BIOLOGICAL ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF TWO DIFFERENT FRUITS OF *MURRAYA KOENIGII*

A dissertation

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Submitted by:

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CANDIDATE'S DECLERATION

I, Kummamuru Laxmi Haritha, (2K22/MSCCHE/48) student of M.Sc. (Chemistry), hereby certify that the work which is being presented in this dissertation entitled *"***Biological activities and phytochemical analysis of two different fruits of *Murraya koenigii***"** which is submitted by me to the Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Masters, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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Date: 30th May 2024 **(2K22/MSCCHE/48)**

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CERTIFICATE

I hereby certify that the dissertation titled "**Biological activities and phytochemical analysis of two different fruits of *Murraya koenigii***" which is submitted by Kummamuru Laxmi Haritha, 2K22/MSCCHE/48 to the Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of science, is a record of the project work carried out by the student under my supervision. This dissertation embodies results of original work, and studies are carried out by the student herself and the contents of this dissertation do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Place: Delhi

Prof. RAJINDER KUMAR GUPTA

Date: 30th May 2024

(Supervisor)

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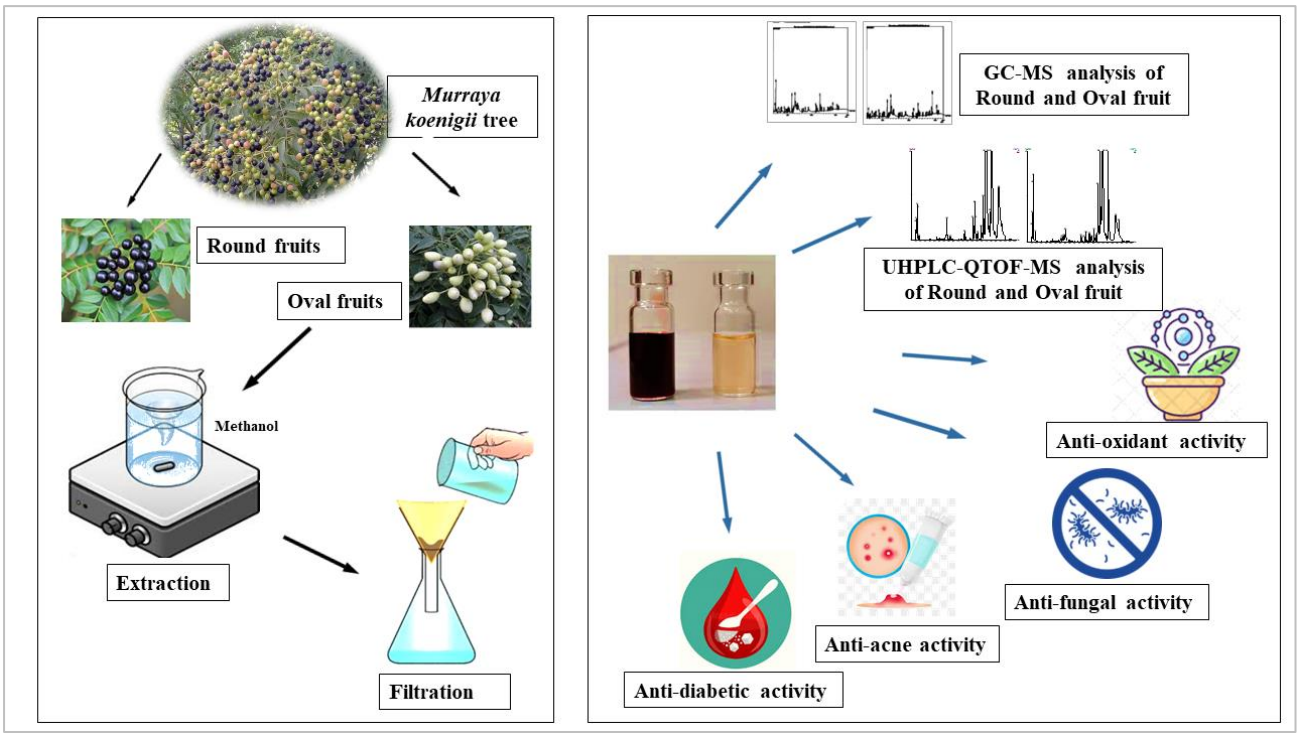
ABSTRACT

Murraya koenigii also known as curry leaves have been known for various traditional medicinal practices. This plant belongs to Rutaceae family. The purpose of this present study is examining the phytochemicals and biological profiling of methanolic extract of two different shaped, oval and round fruits of *Murraya koenigii* plant. The bioactive constituents were investigated using GC-MS. GC-MS determined the presence of pyranone (13.42%) and cis-vaccenic acid (9.68%) in the methanolic extract of oval and round fruits of *Murraya koenigii*. UHPLC-QTOF-MS analysis confirmed the existence of 50 secondary metabolites in the methanolic extract of oval fruit and 26 secondary metabolites in round fruits. These secondary metabolites include, Fosfomycin calcium, 3-Methyl-5-(trifluoromethyl)pyrazole, Dansylamide, N-glycylacrylamide, Thiodan sulfate, Biindoline and so on. In biological activities, extract of both the fruits of *Murraya koenigii* were analysed for antioxidant, antifungal, antiacne and antidiabetic activity. Among the methanolic extract of the two samples, round fruit showed better antioxidant activity than oval fruit sample. In case of antifungal activity of *Murraya koenigii* fruits, round fruits revealed slightly better activity towards *C. Albicans* in comparison to oval fruits. Oval shaped fruits showed higher anti-diabetic activity than the round shaped fruits. Additionally, the round shape fruits were most effective against *Propionibacterium acnes.* Overall, this study suggests that *Murraya koenigii* fruits have the potential to be used as a new medicinal food and can provide a new aspect to develop more potent therapeutics.

GRAPHICAL ABSTRACT



After collection of round and oval fruits, they were washed and cleaned separately. Methanolic extract was prepared of these fruits. Further, they were filtered and concentrated via rotary evaporator. The obtained extracts showed difference in colour. These extracts were used to evaluate the phytochemicals and biological profiling.

CONTENT

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List of Symbols and Abbreviations

S. No	Abbreviations	
1.	*M. koenigii*	*Murraya koenigii*
2.	MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
3.	BHT	Butylated hydroxytoluene
4.	FJMK	Fruit juice of *Murraya koenigii*
5.	DPPH	2,2-Diphenyl-1-picrylhydrazyl
6.	KRPH	Krebs-Ringer-phosphate-HEPES

CHAPTER 1
INTRODUCTION

For thousands of years, people all around the world have used plants as medicine. According to WHO estimates, 80% of people majorly in developing countries continue to rely on plant-based medicines for their primary care (WHO, 1978) [1]. Many distinct plant-based medical systems, including Ayurveda, Siddha, Unani, and regional health customs, are used in India to cure illness in humans and animals. These plants are referred to as medicinal plants. India is rich in many resources of nature and has a long history of practicing traditional medicine. Many physiologically active chemicals found in medicinal plants, including proteins, simple phenolic compounds, terpenoids, flavonoids, oils, and fats, can assist to extend life and ease illness. Natural products continue to be the foundation of the healthcare system, providing synthetic and traditional medicines that are herbal.

As plants contain a variety of components necessary for life, researchers have been looking into the potential applications of these plants in the treatment of chronic wounds and some infectious disorders [1].

Functional foods have grown in popularity in the health and wellness community in recent years. Functional foods, also known as nutraceuticals, are high in nutrients and have been linked to a variety of potent health advantages [2]. They may, for example, prevent disease, avoid vitamin deficits, and support appropriate growth and development. The use of both functional and nutraceutical foods is essential for the effective treatment of many lifestyle-related disorders, such as diabetes, obesity, and heart disease. Plant-based products are growing in popularity because there are so many components that are good for your health available now [3].

Secondary metabolites substances with antioxidant qualities are known as phytochemicals, and they are primarily present in plants. The various phytoconstituents found in medicinal plant extracts were identified through the initial screening of phytochemicals, which also offered insight into potential future medication development and discovery [4]. Since their actions are diverse and includes anti-analgesic, anti-viral, anti-cancer, and anti-microbial qualities, phytochemical components found in plant parts are regarded as physiologically active compounds. alkaloids, glycosides, saponins, oil and resins are examples of phytoconstituents that have a protective or disease-preventive effect. Because of the increasing need for medicinal plants antioxidant qualities, these plants are becoming more and more respected. Antioxidants are employed in the food business to stall the oxidation process [5].

Plants produce Phytochemicals that are bioactive compounds for self-defense. phytochemicals can be found in a wide range of foods, such as whole grains, nuts, fruits, vegetables and herbs. More than one thousand phytochemicals have been found thus far. In addition to having potent antioxidant properties, these phytochemicals have antiviral, antibacterial, anthelmintic, antiallergic, and antidiarrheal properties [6].

Curry tree, or *Murraya koenigii*, is a subtropical to tropical tree in the Rutaceeae family. This tree is indigenous to India, and its leaves have medicinal and culinary benefits. They are incredibly aromatic and have a distinct flavor with citrus undertones [1]. These trees are seen to grow two distinct kinds of fruit, these are green colour fruits which turn purple once they are ripe. These two fruits differ in shape, one is round and the other one is oval shaped. Curry leaves have a wide range of biological properties, including anti-inflammatory, anti-oxidant, and anti-diabetic effects.

