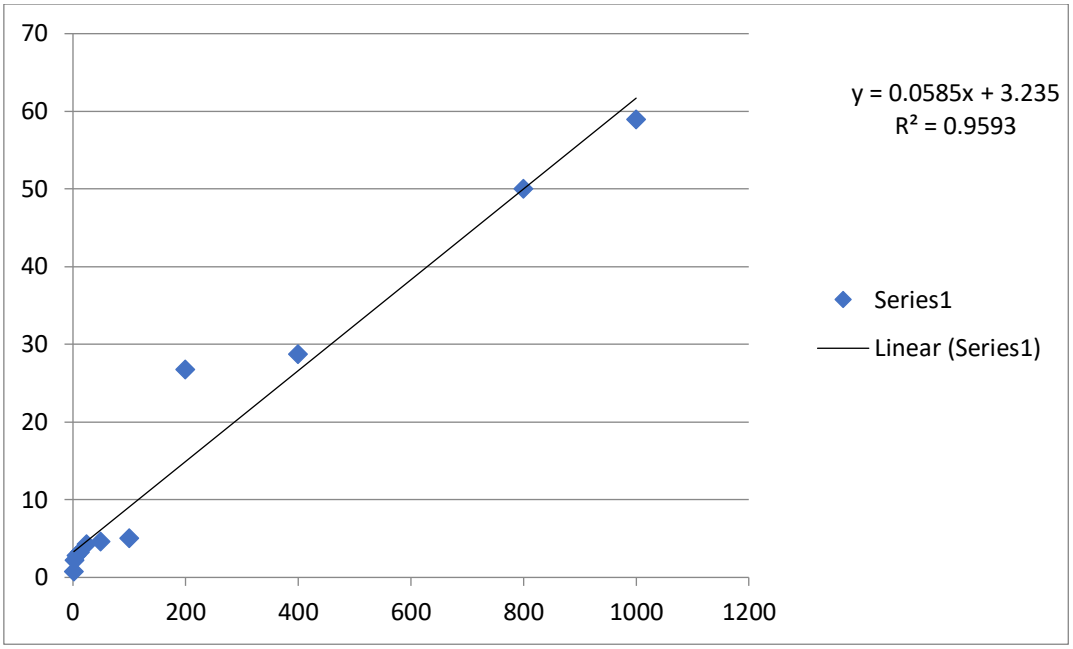


Fig 4: Anti-oxidant Assay of *S. dulcificum* Leaves

The above table and Figure expresses the anti-oxidant activity present in the leaf extract of *Synsepalum dulcificum*. The IC50 value of anti-oxidant activity of *Synsepalum dulcificum* is 806. The extract of *Synsepalum dulcificum* shows 50.03% percentage of inhibition when the concentration is 800 µg/ml. This reveals that the leaves of *Synsepalum dulcificum* have a moderate anti-oxidant property when compared with the standard.

5.2. ANTI-CANCER ASSAY

Table 3:Anti cancer activity leaf extracts of *S. dulcificum* (both standard and test)

	Triplicate 1	Triplicate 2	Triplicate 3	Average
Control	0.597	0.589	0.591	0.592
6.25	0.561	0.555	0.548	0.554
12.5	0.519	0.501	0.512	0.510
25	0.472	0.479	0.461	0.470

50	0.372	0.384	0.396	0.384
100	0.287	0.306	0.294	0.295
Concentration (µg/ml)	**Percentage viability**			**IC 50**
6.25	93.64			
12.5	86.21			
25	79.45			**94.03**
50	64.82			
100	49.91			

