

COMBINATORIAL STUDY OF MURRAYA KOENIGII'S SEED, FRUIT AND TO EVALUATE THE ANTIOXIDANT AND INVITRO ANTIDIABETIC ACTIVITY

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ABSTRACT

Nature products from Plants, animals and minerals have been used for the treatment of human disease. For their primary health care, 80% people in developing countries used traditional medicine based upon plants and animal species. Due to the toxicity and side effects of allopathic medicines, use of herbal medicines becomes more popular. There are around 25000 effective plant based preparation used in folk medicine. Exploration of active constituents from the plant and the pharmacological screening may provide us the basics for the development of novel agents. So the medicinal plants are plays a vital role in pharmacological research and drug development. *Murraya*

Koenigii is commonly known as Curry Leaves or karipatta in Indian dialects they belong to under the family of Rutaceae, and they represent more than 150 genera and 1600 species. And it is one of the valuable plants for its characteristic aroma medicinal value. It plays an important role in exporting from India as it fetches good foreign revenue. In this Research article, research about the seed and the fruit of *Murraya Koenigii* and study about their Pharmacognostical Studies, Phytochemical Studies and to evaluate the Pharmacological invitro studies.

KEYWORDS: *Murraya koenigii*, Pharmacognostical, Phytochemical, Chromatography, Antioxidants.

INTRODUCTION

Plant Profile

Morphology Description

Murraya Koenigii is considered as small shrub of about 2-2.5m height, and it has a dark green and brown stem. The leaves are seen as long, and they have reticulate venation. Flowers are white, funnel shaped, and they have sweet aromatic smell and round shaped fruits are 1.4-1.6cm length. They are found throughout India and cultivated in country including Sikkim, Western Ghats, Assam etc. The moist forest which contains the *Murraya Koenigii* tree, 500-1600 meters in height which is especially presents in S Hainan, Guangdong, Sri Lanka, Nepal, Bhutan, Thailand, Vietnam. Preposition with the South India immigrants, the *Murraya Koenigii* leaves are appeared in Malasia, Reunion Island, South Africa.^[1, 2]



FIG. 1: *Murraya Koenigii* Fruits.

TABLE NO: 1

SCIENTIFIC CLASSIFICATION

KINGDOM	Plantae
SUBKINGDOM	Tracheobionta
SUPERDIVISION	Spermatophyta
DIVISION	Magnoliophyta
CLASS	Magnoliopsida
SUBCLASS	Rosidae
FAMILY	Rutaceae
GENUS	<i>Murraya J. Koenigii</i>
SPECIES	<i>Murraya Koenigii</i>

TABLE NO: 2**VERNACULAR NAMES**

Burmese	Pindosine, Pyim daw thein
Dutch	Kerriebladeren
English	Curry leaves
French	Feuilles de cari, Feuilles de Cury
German	Curryblatter
Indonesian	Daun Kari
Italian	Fogli de Cari
Spanish	Hoja

Traditional Use

Murraya koenigii's essential oils, powdered or dried leaves, and also fresh leaves which are widely used for curries, flavouring fish, soups, egg dishes are ready to use and seasoning other food preparations. In cosmetic aromatherapy and soap industry the essential oils are widely used.^[2] Their leaves are used as first-rate hair tonic for keeping hair as natural hair tenor, and also helps to increasing hair growth. As a single part or the whole plant have a many traditional use as a remedy for antiemetic, blood purifier, anti-inflammatory, anti-diarrhoea, vomiting etc. For the treatment of poisonous animal bite barks and roots of *Murraya Koenigii* have been widely used. The leaves paste and roots juice which helps to relieve boils and relieve pain respectively.^[3,4,5]

MATERIALS AND METHODS**Pharmacognostical Study**

Determination of foreign organic matter, loss on drying, ash and extractive value etc. this gives a clear idea about the specific characteristic of crude drug under analysis. So these evaluated features enable the analyst to know about the nature and characteristic of crude drugs and further evaluation of various parameters which indicate their acceptability **Table No-3.**

Determination of Ash Value**Ash Content**

Ash value is the major criteria for to identify the adulteration of crude drug. After incineration of the residue is the ash content of crude drug, they are simply represents as inorganic salts, and it is normally present in drug or adhering to it or intentionally added to it as a form of adulteration. It will help to judge the purity of crude drug.^[6]

Total Ash

By determination the total ash which is useful for detecting low grade products, exhausted product, excess of sandy, and also earthy matter with drug.^[6]

PROCEDURE

3 grams of air dried fruit and seed powder was accurately weighed in a silica crucible separately. The powder was distribute into fine even layer on the bottom of the crucible and heated by gently increasing the temperature but not exceeding 450°C, until free from carbon. Then it was cooled and weighted for constant weight. The percentage of ash was calculated.

$$\text{Total Ash (\%)} = (\text{weight of ash})/(\text{weight os sample}) \times 100$$