These fruits are also known as curry berries. These fruits are a seasonally foraged fruit that grow in clusters on small trees up to 6 meters in height. They are prized for their unique flavor, which combines sweetness, tanginess, and pepper. Curry berries are a rare fruit that are sometimes obscured by the tree's leaves. They grow from pollinated blooms in the summer. *Murraya koenigii*, commonly called the curry tree, is grown commercially in India for its aromatic greens and is well-known as a home garden plant. The leaves are prized for their intricacy and flavor and are a common culinary component in Indian curries.

Animal models have demonstrated the anti-oxidative, liver-protective, antifungal, antibacterial, anti-inflammatory, and hepatoprotective capabilities of the essential oil extracted from *Murraya koenigii* leaves. The medicinal *Murraya koenigii's* effects are attributed to its chemical contents, including carbazole alkaloids, terpenoids, phenolics, flavonoids, carbohydrates, vitamins, carotenoids and nicotinic acid derived from several portions of the plant. It has gained popularity as a traditional medicine and home remedy. However, few investigations have been undertaken to assess the pharmacological and medicinal effectiveness of *Murraya koenigii* in aiding health and illness treatment [7], [8].

As discussed above, the present article aims to evaluate the bioactive components of *Murraya koenigii* fruit that are found in India by GC-MS and UHPLC-QTOF-MS analysis. Additionally, the in vitro antioxidant activity of the methanolic extract of round and oval fruit was assessed using DPPH radical scavenging activity, antifungal activity using disc diffusion method, antiacne activity against *P. acnes* and *in vitro* anti-diabetic activity which was analysed in multiple steps including MTT assay, α-glucoside, insulin secretion assay, DPP-IV activity assay, α- amylase assay, glucose uptake and reactive oxygen activity.

CHAPTER 2
LITERATURE REVIEW

Murraya koenigii is an underappreciated fruit that grows in the Indian subcontinent. The health advantages of this fruit are unclear, with limited scientific evidence available [9].

Recent years have witnessed the development of research on *Murraya koenigii* fruits. In a study described by Waghmare et al., Phytochemical analysis and in vitro anti-oxidant activity was performed on the fruits of *Murraya koenigii.* The phytochemical examination of extract of fruits of *Murraya koenigii* identified alkaloids, flavonoids, and phenolic compounds. Table 1 shows *Murraya koenigii* fruit extracts: a phytochemical analysis. The study also suggested that the plant's phenolic components enhance its antioxidative activity, making it useful in herbal medicine. The DPPH scavenging assay shows that the extracts of fruits are highly active. This plant extract can limit the generation of ABTS+ by scavenging DPPH, making it suitable for treating radical-related pathological damage, particularly at higher concentrations. The plant's potential for treating human ailments caused by oxidative stress requires more investigation in vivo[10].

Table 1. Phytochemical evaluation of fruit extracts of *Murraya koenigii*

S. No.	**Compound**	**Test**	**Inference**
1.	Flavonoids	Shinoda test	+
2.	Alkaloid	Lead acetate	+
		Wagner	-
3.	Phenolic content	Dragendroffs	+
		Ferric chloride test	+
		Libermann’s test	+

A study conducted by Tembhurne et al., *in vivo*, indicates that prolonged administration (28 days for repeated dose toxicity investigation) of FJMK reduces body weight along with loss of fat under the skin and blood sugar levels, as observed in different dose groups, respectively, while more research on *Murraya koenigii* fruits for biological activities, particularly hypocholesteremic, antidiabetic, and antiobesity, is required for its intended use[11].

Another study performed by Tembhurne et al., analysed the hypoglycemic effects of FJMK. Individual tissue vulnerability to oxidative stress is significantly influenced by its antioxidant content. Oxidative stress may contribute to tissue damage caused by diabetes and its consequences. Fruit juice of *Murraya koenigii*, administered orally, can reduce the effects of alloxan-induced diabetes in mice due to its antioxidant capabilities. This could be owing to the presence of biologically active substances in fruits such as koenimbine, murrayazoline, murrafoline, and pyrayaquinone-A[12].

Madhusudhanan et al. worked with essential oil that was isolated from *Murraya koenigii* (L.) Spreng's raw fruit and suggested that oil might serve as a good candidate for creating herbal-based therapy to treat a variety of ailments. Table 1 and 2 shows analysis of the *M. koenigii* fruit oil's chemical composition and its antioxidant activity *in vitro* [13].

Table 2 Chemical composition analysis of *Murraya koenigii* fruit essential oil

S.No.	**RT**	**RI**	**Compounds**	**%**
1	4.874	928	α-pinene	5.747
2	6.830	1029	β-phellandrene	92.832
3	13.363	1188	Terpineol	1.421
Total				100

RT= Retention time of compound

RI= Retention indices in reference to n-alkanes series

Compounds= Compound identification using MS NIST (2008) library data

%= Percentage of compound in total oil

Table 3 *In vitro* antioxidant activity of *M. koenigii* fruit oil		
***IC_{50} (µg/mL)**		
Assays	**Oil**	**BHT**
DPPH assay	95.858±0.03	140±0.02
Hydroxyl radical scavenging	289.25±0.04	140±0.02
Metal chelation	550.66±0.05	120±0.02
Inhibition of linoleic acid peroxidation	27.83±0.06	145±0.02
Prevention of deoxyribose degradation assay	27.05±0.02	75±0.02
*IC_{50}-inhibitory concentration 50% value expressed as the mean ±standard deviation of three replicates		

It has been demonstrated that the plant has potent anti-inflammatory and wound-healing properties that have been established by the hydroalcoholic liquid extract of fruits of *Murraya Koenigii*, which supports the plant's traditional usage in the treatment of inflammation and exterior wounds. The presence of flavonoids, either by themselves or in combination with other phytoconstituents as triterpenoids and tannins, may be the cause of these activities[14].

In a study by Aroor et al., The DPPH method was utilized to evaluate the antioxidant activity of the berry extract obtained from the berries of the *Murraya koenigii* (L.) Spreng (curry tree). Estimates were also made for total flavonoids, total phenolics, and reducing power. He concluded that Significant amounts of total flavonoids and phenolics are present in the *Murraya koenigii* fruit. These flavonoids and phenolics may be the cause of the extracts' increased capacity to scavenge free radicals and reduce them[15].

RESEARCH GAP

Reviewing the available research work on *Murraya koenigii* fruits, it was concluded that many biological activities such as Antiacne and Antidiabetic has not been explored. Also, a thorough research of the phytochemical compounds has also not been performed.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant material collection and authentication

Fruits of *Murraya koenigii* were gathered from trees on the DTU campus in New Delhi, India. Two varieties of fruits were collected: round and oval-shaped. The botanical specimens of *Murraya koenigii* fruits were verified by the Raw Materials Herbarium, Museum, CSIR NIScPR in Delhi (RHMD), India and the voucher specimen (NIScPR/RHMD/Consult/2023/4617-18) was submitted at the RHMD, Delhi. After collection of fruits, they were washed thoroughly and dried for further analysis.

3.2 Preparation of extracts

50g of two different types of fruits, oval and round shaped, were extracted with the solvent being methanol. The fruits were submerged in 5-6 ml of methanol. Further, it was kept on stirrer for the next 48 hours. The extract was concentrated, filtered and stored at 4º c for further use [16].

3.3 Phytochemical analysis

For the phytochemical analysis following tests were performed [17], [18];

Tannins:

Braymer's Test: 1 mL of the water extract prepared was taken in a test tube. To this, 2 milliliters of distilled water was added. Two to three drops of 5% $FeCl_3$ were added into the aqueous extract.

Observations: Blue-green colour

Flavonoids:

Ferric chloride test: Aqueous extract plus a few drops of a 10% solution of ferric chloride

Observations: A precipitate that is green

Glycosides:

Keller-Killani test:

Combine 1 mL of filtrate, 1.5 mL of glacial acetic acid, 1 drop of 5% ferric chloride solution, and along the test tube's side, conc. H_2SO_4 was added.

Observations: A solution with a blue hue

Terpenoids:

Salkowski test: 5 mL of extract, 2 mL of chloroform, and 3 ml of concentrated H2SO4 (test tube's sidewalls) were added to a test tube.

Observations: The appearance of a reddish-brown hue indicates the presence of terpenoids.

Saponins:

Foam test: In a test tube, 10 milliliters (mL) of distilled water were combined with three milliliters (3 mL) of the extract's aqueous solution. After the test tube was sealed and violently shook for approximately five minutes, it was left to stand for thirty minutes.

Observations: The possible presence of saponins was shown by the honeycomb foam.

Carbohydrates:

Molisch's test: Mix 2mL of extract with 2 drops of alcoholic α-naphthol and 1mL of concentrated H_2SO_4 (around the walls of the test tube).

Observations: A ring that was violet

Coumarins:

NaOH test: Plant extract + 10% NaOH + Chloroform

Observations: A yellow colour

Phenols:

Iodine test: 1 milliliter of extract with a few drops of diluted iodine solution

Observations: A fleeting shade of red

Proteins:

Xanthoproteic test: Extract of plants + a few drops of concentrated nitric acid.

Observations: a solution with a yellow hue.

Anthraquinones:

Ammonium hydroxide test: Isopropyl alcohol plus a drop of concentrated ammonium hydroxide solution are used to dissolve 10 mg of extract.

Observations: red colour formation after two minutes.