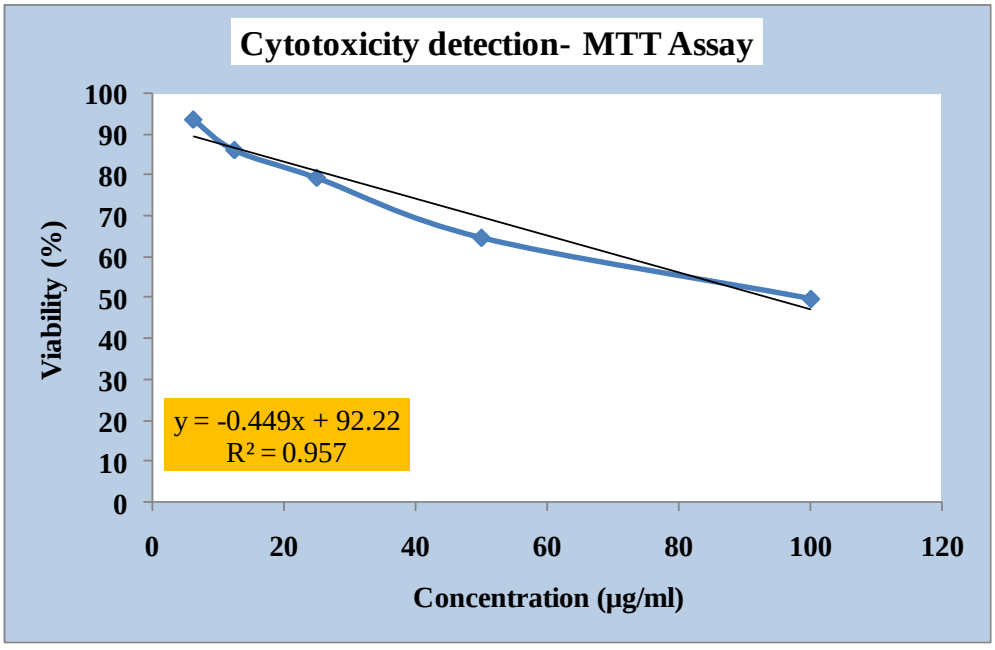


Fig. 5. Anticancer activity of *Synsepalum dulcificum* leaves

Anti-cancer activity was examined by MTT Assay. Table 3 given above reveals the result of anti-cancer activity of leaves of *Synsepalum dulcificum*. At the highest concentration-100µg/ml the extract showed 49.91 percentage viability. The IC50 value is 94.03 (Table 3; Fig.5; Plate 2). The most pescribed anti-cancer drugs was chemotherapy with docetaxel (21%), cyclophosphamide (19%), 5-Fluorouracil (18%) and epirubicin (18%) and hormonal therapies such as letrozole (17%) and tamoxifen (14%) (Bander et.al., 2020). When compared to these, the extracts of *Synsepalum dulcificum* have moderate anti-cancer property. Higher concentration is needed to equalize with docetaxel (21%).

Plants have unlimited capacity to produce substances that attract researchers in the quest for new and novel chemotherapeutics (Reed and Pellecchia, 2005). The results of this study reported similar activities in their study conducted by various groups against tumor. They reported the presence of alkaloids, phenyl propanoides and terpenes (Kintzios, 2006; Park e*t al*., 2008)

5.3. ANTI-BACTERIAL ASSAY

The anti-bacterial activities of the solvent extracts of *S. dulcificum* showed significant variations as shown in the Table 4 (Plates 3-6). All the three extracts tested shows equal anti-bacterial potential in different microorganisms. The maximum activity was observed against *Serratia marcescens* (21±1mm) using chloroform and simultaneously ethanol extract showed zone of inhibition (18±1mm) against *Serratia marcescens*. Distilled water extracts were effective against *Vibrio cholerae* (12±1mm) as well as in *Staphylococcus aureus* (9±0.5mm). The result of distilled water extract reveals that leaves of *Synsepalum dulcificum* can be used by making decoction of the leaves of *Synsepalum dulcificum*, as it have the anti-bacterial potential against *Vibrio cholerae* and *Staphylococcus aureus*. Ethanol extract have the anti-

bacterial potential against *Escherichia coli* (11±0.5mm) and chloroform extract have the anti-bacterial potential against *Vibrio cholerae* (15±1mm).

Table 4: Anti-bacterial Activity of *Synsepalum dulcificum* leaves extract (100µl)

Microorganisms	**Zone of Inhibition (mm in Dia)**			**-ve CONTROL (Gentamycin 160mcg)**	**-ve CONTROL**
	Ethanol (v/v)	**Chloroform (w/v)**	**D. W**		
Vibrio cholerae	-ve	15±1	12±1	30±0.5	-ve
Serratia marcescens	18±1	21±1	-ve	30±0.5	-ve
Escherichia coli	11±0.5	-ve	-ve	28±0.5	-ve
Staphylococcus aureus	-ve	-ve	9±0.5	34±0.5	-ve

(Values are the average of Triplicates; ±sd of the triplicates)

Strains of *Vibrio cholerae* O1, El Tor resistant to multiple antimicrobial agents (Finch et,al., 1988). The chloroform extract shows an inhibition zone of 15±1 which have the antibacterial potential against the strains of *V. cholerae*. Strains of *Serratia marcesens* are resistant to carbapenem and are inherently resistant to colistin (K Moodley, et.al., 2018). The chloroform (21± 1) as well as ethanol (18± 1) extract shows high antibacterial potential against these strains.

Staphylococcus aureus have an antibiotic resistance against vancomycin and methicillin (Cassandra, 2017). Vancomycin was a strong antibiotic once, but now bacteria became resistant to them (Jessica, 2017) while methicillin was a penicillinase-resistant penicillin but there consumption causes interstitial nephritis (Sharon, 2007). To conquer this bacteria, extracts of *Synsepalum dulcificum* can be used to resist the bacterial infections as well as side-effects of drugs.