Water Soluble Ash

It helps to identify the presence of any inorganic salts in the crude drug

PROCEDURE

The total ash is to be taken then they boiled with 25 ml of water for 5 minutes. The insoluble characters are collected by using ashless filter paper and washed with hot water. Then they are ignited for 15 minutes after that the weight of insoluble matter was subtracted from weight of total ash. And producing the difference, represent that the water-soluble ash. The results are presented in the form of **Table No-3**

Water Soluble ash = Total Ash – Weight of water insoluble ash

$$\text{Water Soluble Ash (\%)} = (\text{weight of water soluble ash})/(\text{weight of sample}) \times 100$$

Acid Insoluble Ash

It is used to identify the presence of any earthy matter present in root, rhizomes and leaves.

PROCEDURE

The total ash is to be taken, and then they are boiled for 5 minutes with 25 ml of dilute hydrochloric acid. Then the insoluble mater was collected in a tarred sintered glass crucible. The residue was washed by hot water, dried and weighed. The results are presented in the form of **Table No-3**.

$$\text{Acid Insoluble Ash (\%)} = (\text{weight of acid insoluble ash})/(\text{weight of sample}) \times 100$$

Sulphated Ash

It helps to determination of presence of sulphur in the crude drug

PROCEDURE

1 gram of sample of fruit and seed is taken and moistened with 1 ml of concentrated sulphuric acid and then ignite until all carbonaceous matter has disappeared and then frequently heat till no fumes are evolved. Cooled in a dessicator and weighed. The results are presented in the form of **Table No-3**.

Sulphated Ash (%) = $(\text{weight of sulphated ash})/(\text{weight of sample}) \times 100$

Extractive Value

It helps to determination of active constituents present in the crude drug. They are prepared by treating the crude drug with the solvent so that, most of the constituents gets solublize. So the percentage of active constituents present in the drug indicates the extractive value.^[6]

PROCEDURE

10 gram of powder of fruit and seed of *Murraya Koenigii* was macerated separately with 10ml of petroleum ether in a closed flask for 24 hours. It was frequently shaken for the first 6 hours and allowed to strand for 18 hours. After that the solutions are filtered, the filtrate was evaporated in a tarred flat-bottomed shallow dish, and dried at 105°C and weighted. Then the resulted percentage of petroleum ether soluble extractive value was calculated.

This is the procedure is carried out in various solvents such as Methanol, Hexane, Chloroform, Ethyl Acetate and the results are presented in the form of **Table No-3**.

Extractive value (%) = $(\text{weight of dried extract})/(\text{weight of plant material}) \times 100$

Determination of Foaming Index

This test which is applicable to herbs contains saponins because as these have an ability to form foam when an aqueous decoction is shaken. So this foaming ability of an aqueous decoction of herbal material and their extracts are determined in terms of foaming index.^[7]

PROCEDURE

1 gram of powder of fruit and seed of *Murraya Koenigii* was weighed and transferred in 500 ml of conical flask containing 100ml of boiling water. Then the flask is maintained at moderate boiling at 80-90°C for about 30 minutes. Then it was cooled and filtered into a volumetric flask and add sufficient water through the filter to make up the volume upto 100ml.

10 stoppered 10 ml standard measuring flask was cleaned and notify from 1 to 10. Measured and transferred the portion of 1, 2, 3 upto 10 ml and the volume is adjusted in each tube upto 10 ml. after that the tubes are individually shaken lengthwise for 15 seconds, uniformly and allowed to strand for 15 minutes and measured the length of foam in an individual tube. The results are assessed as fallow.

If the height of the foam in every individual tube was less than 1 cm, the foaming index was less than 100

If the height of the foam of 1 cm was measured in any of test tube, the volume of the plant material decoction in this tube was used to determine the index.

If the height of the foam was more than 1 cm in every test tube, the foaming index was over 1000. The foaming index was calculated by using formula.

FOAMING INDEX = $1000/a$, where **a** is the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm was observed. The results are presented in the form of **Table No-3**.

Determination of Swelling Index

The plant containing gums and mucilage have significant due therapeutic or pharmaceutical unity. The compounds such as pectin or hemicelluloses also show swelling ability. The swelling index is the volume in ml taken up by the swelling of 1 gram of herbal material under specified condition.

The plant material is shaken repeatedly in a glass stoppered measuring cylinder for 1 hour and then they are allowed for strand at required period of time. The volume of mixture in ml is measured.^[7]

PROCEDURE

2 gram of seed is accurately weighed was taken in 25ml of glass stoppered measuring cylinder. Then 25ml of water was added and shaken the mixture thoroughly every 10 minutes for 1 hour. Then they are allowed to stand for 3 hours in room temperature. The volume in ml occupied by the plant material was measured, including sticky mucilage was observed. The results are presented in the form of **Table No-3**.