Phlobatannins:

HCl test: 2mL plant extract plus 2mL of 1% solution of boiled HCl.

Observations: A precipitate that was red.

Anthocyanins:

HCl test: 2 mL of plant extract, 2 mL of 2N HCl, and a few mL of ammonia were added to a test tube.

Observations: Pink-red solution that becomes blue-violet when ammonia is added.

3.4 GC-MS analysis

The quantitative study of volatile compounds in methanol extracts of both of *Murraya koenigii* fruits was performed using the GC-MS method (Shimadzu, GCMS-QP2010) established by Gomathi et al. The carrier gas used was pure helium gas in split mode at 260°C. The ionization method employed was electron ionization (EI), with a column flow

rate of 1.22 mL/min. At the interface, the temperatures and ion source were 220°C and 270°C, respectively. The oven temperature was varied between 50° C and 300° C for 2 min and 17 min after a 3.50-minute solvent delay. The analyses of all the identified peaks were done using NIST library (National Institute of Standards and Technology)[19].

3.5 UHPLC-QTOF-MS analysis

Secondary metabolites were qualitatively determined using the UHPLC-QTOF-MS method, as previously published by Perumal et al. The mass spectroscopic examination was conducted in positive mode (ESI+). The UHPLC system was built with an AD autosampler, and the column was a Waters nano Acquity HSS connected to a QTOF-MS. An Accucore C18 column with dimensions of 100 mm by 3 mm was used as the analytical column. The binary mobile phase, labeled A (water containing 0.1% of formic acid) and B (1% solution of formic acid and acetonitrile), was introduced with 5 µL of methanolic extracts (1 mg/mL) and eluted onto the column. For 25 minutes, A flow rate adjustment of 0.2 mL/min was made with a linear gradient. The components in both samples were identified using the ChemSpider program [20].

3.6 Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of the extracts was determined using the method published by Mensor et al. 5 µL of test sample of different strength (0, 0.78, 1.56, 3.125, 6.25, 12.5, 25 and 50 µg/mL) was incorporated to 0.1mL of 0.1mM DPPH solution in a 96 well plate. After incubation in the dark for 30 minutes, the absorbance at 495 nm of every sample was measured. Control was a reaction mixture with 20 mL of deionized water. The radical scavenging was calculated using the formula below [21].

3.7 Antifungal activity

The disc diffusion method is used to evaluate the antifungal activity in methanolic extract of *Murraya koenigii* fruits. First, 100 µL of Fungal culture, *Candida albicans* was put into sterilized Sabouraud dextrose agar petri plates and then putting 10 µL of different concentrations (0 to 100%) on the discs. One disk with 50 µg of amphotericin B served as the positive control and one disc with solvent served as the vehicle control for each plate. The bacterial strains were grown in an incubator for 24 hours at 37° C. The zone of inhibition was determined after the incubation period [22].

3.8 Antiacne activity

The disc diffusion method is used to determine the antiacne activity in methanolic extract of *Murraya koenigii* fruits. First, 100 µl of Bacterial culture, *Propionibacterium acnes* was put

into sterilized Mueller-Hilton Agar petri plates and then putting 10 µL of different concentrations (0 to 100%) on the discs. One disk with solvent on it served as the vehicle control in each plate, while another disc with 10 µg of ciprofloxacin served as the positive control. The bacterial strains were grown in an incubator for 24 hours at 37° C. The zone of inhibition was determined after the incubation period [23].

3.9 Anti-diabetic activities

3.9.1 α-Amylase assay

α-amylase inhibitory activity of *Murraya koenigii* fruits extract was tested using a slightly changed version of the procedure described by Telagari et al. Sample dilutions in sodium phosphate buffer of various concentrations (0, 0.78, 1.56, 3.12, 6.25, 12.5, 25 and 50 µg/mL) were made. 10mL of Enzyme solution containing 20 µg/mL α-amylase was added to a 96-well plate. The mixture was incubated after adding 10µL of samples, for 10 min. After incubation, 50µL of substrate (0.1% Soluble Starch) was incorporated to start the reaction, and after that, the mixture was incubated for a further fifteen minutes. Further, 100µl of GOD-POD Reagent was added to the mixture after 15 minutes. After 10 minutes of room temperature incubation, the absorbance of the plate was measured at 490 nm. Acarbose, an inhibitor, was used as a control positive at a final concentration of 50 µg/mL. Using formula 2, the α-amylase inhibitory activity was determined [24].

$$\%\text{inhibition} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}} \text{ x } 100 \qquad (2)$$

3.9.2 α-Glucosidase assay

With some slight modifications, the procedure mentioned by Telagari et al., was followed to evaluate the α-glucosidase inhibitory activity of extracts *Murraya koenigii* fruits. The reaction mixture with 10 µl of α-glucosidase and 50 µL of phosphate buffer (100 mM, pH = 6.8) and 20 µL of extract at various doses (0, 0.78, 1.56, 3.12, 6.25, 12.5, 25 and 50µg/mL) on a 96-well plate was preincubated at 37°C for 15 minutes. Next, a substrate of 20 µL of P-NPG (4-Nitrophenyl-β-D-glucopyranoside, 5 mM) was added, and the mixture was incubated for an additional 20 minutes at 7°C. To stop the reaction, 50µL of 0.1M Na_2CO_3 was added. The absorbance of the liberated p-nitrophenol was determined at 405 nm. As a Positive Control, acarbose (1 mg/mL) was used. The α-glucosidase inhibitory activity was calculated using formula 2 [24].

3.9.3 DPP-IV inhibitor activity assay

Using Gly-Pro-pNA as the substrate, the DPP-IV inhibitory activity were evaluated as previously described by You et al. A total of 100µL of Gly-Pro-pNA (0.5 mM Gly-Pro-pNA in 50 mM Tris buffer, pH 8.3, with 0.1% Tween 20) was combined with 10µL of both samples on a 96-well plate. The kinetics of DPP-IV activity was identified by keeping an eye on the rates at which pNA is released from the chromogenic substrate over a 10minute incubating period at 37°C. The absorbance was annalysed at 405 nm. The following formula 3 was used for calculations of DPP-IV inhibitor activity assay [25].

$$\text{DPP} - \text{IV activity} \left(\frac{\text{U}}{\text{mL}}\right) = \Delta\text{OD} \times \frac{\text{V}}{\varepsilon \times \text{v} \times \text{df}} \qquad (3)$$

ΔOD = Change in absorbance per minute at 405 nm.

V = Volume of the reaction mixture

v = Volume of sample

ε = Micromolar extinction coefficient at 405 nm (0.0102 μM^{-1} cm^{-1})

df = Sample dilution factor

3.9.4 Glucose uptake assay

The glucose uptake of extracts from *Murraya koenigii* fruits was determined using a slightly modified technique mentioned by Vishwanath *et al*. The cells were placed at a density of 8 × 104 cells/well in 96-well cell culture plates. Following that, they were put in a regular culture medium and allowed to incubate for 24 hours. Test individuals that served as positive controls were cells. controlling and unfavourable Mastery. Once the cells had been twice washed with Krebs-Ringer-phosphate-HEPES (KRPH) buffer (20 mM HEPES, 5 mM KH2PO4, 1 mM CaCl2, 136 mM NaCl, 4.7 mM KCl (pH 7.4)), they were grown for one hour using glucose-free DMEM (Dulbecco's Modified Eagle Medium).

Further, in a KRPH buffer including 2% (v/v) bovine serum albumin, the cells were treated for 40 min in the presence or 20 min in the absence of 10 mM 2-DG. Following three PBS washes to eliminate exogenous 2-DG, cells were lysed using extraction buffer, centrifuged at 500 rpm for two min and to eliminate endogenous nicotinamide adenine dinucleotide phosphate (NAD(P)), they were heated for 40 min at 85°C. Using the GOD-POD Enzyme Assay Kit, the 2-deoxyglucose-6-phosphate (2-DG6P) concentration of the resultant supernatant was determined and the plate was examined with a microplate reader at 505 nm. To get the blank value, lysates of cells that had not been treated to 2-DG were examined. By comparing the data to a standard, nanomoles of 2-DG were estimated. The standard was 2-

DG6P, 0.1 U/ml of insulin was employed as the control positive and 1 mg of metformin as the control negative [26].

3.9.5 Reactive oxygen activity (ROS) measurement

2'-7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) probe was used to produce ROS. L6 cells were cultured for 24 hours at 37°C after being plated in 6 well plates at a density of 5000–8000 cells/well in 1 mL DMEM medium supplemented with 10% FBS and 1% Antibiotic solution. Then cells were incubated for a further 24 hours at varying concentrations of round shaped fruit (412 µg/mL) and oval shaped fruit (392.3 µg/mL). The medium was withdrawn after incubation, and cells were collected using trypsin-EDTA in a 1.5 ml tube, and then once again washed with 500µL cold PBS. Further, a cell pellet was added to 100 µL of PBS containing 2µM DCFDA, and the samples were collected in an hour using a BD FACS Calibur flow cytometer. The acquired data were examined using the 2.5.1 version of the Flowing program [27].