5.4. PRELIMINARY PHYTOCHEMICAL ANALYSIS

In the preliminary phytochemical analysis, the presence of various phytochemicals present in distilled water, ethanol and chloroform extracts of leaves of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell was examined. The results of preliminary phytochemical analysis are given in the Table 5.

From the table, the results for the presence of various phytochemicals in three different extract of leaves of *S. dulcificum* can be lay down. The result explains that the distilled water extract shows the presence of flavanoid, phenol, tannins, phlobatannins and glycosides. Ethanol expressed the presence of flavanoid, phenol, proteins, quinones, sterols and phytosterols, sugars and terpenoids. Chloroform showed the presence of proteins, tannins, phlobatannins, steroids and phytosterols, sugars and alkaloids.

Table 5: The preliminary phytochemical analysis in various extracts of *S. dulcificum*

Sl. no	**Phytochemicals**	**DW**	**Ethanol**	**Chloroform**
1.	Carboxylic acid	-	-	-
2.	Coumarins	-	-	-
3.	Flavanoids	+	+	-
4.	Phenols	+	+	-
5.	Proteins	-	+	+
6.	Quinones	-	+	-

7.	Resins	-	-	-
8.	Steroid and phytosterols	-	+	+
9.	Tannins	+	-	+
10.	Phlobatannins	+	-	+
11.	Xanthoproteins	-	-	-
12.	Sugars	-	+	+
13.	Glycosides	+	-	-
14.	Alkaloids	-	-	+
15.	Terpenoids	-	+	-
16.	Carotenoids	-	-	-

Positive (+) indicates the presence and negative (-) indicates the absence.

5.5. QUANTITATIVE ANALYSIS

The whole quantitative analysis done are drawn down in the following Table 6 and Fig.6.

5.5.1. Determination of total phenolic content:

Phenolic presence in the plants reveals that they are medicinally imoprtant plants which can be used as medicaments either in raw form or synthetic medicines produced using the extracts. Here, the leaf extracts of *S. dulcificum* was quantified using the method of Malik

and Singh, 1980. The leaf extracts of *S. dulcificum* contains phenolic compounds and the total phenolic compound was estimated as 12.5 mg/g.

5.2.2. Determination of flavanoid content:

Here, the leaf extracts of *S. dulcificum* was quantified using standard methods and the results are calculated. The extract of *S. dulcificum* contains flavanoids which are estimated as 0.152 mg/g.

5.2.3. Determination of protein content:

Protein content was examined using Bradford's method and the crude protein present in the extract of leaves of *S. dulcificum* are estimated as 3.43 mg/g, reveals sufficient amount of protein in the leaves and are much higher when compared to other proteinaceous leaves such as spinach.

5.2.4. Determination of carbohydrates:

Carbohydrate content was examined by using the Anthrone reagent and the solution reveals the presence and amount of carbohydrates present in the leaves of *S.dulcificum* and are evaluated as 6.97 mg/g.

5.2.5. Determination of lipid content:

Lipids are the vital constituent of plants which provides energy in the metabolic activities of the plants. These lipids are examined in the way as in the case of flavanoids and are assessed as 0.24 mg/g.

Table 6: Quantitative analysis of leaf extracts of *S. dulcificum*

SL. NO.	Phytochemical components	Content (mg/g)
1.	Phenol	12.5
2.	Flavanoid	0.152
3.	Proteins	3.43
4.	Carbohydrates	6.97
5.	Lipids	0.24