PHYTOCHEMICAL STUDIES

Phytochemistry is the branch of chemistry concerned with herbs. It deals with variety of secondary metabolite which are produced by plants, their chemical structure, biosynthesis, metabolism, natural distribution and biological function. In pharmacological studies phytochemical evaluation is very important. It is done by qualitative chemical analysis using upon specific reagents are needed for specific constituents followed by confirmation with different chromatographic technique like TLC, HPTLC and HPLC, GC etc. So, complete investigation is needed for characterize the phytoconstituents qualitatively and quantitatively, before proceeding for its pharmacological and toxicological studies.

MATERIALS AND METHODS

About 300 gram of dried powdered of fruit and seed of *Murraya Koenigii* was defatted with 1000 ml ethanol (60-80°C) for 24 hours by maceration. Then the solvent was collected by filtration and the marc was dried. To the dried marc 500 ml of ethanol was added in separate round bottom flask and then extraction was performed by using soxhlet apparatus for 8 hours. Then they are to be filtered, the filtrate was evaporated to cohesive mass using rota vapour. After that the residue was stored at refrigerator and subjected to qualitative chemical analysis. The same procedure was carried out for an aqueous extract. . The results are presented in the form of **Table no- 4**.

Preliminary Phytochemical Screening of Powder And various Extracts of *Murraya Koenigii*

By qualitative screening is useful for the investigation of nature of phytoconstituents present in the powder of seed and fruit of *Murraya Koenigii* and the extracts were carried out in ethanol and aqueous. The chemical tests will be performed by using these various extracts of *Murraya Koenigii* helps for the identification of alkaloids, tannis and phenolic compounds, flavanoids, glycosides and reducing sugar. The results are in the form of table.^[8,9,10,11,12]

Test for Alkaloids

Mayer's Test

To small quantity of extracts of seed and fruit, 2-3ml of Mayer's reagent was added. No cream colour precipitate was obtained, which indicates the absence of alkaloids.

Drangendroff's Test

To small quantity of the extracts and powders of fruit and seed, 2ml of Drangendroff's reagent were added. No Orange brown precipitate was formed, which indicates the absence of alkaloids.

Hager's Test

A small quantity of extracts and powders of seed and fruit was treated with Hager's reagent. No yellow precipitate is formed which indicates the absence of alkaloids.

Wagner's Reagent

A small quantity of extract and powders of seed and fruit was treated with wagner's reagent. Red brown precipitate is formed which indicates the presence of alkaloids.

Tannic Acid Test

A freshly prepared aqueous solution of tannic acid gives precipitate with most of the alkaloids which is soluble in dilute acid or ammonia solution.

Lead Acetate Test

A small quantity of extract and powders of seed and fruit was treated with lead acetate solution. White precipitate is formed which indicates the presence of alkaloids.

Test for Tannins and Phenolic Compounds

Small quantities of various extract were taken separately in water and presence of phenolic compounds and tannins with, 1% solution of gelatin containing 10% NaCl - white precipitate. 10% Lead acetate solution- white precipitate.

Test for Flavonoids**Alkali Test**

A small quantity of powdered drug and extracts of seed and fruit is taken and then mixed with 2ml of 10% aqueous sodium hydroxide solution was added. Yellow orange colour was noticed, which indicates the presence of flavonoids.

Acid Test

To the small quantity of extracts and powders of fruit and seed is mixed with concentrated sulphuric acid was added. Yellow orange colour was formed which indicates the presence of flavonoids.

Test for Glycosides**Test for Anthraquinone Glycoside****Borntrager's Test**

The powdered drug of seed and fruit was boiled with dilute sulphuric acid is filtered then the filtrate was added with benzene and shaken well. The organic layer was separated to which ammonia solution was added slowly. No Pink colour in ammonical layer was formed, which indicates the absence of anthraquinone glycoside.

Modified Borntrager's Test

About 0.1 gram of powdered drug was boiled for 2 minutes with dilute hydrochloric acid and few drops of ferric chloride solution, filtered while hot and cooled. The filtrate was extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to benzene extract. No pink colour in ammonical layer, which indicates the absence of anthraquinone glycoside.

Legal's Test

1ml of pyridine and few drops of sodium nitro prusside solution was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows that the presence of glycosides.

Test for Reducing Sugar

A small quantity of extracts was dissolved separately in 5ml of distilled water and then they are filtered. Then the filtrates were subjected to molisch's test and to detect the presence of carbohydrates.

Molisch's Test

Filtrate was treated with 2-3 drops of 1% of alcoholic alpha naphthol solution and 2ml of con. sulphuric acid was added along the sides of test tube. Appearance of brown ring at the junction of two liquids shown the presence of carbohydrate.

Fehling's Test

The substance heated with dil. hydrochloric acid to hydrolysis a polysaccharide. The reaction mixture is neutralized by addition of sodium hydroxide solution and the Fehling's solution A and B are added. Red precipitate of cuprous oxide is produced on heating in case of reducing sugar.