3.9.6 Insulin secretion assay

Insulin was measured using immunoassay kit from DiaMetra (catalog no: DKO076). A total of 100 µL standard insulin, 100 µL sample, and 100µl Detection antibody (Conjugated Detection Antibody) solution was introduced to each well, the plate was closed and allowed to sit at room temperature for two hours. After incubation, the plate was rinsed four times using wash buffer and the buffer was blotted by vigorously tapping the plate on its side on absorbent paper. Subsequently, after adding 100µL of TMB substrate (3,3',5,5'-Tetramethylbenzidine), it was incubated in the dark for 15 minutes. After adding 100 µL of stopping solution to every well, the reaction was stopped and within 5 mins the absorbance was determined at 450 nm [28].

3.9.7 Cytotoxicity assay

MTT Assay

With few modifications, Cytotoxicity of fruit extracts on L6 cell line was determined by the procedure mentioned by X. In a 96-well plate, the cells (10000 cells/well) were cultured in DMEM (Dulbecco's Modified Eagle Medium) with 10% of FBS (Fetal Bovine Serum) and 1% antibiotic solution in an incubator with 5% CO2 for 24 hours at 37°C. Further, the cells were treated with several concentrations of sample (0, 1, 10, 50, 100, 250, 500 and 1000 µg/mL). After 24h, 10µL of MTT Solution was placed in each well and kept for incubation for an extra two hours. After that, 100 µL of Dimethyl Sulfoxide (DMSO) was incorporated for the removal of the culture supernatant and the dissolution of the cell layer matrix. The sample was then measured at 540 nm and 660 nm using an Elisa plate reader[29].

CHAPTER 4

Results and Discussion

4.1 Phytochemical analysis

The phytochemical analysis of the methanolic extract of *Murraya koenigii's* round and oval fruits revealed the existence of tannins, flavonoids, glycosides, terpenoids, saponins, carbohydrates, coumarins, and phenols, while proteins, anthraquinones, phlobatannins, and anthocyanins were absent, as shown in Table 4.

Table 4 Phytochemical analysis of *Murraya koenigii* fruit extracts

Phytochemicals	Results		
	Test name	Round fruit extract	Oval fruit extract
Tannins	Braymer's test	+	+
Flavonoids	Ferric chloride test	+	+
Glycosides	Keller-Killani test	+	+
Terpenoids	Salkowski test	+	+
Saponins	Foam test	+	+
Proteins	Xanthoproteic test	-	-
Anthocyanins	HCl test	-	-
Carbohydrate	Molisch's test	+	+
Anthraquinones	Ammonium hydroxide test	-	-
Phlobatannins	HCl test	-	-

Coumarins	NaOH test	+	+
Phenols	Iodine test	+	+
"+" = Presence, "-" = Absence			

4.2 Gas chromatography-mass spectrometry analysis

Table 5 lists the chemical components discovered during GC-MS analysis of methanolic plant extracts of *Murraya koenigii* fruits. The GC-MS study of *Murraya koenigii* plant's round fruits verified the presence of 32 components in the methanolic extract. The major components identified were pyranone (13.42%) and cis-vaccenic acid (9.68%). Secondary chemicals include vadex (4.97%) and 2,3-butandiol (3.05%).

The GC-MS examination of the oval-shaped fruits of the *Murraya koenigii* plant revealed the presence of 38 chemicals in the methanolic extract. The major components were identified as 3-Hydroxy-2, 3-dihydromaltol (19.38%) and Hydroxymethylfurfural (14.77%). Cis-9-hexadecenal (10.82%) and 3-Deoxy-d-mannoic lactone (9.52%) are secondary chemicals. These phytochemicals may be responsible for a wide range of pharmaceutical properties, including antioxidant, wound healing, antifungal, and anti-acne effects.

Hydroxymethylfurfural (HMF) is one of the most interesting platform molecules that is capable of being transformed into a wide variety of intriguing compounds[30]. This platform molecule contains a variety of functional groups that has made it a great starting substrate for the synthesis of complex heterocyclic scaffolds, photo-responsive compounds, and therapeutic analogs. The application of this C-6 synthon in innovative synthetic processes is intriguing since it allows the inclusion of renewable sources of carbon into the final products [31].

Cis-vaccenic acid, an ω-7 fatty acid, has been observed to possess antimicrobial properties and a hypolipidemic impact in rats [32].

Table 5 Bioactive compounds discovered in round shaped and oval shaped fruits of *Murraya koenigii* by GC-MS analysis

Sr. No.	**Compound name**	**RT (min)**	**Area%**	**MW ($gmol^{-1}$)**	**MF**
Round shaped fruit extract					
1.	2,3-Butandiol	4.708	3.05	90	$C_4H_{10}O_2$
2.	2-Hydroxypropylacetate	5.500	0.26	118	$C_5H_{10}O_3$
3.	Furfurol	6.136	1.84	98	$C_5H_6O_2$
4.	Methyl 2-ethyl-3-oxobutyrate	6.789	0.28	144	$C_7H_{12}O_3$
5.	1,2-Butanolide	7.250	1.19	86	$C_4H_6O_2$
6.	2-Hydroxycyclopent-2-en-1-one	7.601	0.88	98	$C_5H_6O_2$
7.	2,4-Dihydroxycyclopent-2,5-dimethyl-3(2H)-furanone	8.621	0.84	144	$C_6H_8O_4$
8.	Glutaconic anhydride	8.985	0.40	112	$C_5H_4O_3$
9.	Levulinic acid	9.899	0.36	116	$C_5H_8O_3$
10.	4,5-Dimethyl-1,3-dioxol-2-one	10.075	0.56	114	$C_5H_6O_3$
11.	2,5-Dimethyl-3(2H) furanone	10.329	1.06	128	$C_6H_8O_3$
12.	2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	10.579	0.19	128	$C_6H_8O_3$
13.	2-Propyloxetane	10.829	0.27	100	$C_6H_{12}O$
14.	Pyranone	11.792	13.42	144	$C_6H_8O_4$
15.	2-Methyl-2H-pyran-3,4,5(6H)-trione	12.367	0.41	142	$C_6H_6O_4$
16.	Hydroxymethylfurfural	13.060	4.23	126	$C_6H_6N_3$
17.	1-Methylproline	13.247	2.24	129	$C_6H_{11}NO_2$

18.	Butyl 1-methyl-2-pyrrolidinecarboxylate	13.659	0.74	185	$C_{10}H_{19}NO_2$
19.	4-Vinylguaiacol	14.149	0.75	150	$C_9H_{10}O_2$
20.	Undecylenic acid	16.086	0.12	184	$C_{11}H_{10}O_2$
21.	ɣ-Lactone	16.601	0.66	132	$C_5H_8O_4$
22.	Palmitic acid	21.743	2.22	256	$C_{16}H_{32}O_2$
23.	Dextrose	22.082	4.97	180	$C_6H_{12}O_6$
24.	Cis-vaccenic acid	23.467	9.68	282	$C_{18}H_{34}O_2$
25.	Vanicol	23.647	0.81	284	$C_{18}H_{36}O_2$
26.	Linoleic	23.845	0.71	280	$C_{18}H_{32}O_2$
27.	Ricinoleic acid	25.143	0.85	298	$C_{18}H_{34}O_3$
28.	Erucic acid	26.834	0.34	338	$C_{22}H_{42}O_2$
29.	1,3-Docosenoic acid	27.289	0.08	410	$C_{25}H_{50}O_2Si$
30.	Oleoly chloride	27.801	0.56	300	$C_{18}H_{33}ClO$
31.	9-Octadecenamide	28.608	0.12	281	$C_{18}H_{35}NO$
32.	Cis-1,3-docosenoyl chloride	31.015	0.30	356	$C_{22}H_{41}ClO$
Oval shaped fruit extract					
Sr. No.	**Compound name**	**RT (min)**	**Area%**	**MW ($gmol^{-1}$)**	**MF**
1.	Isopropyl propionate	4.277	0.29	116	$C_6H_{12}O_2$
2.	Acetol	4.344	0.53	74	$C_3H_6O_2$
3.	Diethoxymethyl acetate	4.634	3.78	162	$C_7H_{14}O_4$
4.	1-Methylurea	5.442	0.41	74	$C_2H_6N_2O$
5.	2-Furanmethanol	6.078	1.07	98	$C_5H_6O_2$
6.	Methyl 2-ethyl-3-oxobutanoate	6.723	0.37	144	$C_7H_{12}O_3$

7.	1,4-Butanolide	7.215	0.91	86	$C_4H_6O_2$
8.	Cyclohexenol	7.422	0.16	98	$C_6H_{10}O$
9.	2-Hydroxycyclopent-2-en-1-one	7.562	0.72	98	$C_5H_6O_2$
10.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	8.608	1.49	144	$C_6H_8O_4$
11.	Glutaconic anhydride	8.952	0.57	112	$C_5H_4O_3$
12.	Levulinic acid	9.884	0.45	116	$C_5H_8O_3$
13.	2,5-Dimethylfuran-3,4(2H,5H)-dione	10.323	1.19	128	$C_6H_8O_3$
14.	2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	10.569	0.29	128	$C_6H_8O_3$
15.	3-Hydroxy-2,3-dihydromaltol	11.808	19.38	144	$C_6H_8O_4$
16.	Dihydro-4-hydrxy-2-(3H)-furanone	12.244	1.15	102	$C_4H_6O_3$
17.	Hydroxymethylfurfural	13.113	14.77	126	$C_6H_6O_3$
18.	Glycerol 1-monoacetate	13.385	3.56	134	$C_5H_{10}O_4$
19.	4-Vinylguaiacol	14.147	0.71	150	$C_9H_{10}O_2$
20.	Undecylenic acid	16.080	0.20	184	$C_{11}H_{20}O_2$
21.	ɣ-Lactone	16.427	1.49	132	$C_5H_8O_4$
22.	2,4-Bis(1,1-dimethylethyl)phenol	16.736	0.22	206	$C_{14}H_{22}O$
23.	3-Deoxy-d-mannoic lactone	18.761	9.51	162	$C_6H_{10}O_5$
24.	Methyl3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoate	21.411	0.51	292	$C_{18}H_{28}O_3$
25.	Palmitic acid	21.741	2.86	256	$C_{16}H_{32}O_2$
26.	13-Hexyloxacyclotridec-10-en-2-one	22.775	0.20	280	$C_{18}H_{32}O_2$
27.	Trans-phytol	23.126	0.16	296	$C_{20}H_{40}O$
28.	Cis-9-hexadecenal	23.471	10.82	238	$C_{16}H_{30}O$