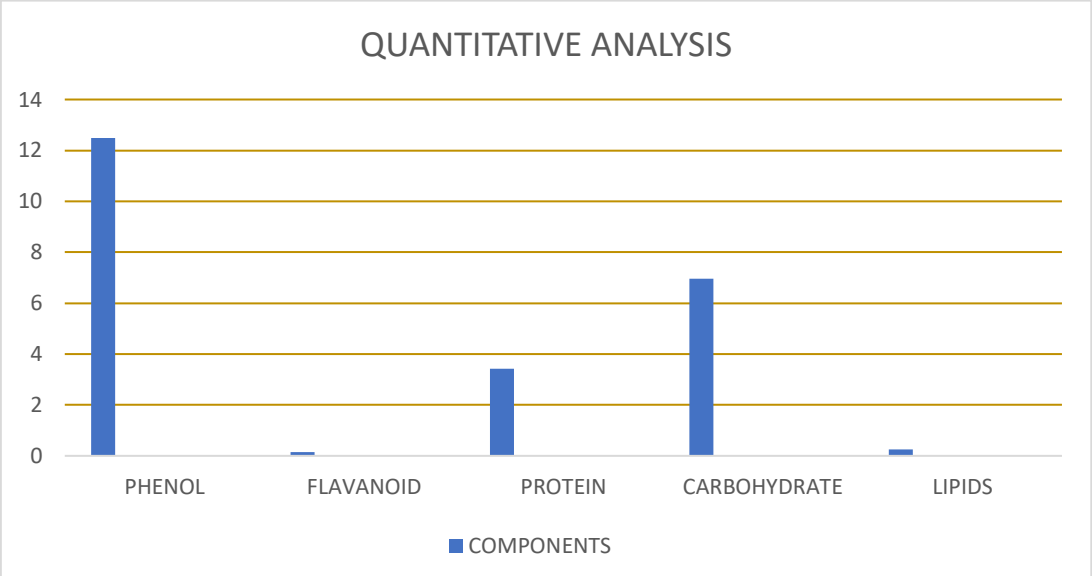


Fig. 6: Quantitative analysis of phytochemical contents in *S. dulcificum*

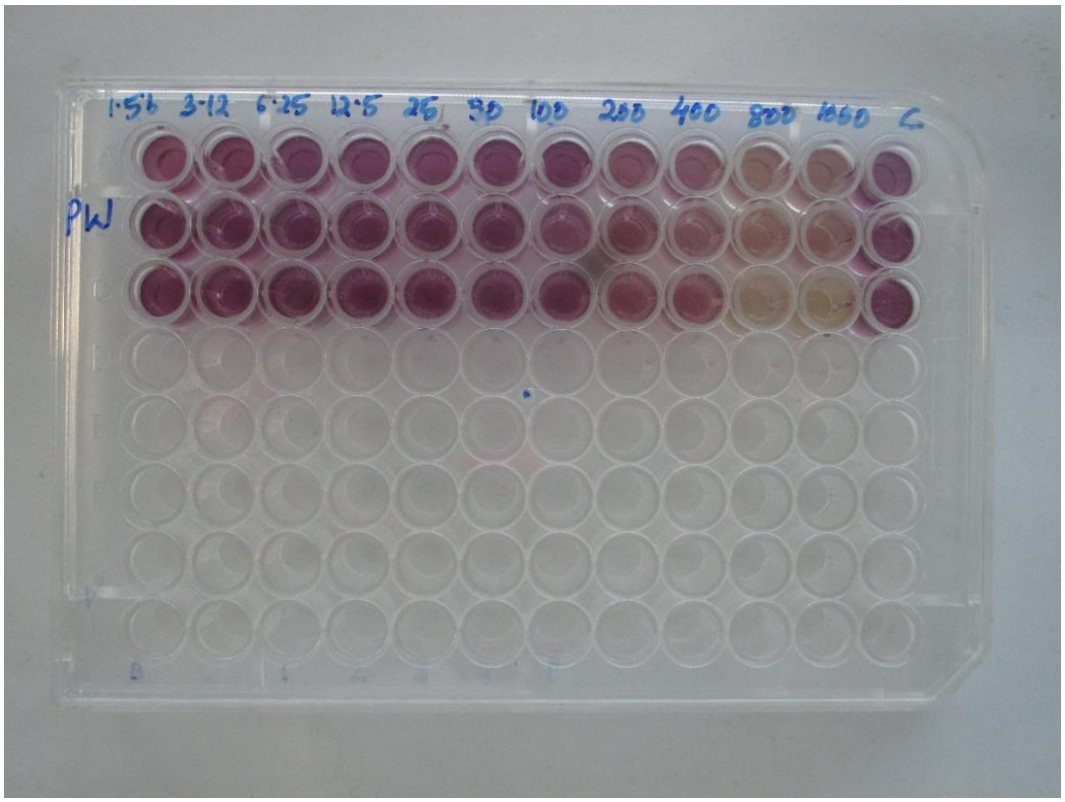
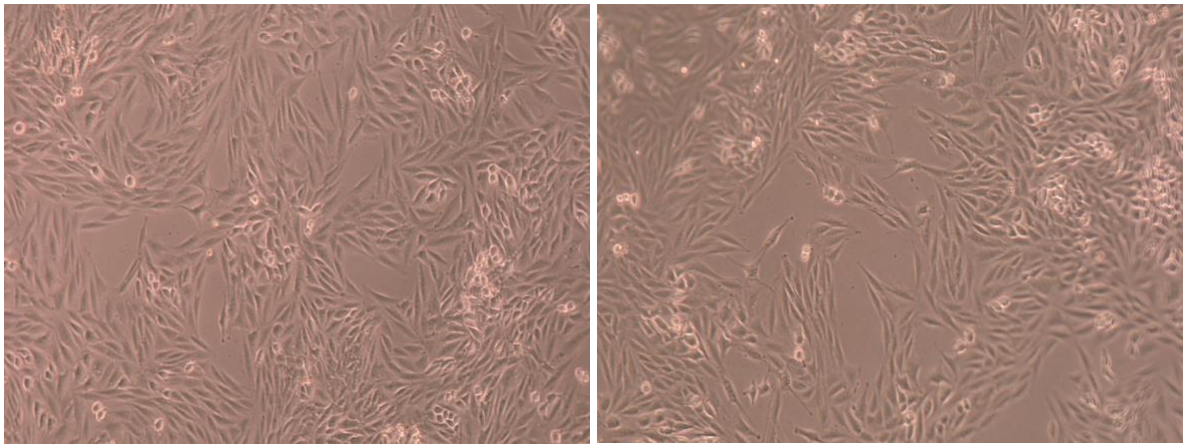
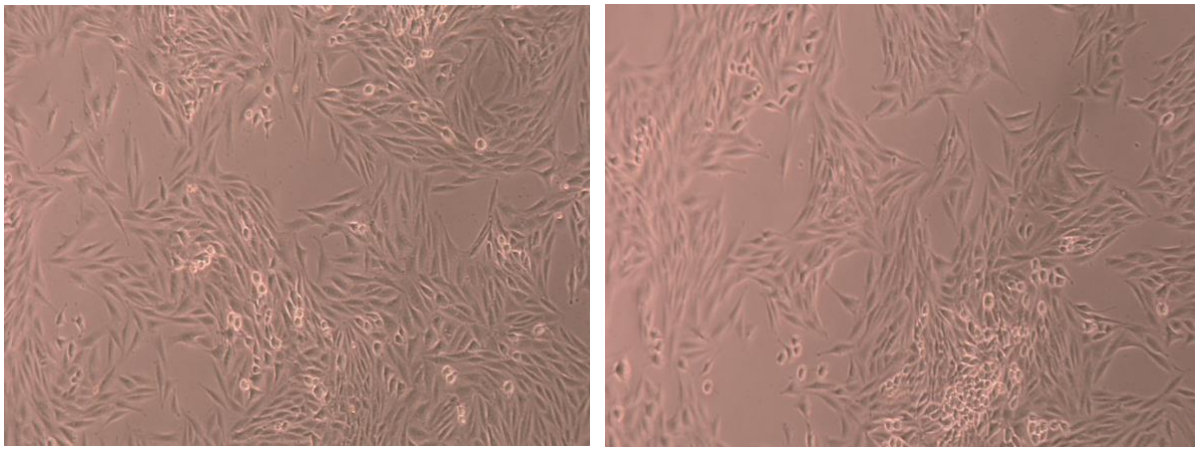