Chromatography

Chromatography methods are important analytical tool for separation, identification, and also estimation of different components presents in crude plant mixture or extract. In these various chromatography methods are available namely paper chromatography, thin layer chromatography, gas chromatography, high performance thin layer chromatography and also high performance liquid chromatography.^[13, 14, 15]

Thin Layer Chromatography

Thin layer chromatography is a simple, quick and inexpensive method. It is also called planner chromatography.

Principle

Thin layer chromatography is a technique used for separation, identification and estimation of single or mixture of compound present in various extracts and they are based on the principle of adsorption. In which the solute undergo distribution between two phases that is stationary phase and mobile phase. The separation is mainly based on affinity that occurs when a solvent flows along the thin layer of stationary phase.

TLC Plate Preparation

The plates are prepared by using TLC spreader. 40 gram of silica gel G was mixed with 85 ml of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates was prepared and air dried. Then, the plates were kept at 110°C for 30 minutes and then kept in dessicator.

Selection of Mobile Phase

The mixture of solvent was selected by basics of phytoconstituents present in each extract. Solvents were analysed in order of increasing polarity. Some factors such as nature of components, stationary phase and mobile phase polarity influence the rate of separation with the maximum number of components.

Solvent System

Solvent system for fruit – Chloroform:Methanol (9:1)

Solvent system for seed – Chloroform:Methanol (9:1)

Solvent Preparation

The aqueous and ethanol extract of fruit and seed of *Murraya Koenigii* was dissolved in particular solvent to get a concentration of 100mg/ml and 0.2µl of this solution was applied on the activated TLC plate in 2 cm above using capillary tube.

Detection

Visible and UV light at 254 and 366nm

Rf Value

The Rf value of the spots obtained were calculated using formula

Rf value = *(Distance travelled by the solute)/(Distance travelled by the solvent)*

The result which is obtained from TLC analysis is presented in the **TABLE NO-5**

PHARMACOLOGICAL EVALUATION

Section - A

Hydrogen Peroxide Scavenging Assay for Fruit and Seed

During past decades, the most degenerative diseases are related with Reactive Oxygen Species such as superoxide anion radicals, hydroxyl radical and hydrogen peroxide.^[16] When our body undergoes such stress condition they produce more ROS which creates imbalance of homeostatis and generate oxidative stress and causes cell death and tissue injury.^[17] In these condition Anti oxidants play a very important role in biological system^[18] by suppressing the formation of active oxygen species thereby reducing hydroperoxides, and H₂O₂ and scavenging free radicals among others.

Murraya Koenigii and their family is Rutaceae which is commonly known as Curry leaf, and it is a native of India, Sri Lanka, and other South Asian Countries and sometimes they act as a natural flavouring agent.^[19] The Plant has been examined to intensive investigation and possesses various biological activities.^[20]

MATERIALS AND METHODS

Materials

Powder of fruit and seed of various extracts that is (ethanol), Sodium Dihydro Ortho Phosphate, ascorbic acid, distilled water 1ml and 5ml pipette, volumetric flask, Hydrogen peroxide, after the results they taken under UV spectrophotometer in 265nm for absorbance.

PROCEDURE

1 gram powder of seed and fruit of various extracts of alcohol.

Alcohol Extract of Fruit and Seed

1 gram powder of aqueous seed and fruit extracts which is first dissolved in 100ml ethanol, now 10mg is present in 1ml. After that 5ml is sucked from the first dilution then it is make up into 50ml, now 1mg of sample is present in 1ml. After from that of second dilution we started the serial dilution that is 1 ml is sucked from second dilution makeup that with 10 ml of ethanol, 2 ml is sucked from second dilution and makeup with 10ml of ethanol. This process will continue up to take 5ml from second dilution and make with ethanol. From that serial dilution 2ml is taken from each, then it is make up with 7.6ml buffer solution of Sodium Dihydro Ortho Phosphate with P^H 7.4 and the add 0.4ml of hydrogen peroxide these are make up with each of five serial dilutions. Then they are taken to seen under UV spectrophotometer in 265nm, and the control are taken to zero.^[21] The results are presented in the form of **Table no-6 and 7** % Scavenged $[H_2O_2] = [(Ac - As)/Ac] \times 100$ Where Ac is the absorbance of the control and As is the absorbance in the presence of sample of *Murraya Koenigii* extracts of fruit and seed or standard.

Section - B

To Determine the Anti-diabetic Activity by Invitro Method

Diabetes is one of the major causes of death in worldwide. Every day a person dies due to diabetes related causes mainly from cardiovascular complication. Dyslipidemia and diabetes are commonly called as metabolic disease and it contributes a major emerging health crisis in the world. It is diagnosed by blood plasma hyperglycemia, has been related to lipid overload and abdominal obesity and these causes to promote negative clinical outcomes.