29.	Steric acid	23.645	0.56	284	$C_{18}H_{36}O_2$
30.	Linoleic acid	23.840	0.56	280	$C_{18}H_{32}O_2$
31.	Cyclohexadecan	25.189	0.07	224	$C_{16}H_{32}$
32	2-Monopalmitin	26.594	0.21	330	$C_{19}H_{38}O_4$
33.	3-tert-butyl-1-methyl-2-phenyl-1H indole	26.786	0.34	263	$C_{19}H_{21}N$
34.	Cis-13-docosenoic acid, trimethylsilyl	27.296	0.12	410	$C_{25}H_{50}O_2Si$
35.	Oleoyl chloride	27.796	0.46	300	$C_{18}H_{33}ClO$
36.	9-Octadecenamide	28.615	1.42	281	$C_{18}H_{35}NO$
37.	(14Z)-14-tricosenyl Formte	31.010	0.25	366	$C_{24}H_{46}O_2$

RT= Retention time

MF=Molecular formula

MW=Molecular weight

4.3 UHPLC-QTOF-MS analysis

The chemical compunds found by UHPLC-QTOF-MS analysis of methanolic plant extract of *Murraya koenigii* fruits were listed in Table 6 and Table 7. UHPLC-QTOF-MS analysis identifies secondary metabolites that are present in the methanolic extract of fruits of *Murraya koenigii*. Few of the secondary metabolites that are identified by UHPLC-QTOF-MS are Fosfomycin calcium, 3-Methyl-5-(trifluoromethyl)pyrazole, Dansylamide, N-glycylacrylamide, Thiodan sulfate, Biindoline and so on.

Fosfomycin calcium is a novel structural fosfomycin antibacterial medicine. After oral administration, the medicine is absorbed and removed unchanged via the kidneys, without being processed in the body. As a result, it is recommended for the treatment of urinary tract infections such as cystitis and pyelonephritis [33]**.**

Table 6 - Bioactive compounds discovered in oval shaped fruits of *Murraya koenigii* by UHPLC-QTOF-MS analysis

Peak No.	Tentative Metabolites	RT(min)	MF	MM (gmol-1)	[M-H]+	Error (ppm)	Compound ID
1.	Chloropivaloylpalladium	1.94	C_5H_8ClOPd	185.831	186.8388	3.7	CSID25935213
2.	1-{[Brom(difluor)methyl]sulfinyl}-4-nitrobenzol	1.94	$C_7H_4BrF_2NO_3S$	297.898	298.9063	2.8	CSID29325707
3.	Bis(2-phenylethanaminium)tetrabrommercurat(2-)	2.08	$C_{16}H_{24}Br_4HgN_2$	698.742	699.7498	-3.5	CSID26667012
4.	Tri-2,2'-bithiophen-2(3h)-ylindium	2.08	$C_{24}H_{21}InS_6$	552.804	553.8126	-5.1	CSID29323411
5.	Fosfomycin calcium	2.08	$C_3H_5CaO_4P$	112.859	113.8671	-1.4	CSID84044
6.	Azobenzenethiol	2.28	$C_{12}H_{10}N_2S$	213.048	214.0564	3.9	CSID24769776
7.	Thienoindole	2.28	$C_{10}H_7NS$	172.022	173.0299	4.8	CSID25933318
8.	Dansylamide	2.28	$C_{12}H_{14}N_2O_2S$	249.069	250.0775	3.4	CSID58587
9.	3-Chlor-2-[4-(3-fluorphenoxy)-	2.53	$C_{16}H_{15}ClFN$	350.0	351.0	0.3	CSID70669

	1-piperidinyl]-5-nitropyridin		$_3O_3$	70	785		017
10.	Rhodium(ii) octanoate, dimer	2.53	$C_{32}H_{60}O_8Rh_2$	739.141	740.1493	-0.1	CSID9796682
11.	4-Acetotoluide	3.49	$C_9H_{11}NO$	148.076	149.0840	-2.9	CSID10243171
12.	N-glycylacrylamide	12.28	$C_5H_8N_2O_2$	105.068	106.0766	-3.5	CSID10739518
13.	3-Methyl-5-(trifluoromethyl)pyrazole	12.28	$C_5H_5F_3N_2$	149.032	150.0404	-2.7	CSID122666
14.	3,5-Difluorobenzeneacetohydrazide	12.28	$C_8H_8F_2N_2O$	185.05	186.0604	3.8	CSID21468956
15.	Imidazolidin-1-iumtrifluoracetat	12.28	$C_5H_9F_3N_2O_2$	185.053	186.0616	-2.2	CSID26668587
16.	Bis(9h-fluoren-9-ylmethyl)phosphonat	12.28	$C_{28}H_{23}O_3P$	437.130	438.1389	5.7	CSID29325371
17.	3,3'-[(Diphenylsilandiyl)di-4,1-phenylen]dipyridin	12.28	$C_{34}H_{26}N_2Si$	467.196	468.2046	0.2	CSID26946994
18.	8-(Diphenylphosphino)-2-methylquinoline	13.04	$C_{22}H_{18}NP$	326.109	327.1176	-1.0	CSID9620775
19.	Calciumhydrid-2-isopropoxypropan	13.49	$C_6H_{16}CaO$	143.074	144.0827	-2.3	CSID29336834
20.	Aluminiumnatriumtetrahydroborat	13.49	$H_{16}AlB_4Na$	71.035	72.0432	4.7	CSID29434312
21.	Methyl-2-fluor-3,3-	13.49	$C_7H_{13}FO_2$	147.0	148.0	1.2	CSID91068

	dimethylbutanoat			82	899		25
22.	Methyll-γ-glutamylglycinat	13.49	$C_8H_{14}N_2O_5$	195.100	196.1083	1.1	CSID10148990
23.	1-Dimethylarsinoylpentadecane	13.49	$C_{17}H_{37}AsO$	293.107	294.1156	1.1	CSID25933773
24.	Parabenzlactone	13.49	$C_{20}H_{18}O_7$	347.115	348.1233	-5.9	CSID147581
25.	6-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)naphtho[2,3-d][1,3]dioxol	14.14	$C_{17}H_{19}BO_4$	259.039	260.0471	-5.6	CSID116079356
26.	Glycylserylglycylarginylserylalanylglycin	14.14	$C_{21}H_{38}N_{10}O_{10}$	567.287	568.2953	-1.5	CSID26998920
27.	1-(4-Amino-2-chlorphenyl)-n-[3-(trifluormethyl)phenyl]-4-piperidinamin	14.33	$C_{18}H_{19}ClF_3N_3$	367.106	369.1219	-5.4	CSID116081112
28.	N-nitrosoguanidine	14.90	CH_4N_4O	87.030	88.0385	-4.5	CSID12139
29.	4,5-Dihydro-3h-1,2,3,5-diazadiborol	14.90	$CH_4B_2N_2$	43.066	44.0741	1.6	CSID29320481
30.	Formic-d acid	14.90	$CHDO_2$	46.003	47.0117	-4.1	CSID9438478
31.	Di-tert-butyl(dimethyl)stannane	15.15	$C_{10}H_{24}Sn$	223.983	224.9916	-4.0	CSID110045
32.	13-(5-Brompentyl)-3,6,9,12,14,17,20,23-	16.15	$C_{22}H_{45}BrO_{10}$	509.121	510.1291	4.8	CSID25936423

	octaoxapentacosan-1,25-diol						
33.	4-[2-[4-(Dimethylamino)phenyl]diazenyl]benzenesulfonic acid	17.16	$C_{14}H_{15}N_3O_3S$	304.075	305.0834	3.9	CSID10569
34.	8-Methyl-6-(1-propyn-1-yl)-2-[(2-pyrimidinylsulfanyl)methyl]-4(3h)-quinazolinone	17.67	$C_{17}H_{14}N_4OS$	321.081	322.0888	-0.1	CSID11608 1997
35.	Methyl-(2e)-2-azido-3-(2,5-dimethoxy-3-pyridinyl)acrylat	17.67	$C_{11}H_{12}N_4O_4$	241.096	242.1039	-0.5	CSID25937 563
36.	N-hydroxy-n-propyl-1h-1,2,3-triazol-4-amine	18.09	$C_5H_{10}N_4O$	141.077	142.0854	1.4	CSID24771 399
37.	Ethyl propiolate	18.09	$C_5H_6O_2$	97.0289	98.0367	2.3	CSID11682
38.	2-Methyl-2-propanyl-(4s,5r)-4,5-bis(benzyloxy)-3-hydroxy-3-(hydroxymethyl)-1-piperidincarboxylat	19.67	$C_{25}H_{33}NO_6$	381.142	382.1505	-0.4	CSID29326 889
39.	1-Vinyl-3-octadecylimidazolium bromide	21.22	$C_{23}H_{43}BrN_2$	425.253	426.2609	-3.2	CSID24770 545
40.	2-(1,3-Benzothiazol-2-yl)pyrrolidinium	24.27	$C_{11}H_{13}N_2S$	181.081	182.0896	-2.7	CSID35523 077
41.	2,4-Bis{5-[4-(2-methyl-2-propanyl)phenyl]-1,3,4-oxadiazol-2-yl}pyridin	24.75	$C_{29}H_{29}N_5O_2$	417.144	418.1519	-2.3	CSID21143 386