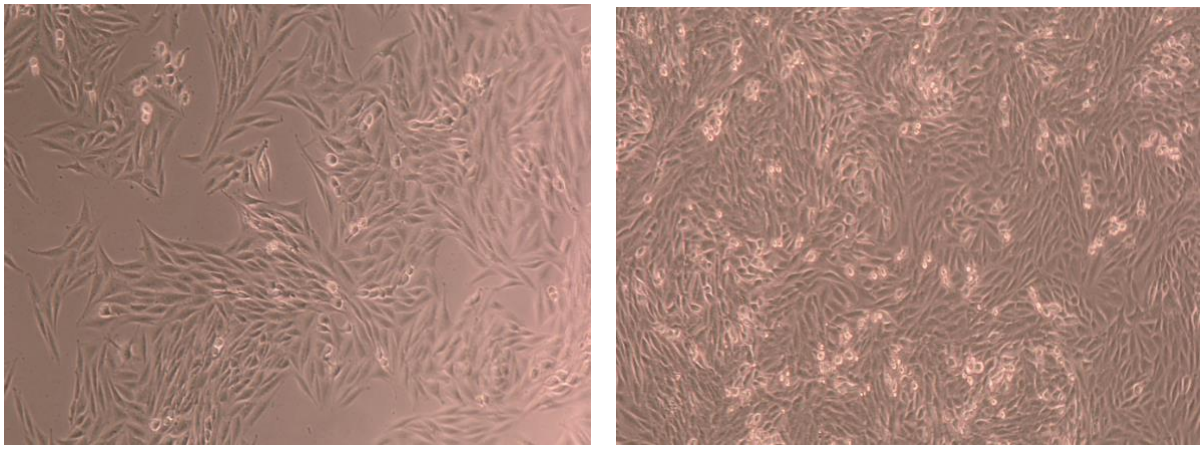
Plate 1. Antioxidant assay using DPPH radical scavenging method