Requirements

- Glucose
- Test tubes
- Distilled water
- Pipette
- Calorimeter
- DNSA reagent
- Sample

- Test tube stand
- Water bath

Procedure

First to take 6 test tubes and give a number individually, then to take 0.2, 0.4, 0.6, 0.8 and 1ml of stock solution of glucose in test tubes and the blank is prepared by without taking stock solution and then same procedure is carried out, then they are make up to 2ml by using distilled water by pipette. After that to add 1ml of DNSA reagent to each of the test tubes, DNSA reagent is prepared by using sodium hydroxide solution because to understand that the reaction will be taken on alkaline medium. Now, we have to keep all the test tubes for heating for 15 minutes by using water bath. After that, add 7 ml of distilled water to each of the test tubes at that time the color of solution is gradually increased to each of the test tubes. Now by using colorimeter each samples are observed at 540nm and the results are noted. The results are presented in the form of **Table no-8**.

RESULT AND DISCUSSION

Pharmacognostical Study

The various Physio-chemical parameters such as moisture content, ash value, extractive value, foaming index, swelling index were determined for *Murraya Koenigii* seed and fruit powder. The results obtained for physio-chemical parameters are presented.

Table-3.

S.NO	Parameters	Value expressed As %	
		Fruit	Seed
1.	Ash values		
	TOTAL ASH	9	8.6
	ACID INSOLUBLE	1.3	5
	WATER SOLUBLE	8.3	6.6
	SULPHATED ASH	11	22
2.	Extractive value		
	CHLOROFORM	40	5
	ETHYL ACETATE	25	10
	ETHANOL	10	20
	METHANOL	75	5
	PET.ETHER	25	30
	HEXANE	20	20
	AQUEOUS	41.5	48
3.	FOAMING INDEX	Less than 100	Less than 100
4.	SWELLING INDEX	-	0.2ml
	INITIAL VOLUME	-	2.5ml

	FINAL VOLUME	-	2.7ml
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PHYTOCHEMICAL STUDY

Qualitative Chemical Test For the *Murraya Koenigii*'s Fruit and Seed Powder

From the qualitative phytochemical screening study it has been observed that the powder of seed and fruit of MURRAYA KOENIGII contain all the necessary primary and secondary metabolite such as alkaloids, tannis and phenols compound, flavanoids, glycosides, and test for reducing sugar.

Table NO: 4

Chemical Test	Fruit		Seed	
	Aqueous	Alcohol	Aqueous	Alcohol
1. Test for Alkaloids:				
Mayers Test	+	+	—	—
Dragndraffs Reagent	—	+	+	—
Hagers Reagent	—	—	+	+
Wagners Reagent	+	—	+	+
Tannic acid	—	+	—	—
Lead acetate	—	+	+	+
2. Test for Tannis and Phenols Compounds:				
Lead acetate	—	+	+	+
NaCl	—	+	+	—
3. Test for Flavonoids:				
Alkali test	—	+	+	—
Acid test	—	+	+	—
4. Test for Glycosides:				
Borntager's test	—	—	—	—
Modified borntagers test	—	+	+	+
Legals test	+	+	—	—
5. Test for Reducing sugar:				
Benetics test	+	+	—	—
Fellings A and B	+	+	—	—

Thin Layer Chromaography

The TLC studies were carried out using solvent compositions for the extracts of MURRAYA KOENIGII fruit and seed powder (aqueous and ethanol).

Solvent System- Chloroform:Methanol (9:1).

The optimized chamber saturation time for mobile phase was 10 min at room temperature (25±1°C). The results obtained in different extracts of *Murraya Koenigii* (aqueous and ethanol) by TLC analysis with mobile phase [chloroform:methanol(9:1)] were given in the table. The R_f values were calculated and represented in.

Table NO-5

Colour of Spot Under UV light

Sample	Sl. NO	Name Of Extract	Solvent System	Number Of Spot	Rf Value
Fruit	1	Aqueous	Chloroform :	1	2.6
	2	Alcohol	Methanol (9:1)	1	2.9
Seed	1	Aqueous	Chloroform :	1	2.3
	2	Alcohol	Methanol (9:1)	1	2.4