42.	5-(8-Hydroxyoctyl)-2-(4-thiomorpholinylmethyl)phenol	25.09	$C_{19}H_{31}NO_2S$	314.217	315.225	4.3	CSID11608 1422
43.	2,2',2''-[(2,4,6-Triethylbenzol-1,3,5-Triyl)Trimethylen]Tris(1h-Isoindol-1,3(2h)-Dion)	25.60	$C_{39}H_{33}N_3O_6$	616.247	617.2550	-2.4	CSID26995 551
44.	Methyl behenate	26.02	$C_{23}H_{46}O_2$	315.251	316.2592	-1.6	CSID12995
45.	3-Methyl-1-(methoxyethoxycarbonylmethyl)imidazolium	26.99	$C_9H_{15}N_2O_3$	136.012	137.0200	-4.1	CSID25946 513
46.	3,5,5-Triallyl-1,3-thiazolidin-2,4-dion	28.23	$C_{12}H_{15}NO_2S$	213.084	214.0926	5.6	CSID26996 189
47.	Methyl epoxystearate	29.13	$C_{19}H_{36}O_3$	311.258	312.2664	5.3	CSID10806 913
48.	1,3-Dimethylguanine	30.68	$C_7H_{11}N_5O$	119.008	120.0161	-2.3	CSID20169 871
49.	1-Allyl-3-methylimidazolium tetrafluoroborate	31.64	$C_7H_{11}BF_4N_2$	186.097	187.1054	3.1	CSID26666 441
50.	5-(3,5-Dimethoxyphenyl)-2-n-propylthiazole	34.16	$C_{14}H_{17}NO_2S$	239.100	240.1008	0.9	CSID25937 196
MM=Monoisotopic mass							

Table 7 - Bioactive compounds discovered in round shaped fruits of *Murraya koenigii* by LC-MS analysis.

Peak No.	Tentative Metabolites	RT	MF	MM	$[M-H]^+$	Error (ppm)	Compound id
1.	Fosfomycin calcium	2.08	$C_3H_5CaO_4P$	112.8593	113.8671	-1.4	CSID84044
1.	Thiodan sulfate	2.17	$C_9H_6Cl_6O_4S$	357.7237	358.7315	2.0	CSID 16736500
3.	4-Chlorophenyl acetate	2.25	$C_8H_7ClO_2$	143.9254	144.9332	-0.8	CSID12836
4.	4,4-Bis(ethylthio)-1,3-diphenylbut-3-en-2-one	2.31	$C_{20}H_{22}OS_2$	338.0051	339.0129	-1.7	CSID24770450
5.	Lysergene	2.48	$C_{16}H_{16}N_2$	235.1235	236.1313	1.0	CSID10390293
6.	Dichloro(di-3-octanyl)germane	2.53	$C_{16}H_{34}Cl_2Ge$	347.1352	348.1430	-4.3	CSID29542515
7.	(Pentafluorphenyl)(diphenyl) phosphin	3.21	$C_{18}H_{10}F_5P$	312.9457	313.9535	1.3	CSID265587
8.	Biindoline	3.46	$C_{16}H_{16}N_2$	196.0252	197.0330	-2.8	CSID24770622
9.	Importazole	11.91	$C_{20}H_{22}N_4$	279.0861	280.0939	5.5	CSID2222915
10.	4-(4-Pyridyl)-1,3-dithiol-2-one	12.16	$C_8H_5NOS_2$	171.9915	172.9993	-5.0	CSID25940928
11.	Diphenylethylenediamine	13.4	$C_{14}H_{16}N_2$	211.1	212.13	-3.6	CSID10659

		0		235	13		547
12.	Reticulin	14.53	$C_{21}H_{39}N_7O_{13}$	558.1622	559.1700	-1.8	CSID21759
13.	Sanguinamide b	14.84	$C_{33}H_{42}N_8O_6S_2$	687.2771	688.2849	2.2	CSID27024096
14.	Di-tert-butyl(dimethyl)stannane	15.15	$C_{10}H_{24}Sn$	223.9838	224.9916	-4.0	CSID110045
15.	Triphenyleno[1,2-i]pyranthren	16.15	$C_{46}H_{24}$	537.0895	538.0973	4.5	CSID29436178
16.	1-Phenyl-6-(10-phenyl-9-anthryl)pyrene	16.57	$C_{42}H_{26}$	507.2137	508.221	5.6	CSID29372271
17.	Arthpyrone a	17.70	$C_{24}H_{31}NO_7$	422.2203	423.2281	-5.0	CSID34452006
18.	1-Methylimidazolium tetrafluoroborate	20.23	$C_4H_7BF_4N_2$	169.0560	170.0638	1.4	CSID9963197
19.	L-valyl-l-prolyl-l-seryl-l-alanyl-l-glutaminyl-l-serinamid	26.22	$C_{24}H_{42}N_8O_9$	547.2091	548.2169	3.4	CSID68006739
20.	3-Methyl-1-(methoxyethoxycarbonylmethyl)imidazolium	26.99	$C_9H_{15}N_2O_3$	485.1211	486.1289	-4.1	CSID25946513
21.	8-(N-maleimidyl)-3,6-dioxaoctan-1-ol	27.10	$C_{10}H_{15}NO_5$	228.0872	229.0950	5.5	CSID14454565
22.	3,5,5-Triallyl-1,3-thiazolidine-2,4-dione	28.23	$C_{12}H_{15}NO_2S$	213.0848	214.0926	5.6	CSID26996189

23.	Tirazone	29.78	$C_7H_6N_4O_2$	177.0412	178.0490	-2.2	CSID10437748
24.	1,3-Dimethylguanine	30.68	$C_7H_{11}N_5O$	119.0083	118.0005	-2.3	CSID20169871
25.	1-Allyl-3-methylimidazolium tetrafluoroborate	31.64	$C_7H_{11}BF_4N_2$	186.0976	187.1054	3.1	CSID26666441
26.	2-Ethylimidazoline	33.99	$C_5H_{10}N_2$	97.0765	98.0843	-4.7	CSID13001
MM=Monoisotopic mass							

4.4 Antioxidant activity

DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical that is stable and has been used for long to examine antioxidant free radical-scavenging characteristics (DPPH stable). Fig. 1 shows the capability of both extracts. The findings revealed that the extracts exhibit concentration-dependent activity against free radical species. Round fruit (IC_{50}= 1.53 ± 0.039 µl/ml) performed better than oval fruit (IC_{50}= 1.808 ± 0.035 µl/ml). The IC_{50} value of round fruit was found to be equal to the standard of ascorbic acid (IC_{50}= 1.12 ± 0.32µg/ml).

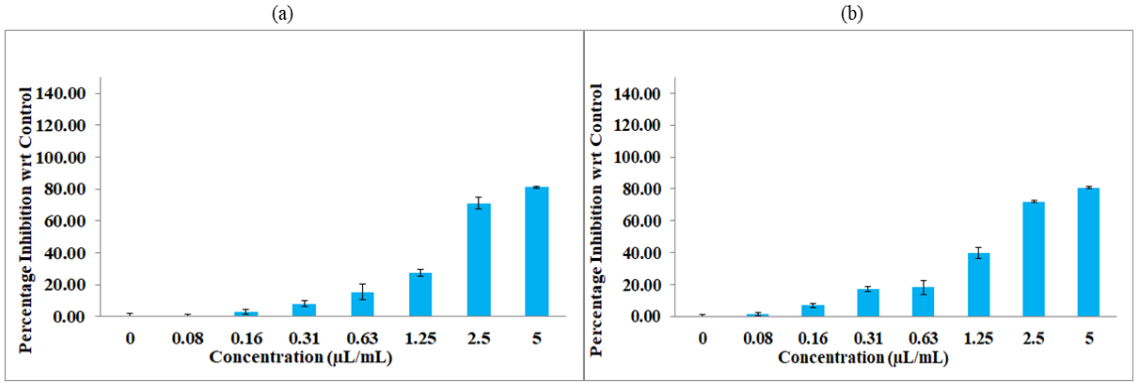

Fig. 1: Antioxidant activity of methanolic extract of *Murraya koenigii* (a) Oval fruit (b) Round fruit

4.5 Antifungal activity

The disc diffusion method was used to assess the antifungal activity of *Murraya koenigii*

fruits against *Candida albicans*. Fig. 2 show the inhibitory zones surrounding each disc in the presence of different percentages of concentrations (0, 6.25, 12.5, 25, 50, and 100% dose) of both the round and oval fruits, respectively. In both cases, there was no inhibitory zone around the disc in the absence of extract. The antifungal activity of *Murraya koenigii* fruits is shown to be concentration dependent. In every case, the inhibitory regions grow in proportion to the concentration of *Murraya koenigii* fruits. Fig. 2 shows that the minimum zone of inhibition is 7.3mm obtained for 6.25 % dose in the case of round fruit and 7mm in the case of oval fruit. The largest zone of inhibition is found to be 9.6mm for 100% dose in case of round fruit and 8.6mm in the case of oval fruit. Round fruit showed slightly better activity towards *C. Albicans* in comparison to oval fruit.