A **B**



C **D**



E **F**

Plate 2: Anticancer assay using MTT method. (A- 6.25, B-12.5, C-25, D-50, E-100 and F-Control)

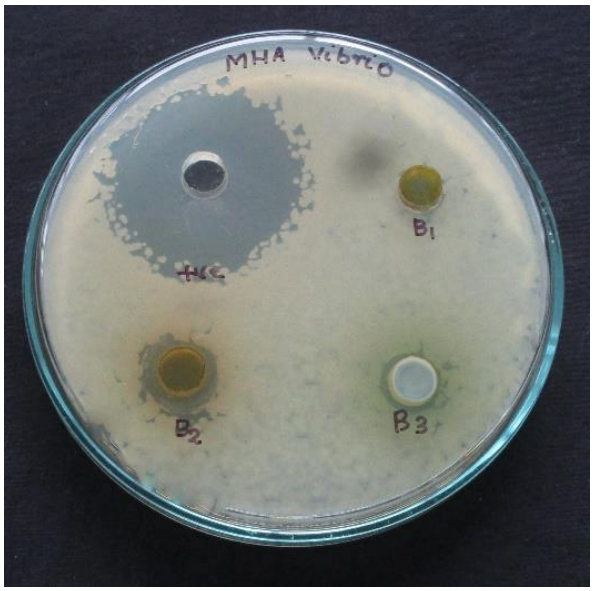


Plate 3. Antibacterial assay of *S. dulcificum* against *Vibrio cholerae*. B_1- ETHANOL EXTRACT, B_2- CHLOROFORM EXTRACT and B_3- DISTILLED WATER EXTRACT.

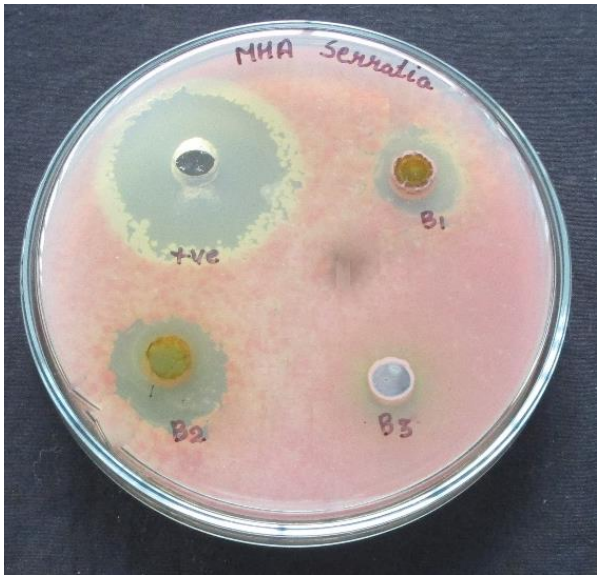
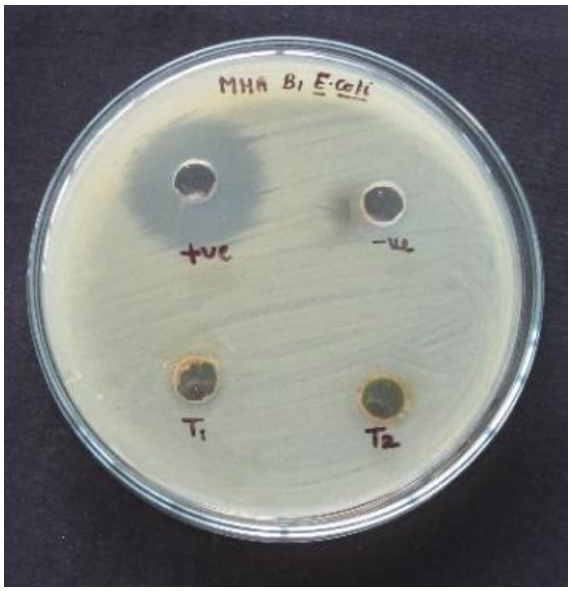
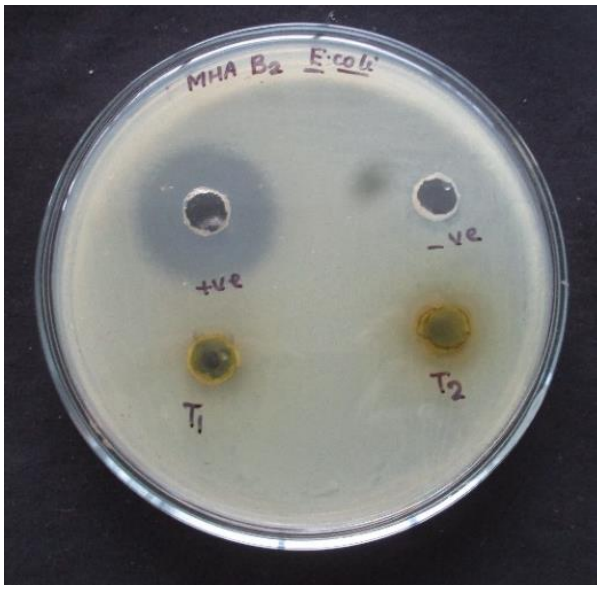
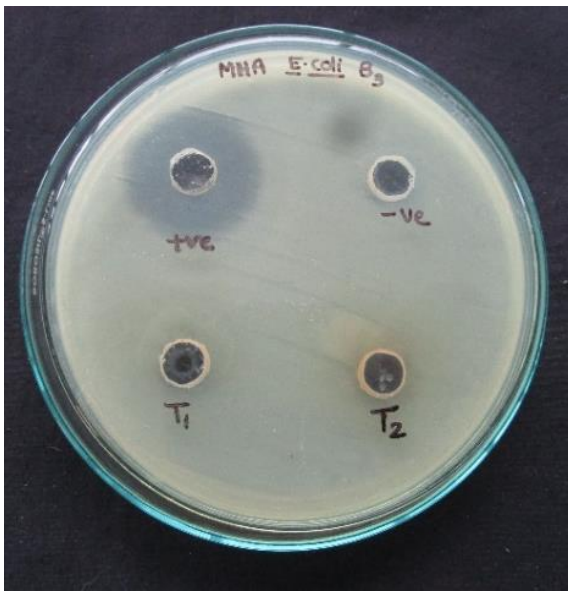