HYDROGEN PEROXIDE SCAVENGING ASSAY FOR FRUIT AND SEED

Table NO - 6

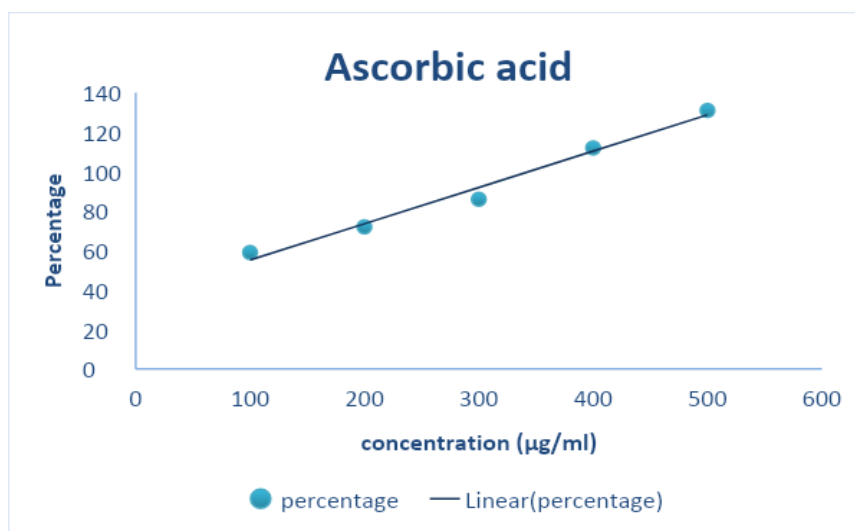
Alcohol Extraction of *Murraya Koenigii*'s Fruit and Seed Powder.

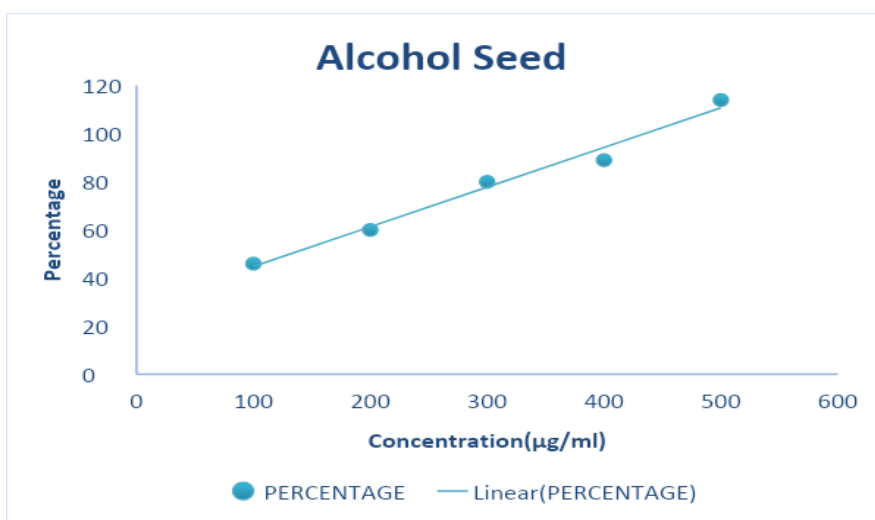
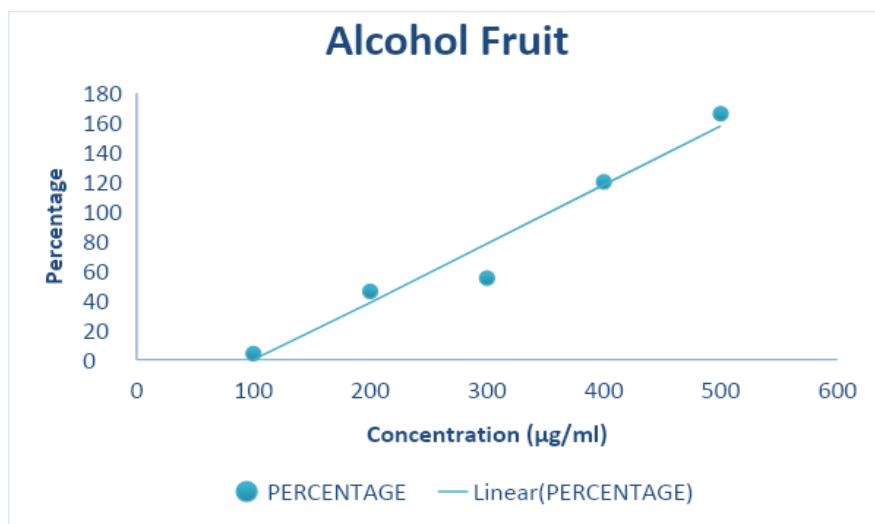
S.NO	CONCENTRATION	%OF INHIBITION		IC ₅₀ µg/ml	
		FRUIT	SEED	FRUIT	SEED
1	100	2	46	22.11µg/ml	131.52µg/ml
2	200	46	60		
3	300	55	80		
4	400	120	89		
5	500	166	114		