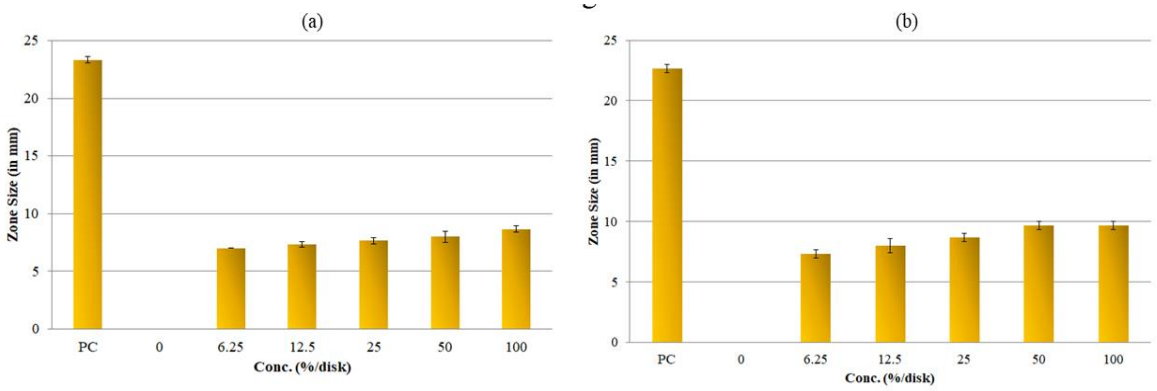

Fig. 2: Antifungal activity of methanolic extract of *Murraya koenigii* (a) Oval fruit (b) Round fruit

4.6 Antiacne activity

In this study, the disc diffusion method was used to assess the antiacne activity of *Murraya koenigii* fruits against *Propionibacterium acnes. P. acnes* is a comparatively slow-growing, aerotolerant anaerobic Gram-positive bacteria (rod form) connected with the acne skin disease. Fig. 3 depict the inhibitory zones encircling each disc in the presence of various percentages of concentrations (0, 6.25, 12.5, 25, 50, and 100% dose) of both round and oval fruits. When the test organism was treated with varying amounts of sample on an agar plate, it was discovered that Sample Round fruit was active with Max zone of inhibition 7 mm at 100% dose while oval fruit with No zone of inhibition is not active against the test organism *P. acnes* as compared to the positive control (Max zone of inhibition 36mm at 10 µg dose).

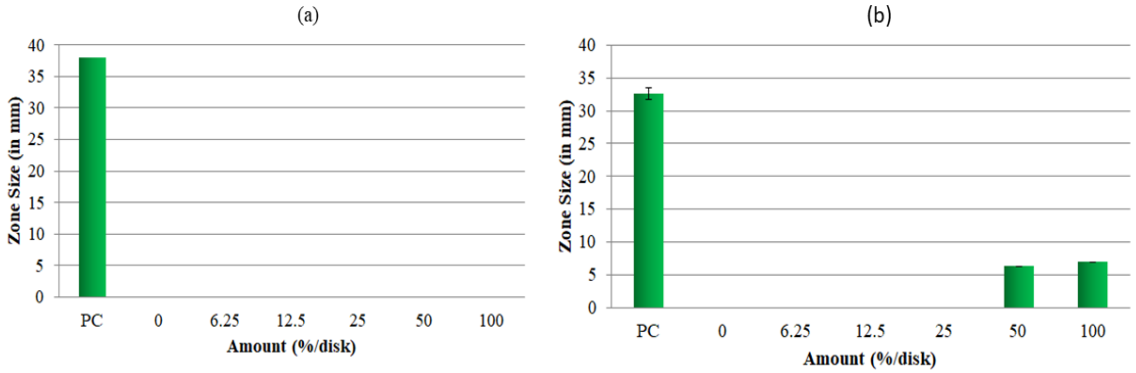

Fig. 3: Antiacne activity of methanolic extract of *Murraya koenigii* (a) Oval fruit (b) Round fruit

4.7 Antidiabetic activity

4.7.1 α-Amylase inhibitory assay

The antidiabetic efficacy of methanol extracts from both varieties of *Murraya koenigii* fruits was studied to corroborate its historic use in diabetes management. α-Amylase, found mostly in saliva and pancreatic fluid, plays a crucial role in digestion. Targeting and blocking this enzyme is a potential method for preventing high postprandial blood glucose levels.

Our findings also support its use by demonstrating substantial antidiabetic activity against various diabetes enzymes. The methanolic extract of both types of fruits showed strong α-amylase inhibitory action. When compared to the round fruit extract, the oval fruit extract showed encouraging results. The methanol extract of oval fruits demonstrated the highest inhibition at 0.97 µL/mL, while round fruit extract had an IC_{50} value of 9.69 µL/mL. Acarbose was employed as a positive control and had an IC_{50} value of 0.60 µL/mL.

4.7.2 α-Glucosidase inhibitory assay

The α-glucosidase enzymes are crucial for digestion and can be found in the small intestine's mucosal brush boundary. Its job is to convert and degrade complicated carbs into smaller, simpler, and more absorbable ones. Its blockage is an efficient way to postpone glucose absorption while also preventing excessive postprandial blood sugar levels, which may slow diabetes progression.

Methanolic extracts from *Murraya koenigii* oval and round fruits inhibited α-glucosidase enzyme activity, as demonstrated in Fig. 4. The inhibition of α-glucosidase enzyme is dose-dependent, as the concentration of both extracts significantly impacts the quantity of enzyme inhibited. The IC_{50} values for oval and round fruit extracts are 1.62 µL/mL and 3.90 µL/mL, respectively. This suggests that oval fruits methanolic extract has a higher inhibitory potential

than round fruits methanolic extract. Acarbose was utilized as a positive control and had an IC_{50} value of 2.50 µL/mL.

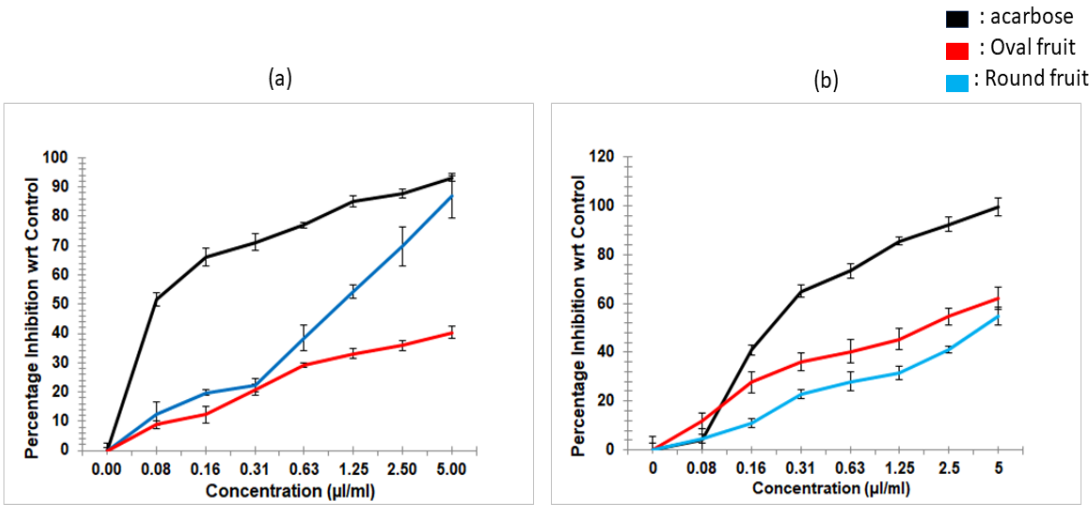

Fig. 4: Inhibitory assay of both sample extracts of *Murraya koenigii* (a) α-Amylase (b) α-Glucosidase

4.7.3 DPP-IV inhibitor activity assay

Fig. 5 shows the inhibitory activity of DPP-IV in both methanolic extracts of *Murraya koenigii*. Round fruit extract had the most inhibitory efficacy, with a percentage inhibition value of 1.37%, compared to oval fruits, which had a percentage inhibition value of 0.74%, while the positive control showed 2.23% inhibition. The results show that methanolic extracts of round fruits are more potent against the DPP-IV enzyme than oval fruits.