Plate 4.: Antibacterial activity of *S. dulcificum* against *Serratia marcescens*. B_1- ETHANOL EXTRACT, B_2- CHLOROFORM EXTRACT and B_3- DISTILLED WATER EXTRACT.



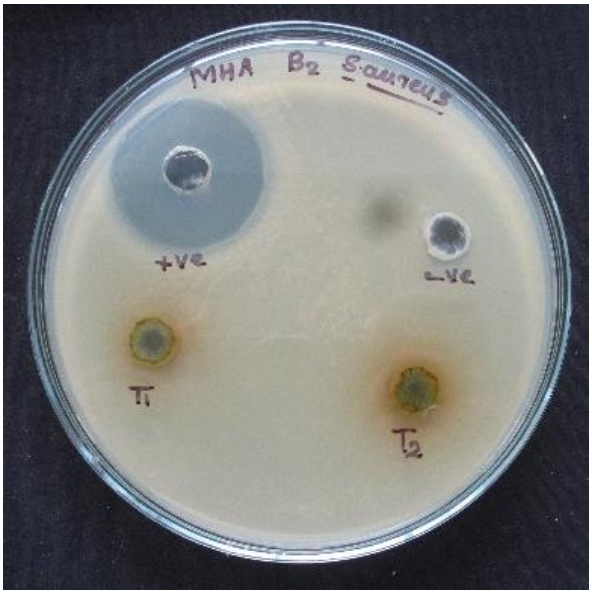


A- ETHANOL EXTRACT **B- CHLOROFORM EXTRACT**

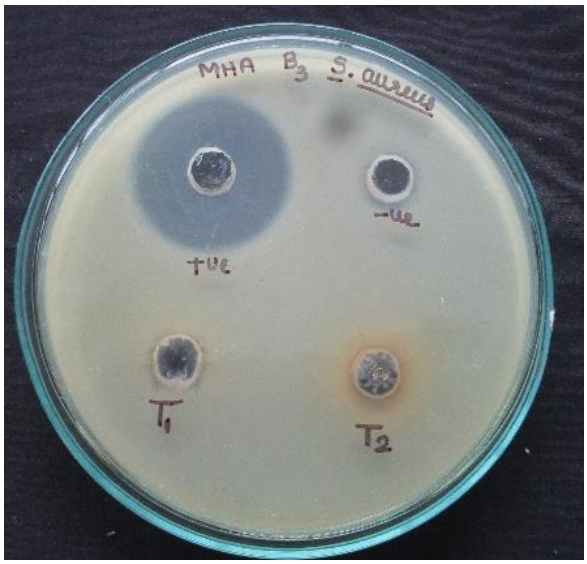


C- DISTILLED WATER EXTRACT

Plate 5: Antibacterial activity of *S. dulcificum* extracts against *Escherichia coli.*



A- ETHANOL EXTRACT **B- CHLOROFORM EXTRACT**



C- DISTILLED WATER EXTRACT

Plate 6: Antibacterial activity of *S. dulcificum* extracts against *Staphylococcus aureus*.