Table -7

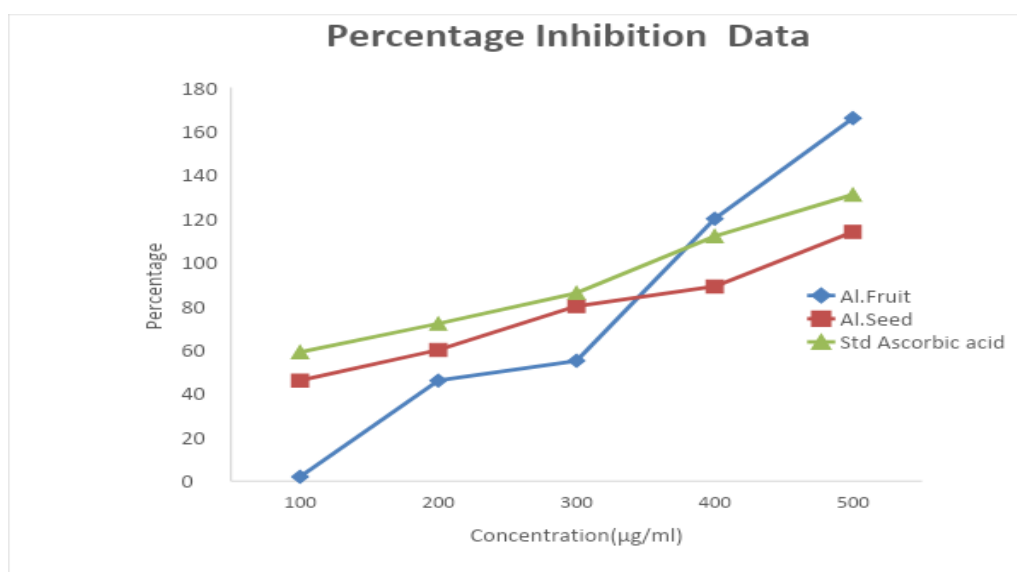
S.NO	CONCENTRATION	% OF INHIBITION STANDARD	IC ₅₀ µg/ml
1	100	59	71.73µg/ml
2	200	72	
3	300	86	
4	400	112	
5	500	131	

Regression Data



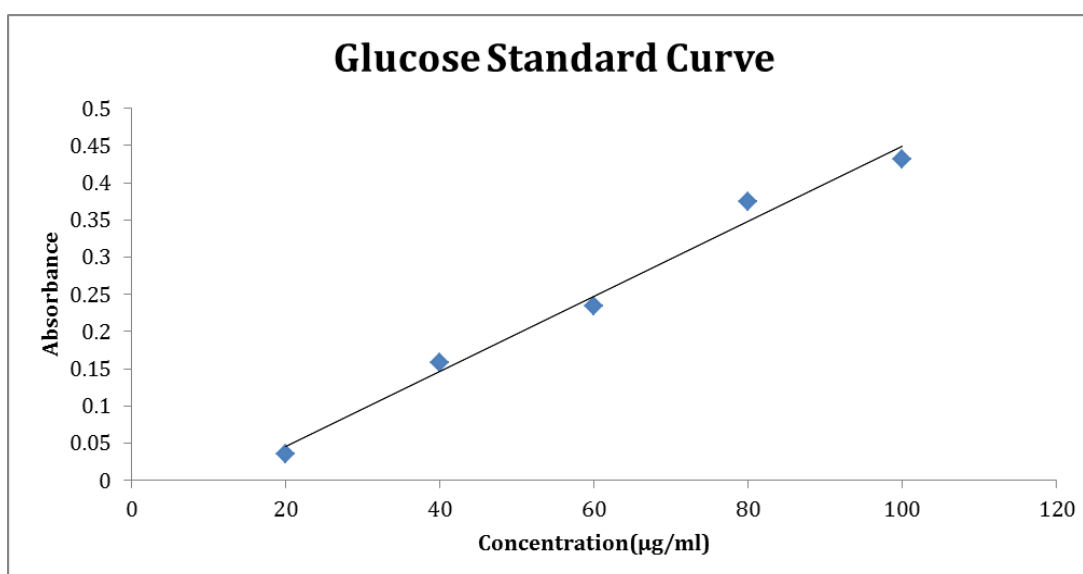


Percentage Inhibition Data



TO DETERMINE THE DIABETIC ACTIVITY BY INVITRO METHOD**Table-8**

GLUCOSE	D/W	DNSA	DISTILLED WATER	ABSOBANCE 540NM	ABSORBANCE
0.2ml	1.8ml	1ml of DNSA Reagent Heat 15 Mints	7ml of distilled water to make up	540nm	0.036 0.158 0.234 0.374 0.432 0.109
0.4ml	1.6ml				
0.6ml	1.4ml				
0.8ml	1.2ml				
1ml	1ml				
UN-1ml Extract of Aq.fruit	1ml				