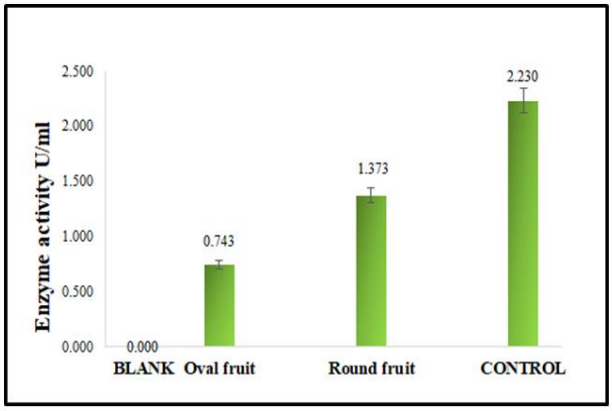

Fig. 5: DPP-IV inhibitor activity assay of both sample extracts of *Murraya koenigii* (a) Oval fruit (b) Round fruit

4.7.4 Glucose uptake assay

An *in vitro* investigation was carried out to assess the glucose utilisation of both of the *Murraya koenigii* methanolic extracts in L-6 cells. Fig. 6 shows the results. The results were compared to metformin (1mM) and insulin (0.1 U/mL), which are basic anti-diabetic medications. As expected, insulin and metformin greatly boosted glucose uptake in the L6 cell line, the best cellular model for glucose uptake. The oval and round fruit extracts of *Murraya koenigii* reduced glucose absorption. The results show that *Murraya koenigii* fruit extracts improve glucose uptake by 196.15 µg/mL and 206 µg/mL compared to the control.

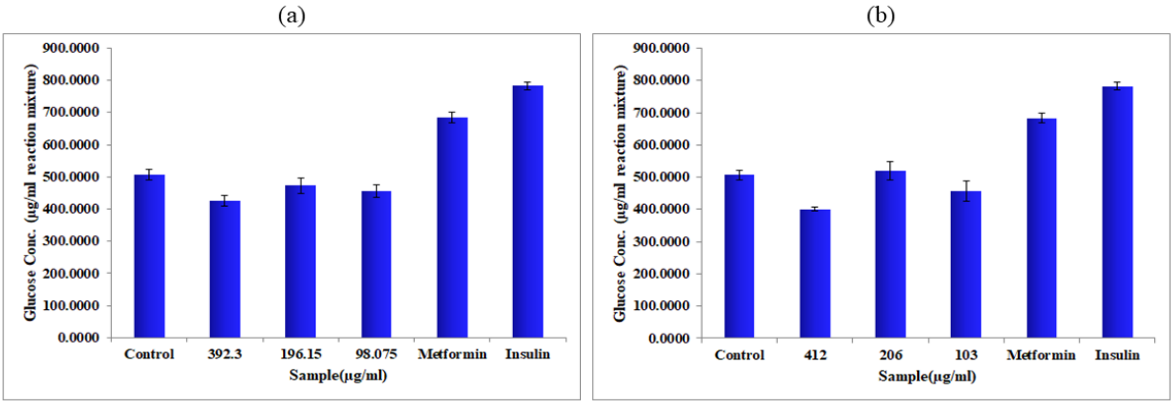

Fig. 6 Glucose uptake assay of *Murraya koenigii* (a) Oval fruit (b) Round fruit

4.7.5 ROS measurement

Figure 7 depicts the ROS measurement results in the L6 cell line after exposure to both fruit kinds of *Murraya koenigii* methanolic extract. ROS levels were shown to increase in a dose-dependent manner. ROS generation was highest in the methanol extract of round fruits (6.28%) compared to oval fruits (5.99%).

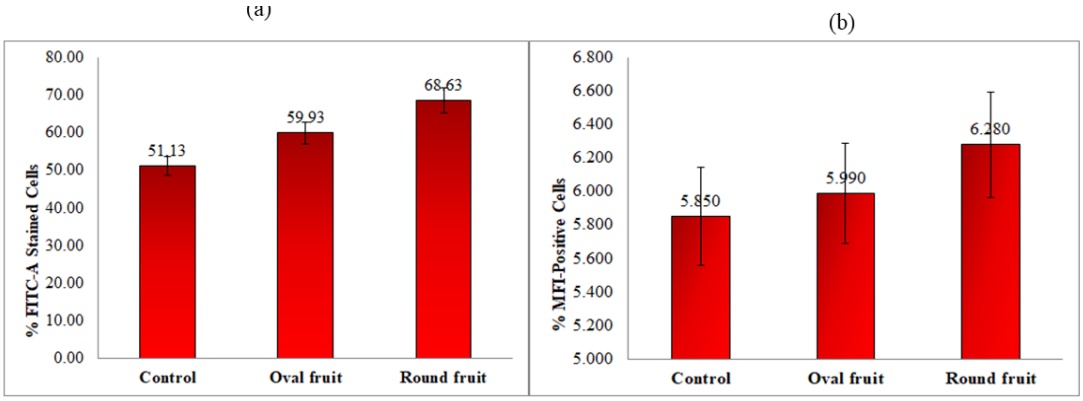

Fig. 7: ROS measurement of methanolic extract of both sample of *Murraya koenigii* (a) % FITC-A Stained Cells (b) MFI-Positive Cells

4.7.6 Insulin secretion assay

The findings of the investigation revealed that different insulin concentrations were found in both *Murraya koenigii* samples, as illustrated in Fig. 8. When compared to the control, the round fruit sample had the greatest concentration (272.55 U/ml Lysate) and the oval fruit sample had the lowest (265.09 U/ml Lysate).

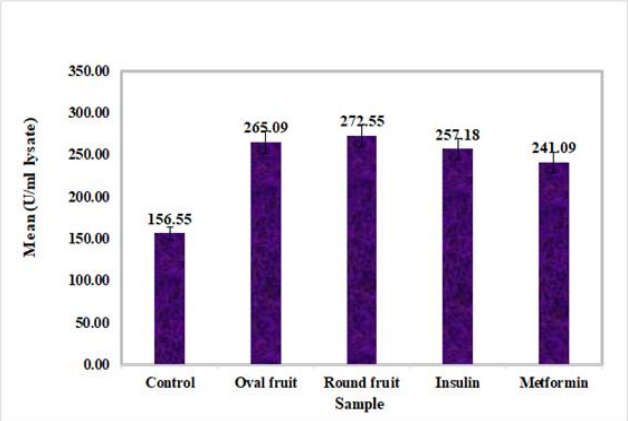

Fig. 8 Quantitative estimation of Insulin

4.7.7 Cytotoxicity assay

The cytotoxic impact of methanol extracts of both varieties of *Murraya koenigii* fruits on the L6 cell line (myoblast cell line) is depicted in Fig. 9. Both fruit extracts exhibited substantial differences in percentage cell viability at varied concentrations. At 1000 µg/mL, oval fruit extract showed 42.27% viability while round fruit extract showed 43.765 viability on the L6 cell line. However, at 1 µg/mL concentration, the oval fruit extract showed 87.19% vitality, whereas the round fruit extract showed 83.70%. The reduction in cell viability was concentration-dependent. The MTT assay yielded inhibitory concentrations (IC_{50}) of 392.2 ± 0.16 µg/mL for oval fruits and 412.2 ± 0.13 µg/mL for round fruits. Interestingly, both the fruit extracts did not show any harmful impact at lower doses, and reduced cytotoxicity was discovered with the increased concentration of oval fruit extract above 500 µg/mL, while round fruit extract was above 250 µg/mL. As a result of the findings, it is possible to conclude that both fruits are biocompatible and not dangerous to humans or other living organisms when consumed in moderation.

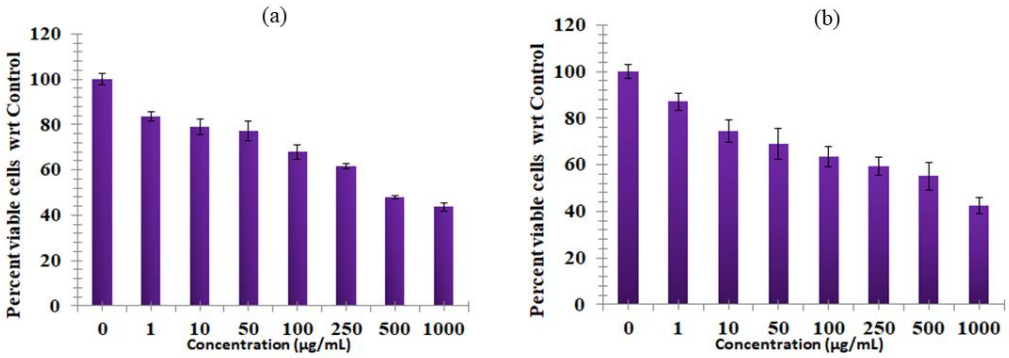

Fig. 9: Cytotoxicity assay of methanolic extract of *Murraya koenigii* (a) Oval fruit (b) Round fruit

CHAPTER 5

CONCLUSION

This study explored the phytochemical composition and *in-vitro* biological profiling of two distinct types of *Murraya koenigii* fruits; Round and Oval shaped, that are grown in India. Both the fruits exhibited different results against various examination. The presence of various bioactive compounds with potential therapeutic activity was confirmed by GC-MS and UHPLC-QTOF-MS analysis. The extracts were analysed for anti-oxidant and anti-fungal activities which revealed that methanolic extract of round fruit is better anti-oxidant and anti-fungal agent. Strong anti-acne activity against *C. Albicans* was exerted by methanolic extract of round fruit. The methanolic extract of both the fruits were also investigated for various *in-vitro* anti-diabetic activities, which revealed that oval fruit extract is more effective than round fruits in diabetes management. Overall, these findings suggest that oval and round shaped fruits of *Murraya koenigii* could be a potential source of bioactive metabolites and may be a natural source of anti-oxidants, anti-fungal, anti-acne, and antidiabetic agent. The results suggest traditional use of both the fruits for the management of diabetes. A comprehensive *in-vivo* study evaluating the therapeutic potentials of both types of fruits of this plant is strongly recommended.

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