CHAPTER- 6

SUMMARY AND CONCLUSION

The project entitiled "Anti-oxidant, Anti-cancerous, Anti-bacterial Properties and Phytochemical Analysis of Leaves of The Miracle Fruit Plant, *Synsepalum dulcificum*", the leaves of *S. dulcificum* was analysed using different organic solvents (ethanol, chloroform) and distilled water for their anti-oxidant, anti-cancerous, anti-bacterial activities and qualitative and quantitative phytochemical analysis. Anti-oxidant analysis were done by DPPH radical scavenging assay using Ascorbic acid as standard, anti-cancerous by MTT assay, anti-bacterial by Agar Well Diffusion method. The major findings were summarized as below:

- In the analysis of anti-oxidant activity of extracts of leaves of *S. dulcificum*, Ascorbic acid is used as the standard. The IC50 value of anti-oxidant activity of *Synsepalum dulcificum* is 806. The extract of *Synsepalum dulcificum* shows minimal percentage of inhibition when the concentration is 800 µg/ml. This reveals that the leaves of *S. dulcificum* have a moderate anti-oxidant property when compared with the standard.
- In the analysis of anti-cancerous activity of extracts of leaves of *S. dulcificum*, the highest concentration- 100µg/ml the extract showed 49.91 percentage viability. The IC50 value is 94.03.
- The study shows the results of anti-bacterial activity of leaves of *S. dulcificum* against four various bacteria, *Escherichia coli, Vibrio cholerae*, *Serratia marcescens* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria). All the three extracts tested shows equal anti-bacterial potential in different microorganisms. The largest zone of inhibition were observed for chloroform against *Serratia marcescens* (21±1mm) simultaneously ethanol extract showed zone of inhibition (18±1mm) against

Serratia marcescens. Distilled water extracts were effective against *Vibrio cholerae* (12±1mm) as well as in *Staphylococcus aureus* (9±0.5mm). The result of distilled water extract reveals that leaves of *S. dulcificum* can be used by making decoction as it have the anti-bacterial potential against *Vibrio cholerae* and *Staphylococcus aureus*. Ethanol extract have the anti-bacterial potential against *Escherichia coli* (11±0.5mm) and chloroform extract have the anti-bacterial potential against *Vibrio cholerae* (15±1mm).

- The qualitative phytochemical analysis reveals the presence of various phytochemical components in three different organic solvents. The distilled water extract shows the presence of flavanoid, phenol, tannins, phlobatannins and glycosides. Ethanol expressed the presence of flavanoid, phenol, proteins, quinones, sterols and phytosterols, sugars and terpenoids. Chloroform showed the presence of proteins, tannins, phlobatannins, steroids and phytosterols, sugars and alkaloids.
- The quantitative analysis reveals the amount of protein (3.43 mg/g), carbohydrates (6.97 mg/g), phenol (12.5 mg/g), flavonoid (0.152 mg/g) and lipid (0.24 mg/g) in the crude extracts of leaves of *Synsepalum dulcificum*.

In conclusion, the result of the present study was on the anti-oxidant, anti-cancerous, anti-bacterial effect and scientific validation of the miracle fruit plant, *Synsepalum dulcificum*. The extracts of leaves have moderate anti-oxidant activity when compared to the standard (Ascorbic acid) with IC50 value of 806 and they also have anti-cancer potential at an optimum concentration of 100µg/ml with a percentage viability of 49.91 and IC50 value of 94.03. The antimicrobial activity of chloroform extracts showed maximum zone of inhibition against *Serratia marcescens*. Simultaneouly, it reveals that the distilled water have anti-bacterial potential against *V. cholerae* and *S. aureus*, therefore it can be consumed by making decoction. It also revealed the presence of many phytochemical constituents like phenols, flavonoids, proteins, aminoacids, glycosides, carbohydrates, lipids which may be responsible for their anti-

oxidant, anti-cancerous and anti-bacterial activities. Hence, the study justifies their use as a traditional medicine which is easily available, cost effective and no side effects.

CHAPTER-7

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#Originals not referred

APPENDIX

- COMPOUNDS USED FOR EXTRACTION

1. Ethanol
2. Chloroform
3. Distilled water

- PHYTOCHEMICAL COMPOUNDS

1. Alkaloids
2. Carboxylic acid
3. Coumarins
4. Flavonoids
5. Saponins
6. Phenol
7. Proteins

- OTHER COMPOUNDS USED FOR PHYTOCHEMICAL ANALYSIS

1. Dil. HCl
2. Dragendoff’s reagent
3. Sodium bicarbonate
4. NaOH
5. Anthrone reagent