Absorbance of unknown sample	0.109
Concentration of unknown sample	33.01943965

CONCLUSION

By the Physio-chemical parameters of *Murraya Koenigii* seed and fruit play an very important role in the authentication of crude drug and will be useful for determination of quality and recognize the plant for future work and the Phytochemical evaluation deals with the preliminary phytochemical screening and quantitative estimation of phytoconstituents present in powder and different extracts of *Murraya Koenigii* which confirms the presence of secondary metabolites like Flavonoids, terpenoids, tannis and alkaloids these are present in fruit and seed powder of *Murraya Koenigii* extracts of aqueous and alcohol, these secondary metabolite is responsible for pharmacological activity. Chromatographic separation are therapeutically important phytoconstituents there was carried out by TLC and the R_f value

obtained which coincides with the standard Rf value of alkaloid. In Pharmacological evaluation the invitro method of Antioxidant activity which states that ethanolic extract of fruit and seed powder of *Murraya Koenigii*, and the antioxidant activity is greater in ethanolic extract of fruit when compared to the ethanolic extract of seed, based on IC50 value it possess Moderate Activity, because of moderate activity they also may have an anti-inflammatory and anti-microbial activity. Then the invitro method of anti-diabetic activity by using DNSA reagent states that their activity is present in aqueous extract of fruit and to evaluate the concentration of unknown sample.

Author Contribution

Corresponding author had collected all the study material, analyzed, and prepared the complete manuscript.

Declaration

Competing Interest

The author declare that there is no conflict of interest.

Ethics approval and consent to participate

Not applicable.

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I hereby acknowledge that I prepared the project. And all the details are correct to the best of my knowledge.

REFERENCES

1. Jain V, Momin M, Laddha K. *Murraya koenigii*: an updated review. International Journal of Ayurvedic and Herbal Medicine, 2012 Aug 19; 2(04): 607-27.
2. Singha S, Omreb PK, Mohan SM. Curry leaves (*Murraya koenigii* Linn. Sprengal)-a mircale plant. Indian Journal of Science Researches, 2014; 4(1): 46-52.
3. Gupta GL, Nigam SS. Chemical examination of the leaves of *Murraya koenigii*. Planta Medica, 1970 Oct; 18(05): 83-6.
4. Adebajo AC, Olayiwola G, Eugen Verspohl J, Iwalewa EO, Omisore NO, Bergenthal D, Kumar V, Kolawole Adesina S. Evaluation of the Ethnomedical Claims of *Murraya koenigii*. Pharmaceutical Biology, 2005 Jan 1; 42(8): 610-20.

6. Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S, Ravi Kumar A. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. *Evidence-Based Complementary and Alternative Medicine*, 2010 Sep 23; 2011.
7. Choudhury, DR. Antara. *Quality Control and Standardization of Herbals*. Pune : Nirali Prakashan, 2021 Jan.
8. Gokhale S.B, DR.Kalaskar M.G, DR.Kulkarni, DR.Yele S.U. *A Practical Book of Pharmacognosy and Phytochemistry-1*. Pune : Nirali Prakashan, 2018 Nov.
9. JB, Harbone. *Phytochemical methods- A guide to modern techniques of plant analysis*. 2nd edition. Chapman and Hall, London. New York, 1994; pp 1-35.
10. Drivers, Bentley. *Text book of Pharmaceutical Chemistry*, 8th edition Oxford University Press. London, 1983; 13.
11. IL, Finar. *Organic chemistry*, vol 2, ELBS, 5th edition. London: s.n, 1996; 771.
12. GR, Chatwal. *Orgaic chemistry*, 1st edition. Mumbai : Himalaya Publishing Home, 2000; 2: 539.
13. Wadher SJ, Yeole PG, Gaikwad NJ. Pharmacognostical and physicochemical studies of heartwood of *Pterocarpus marsupium*. *Hamdard Medicus*, 2009; 52(2): 97-101.
14. JB, Becket AH and Stenlake. *Practical Pharmaceutical Chemistry*. Vol-2,4 edition. New Delhi: CBS Publication, 1997.
15. E, Stahl. *Thin Layer Chromatography*. 2nd edn. New york : Springer-verlag. Pp.30-160, 1969.
16. Wagner H, Bladt S. *Screening of unknown commercial drugs. Plant drug analysis: a thin layer chromatography atlas* 2nd ed.
17. Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. *Neuropharmacology*, 2001 Jun 1; 40(8): 959-75.
18. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. *Food and bioproducts processing*, 2011 Jul 1; 89(3): 217-33.
19. Cheeseman KH, Slater TF. *An introduction to free radical biochemistry*. British medical bulletin, 1993 Jan 1; 49(3): 481-93.
20. Hiremath SM, Madalageri BB, Basarkar PW. Composition of curry leaf (*Murraya koenigii* Spreng) oil during leaf growth. *Indian Perfumer*, 1998; 42(2): 58-9.

21. Bonde SD, Nemade LS, Patel MR, Patel AA. *Murraya koenigii* (Curry leaf): Ethnobotany, phytochemistry and pharmacology-A review. *Int J Pharm Phytopharmacol Res*, 2011; 1: 23-7.
22. Keser S, Celik S, Turkoglu S, Yilmaz O, Turkoglu I. Hydrogen peroxide radical scavenging and total antioxidant activity of hawthorn. *Chem J*, 2012; 2(1): 9-12.