IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Quantitative Analysis of Phytochemical constituents of Mimusops balata (Fruit) Extract by using UV-Visible Spectrophotometer

Authors: [1]Sagi Sambamurthy Raju, [2] Uppalapati Vidyamani,[3]Pakala Subhashini

KVRKVR&MKR COLLEGE,

Department of PG Chemistry

Khajipalem

Abstract:

Phytochemical compounds are found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. *Mimusops balata* plant is considered one of the most promising medicinal properties for the prevention and treatment of many diseases like. The present study is conducted to investigate the phytochemicals reason for its different medical properties. Different solvents have been used for extraction of the lemon plant according to their polarity. Majorly Methanol and water solvents are used for extraction and further screening studies were applied to the crude extract. Flavanoids and Phenoilc compounds are identified in the methanolic extract. Steroids are identified in the chloroform extract. Methanol extracts shows +ve results for much number of chemical compounds. The methanol extract of mimusops balata contain 0.191mg/gram of phenolic compounds, 0.076 mg/gram of steroids and 0.549mg/gram flavonoids. This confirms the high amounts of various antioxidents are present in the mimusops balata plant which confirms its presence of various antioxidant chemicals may be reason for its high medicinal properties.

Key words: Mimusops balata fruit, pytochemical analysis, UV-Visible spectrometer.

1) Introduction:

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals. Phytochemicals under research can be classified into majorcategories, such as carotenoids and polyphenols, which include phenolic acids, flavonoids, and stilbenes/lignans. Phytochemists study phytochemicals by first extracting and isolatingcompounds from the origin plant, followed by defining their structure or testing in laboratory model systems, such as cell cultures in that field include isolating specific compounds and determining their structures, which are often complex and identifying what specific Phytochemical is primarily responsible for any given biological activity. The phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

Mimusops balata is a species of flowering plant in thefamily Sapotaceae. It is native to Mauritius and Reunion. Mimusops balata belongs to the Sapotaceae family. Species from the Sapotaceae family of chemical constituents such as saponins, flavonoids and polyphenolic compounds. The present study was undertaken to evaluate the phytochemical analysis, with quanitative analysis of Mimusops balata fruit extracts.

2. Materials &Methods:

2.1) Collection of plant materials:

The plant Mimusops balata was collected from kvrkvr and mkr college campus.The fruits of the plant were collected from the tree. The collected fruit was cleaned and was shade dried. After complete dry, the fruit was powdered and was preserved in air tight container and was used when required.

2.2) Reagents & Chemicals:

All reagents and chemicals used in the experiments were of analytical grade and solvents used for extraction of plant material were of analytical grade

2.3) Extraction procedure:

The powdered material was weighed in a selected quantity and is subjected to extraction using chloroform and Methanol in successive mode respectively for 48h. The solvent was then recovered using distillation apparatus and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical screening and quantification of phytochemicals.

2. 4) Preparation of reagent:

2.4.1) Bromocresol green solution:

Solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.

2.4.2) Phosphate buffer solution (pH 4.7):

Buffer solution was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

2.4.3) *Folin- Ciocalteu's (FC) reagent:*

10ml of ofFolin- Ciocalteu's solution was dissolved in 90ml of double distilled water.

2.4.4) Iron (III) chloride solution:

500mg of ferric chloride was weighed and was dissolved in 100ml of distill water.

2.4.5) Potassium hexacyanoferrate (III) solution:

500mg of potassium hexacyanoferrate was weighed and was dissolved in 100ml of distillwater.

2.5) Preliminary phytochemical screening:

2.5.1) Test for steroids:

2.5.1.1) Salkowski Test:

Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in colour.

2.5.1.2) Liebermann Burchard's Test:

To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers.

2.5.2) TEST FOR TRITERPINOIDS:

2.5.2.1) Salkowski Test:

Few drops of concentrated sulphuric acid is added to the extract, shaken and on standing, lower part turns golden yellow colour.

2.5.2.2) Lieberman Burchard's Test:

To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a red ringindicates triterpenes.

2.5.2.3) Ischugajiu Test:

Excess of acetylchloride and pinch of zinc chloride are added to the extract solution, Keptaside for reaction to subside and warmed on water bath, cosin red colour is produced.

2.5.2.4) Brickorn and Brinar Test:

To the extract, few drops of chlorosulfonic acid in glacial acetic acid (7:3) are added, redcolour is produced.

2.5.3) Test for Saponins:

2.5.3.1) Foam Test:

Small amount of extract is shaken with little quantity of water; the foam producedpersists for 10 minutes. It confirms the presence of saponins.

2.5.3.2) Haemolysis Test:

To 2ml of 1.8% Sodium chloride solution in two test tubes, 2ml distilled water is added to one and 2ml of 1% extract to the other, 5 drops of blood is added to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the

Tube containing the extract indicates the presence of saponins.

2.5.3.3) Test for Steroidal Saponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform.The chloroformlayer is tested for steroids.

2.5.3.4) Tests for TriterpenoidalSaponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform.The chloroformlayer is tested for triterpenoids.

2.5.4) Tests for Alkaloids:

Mayer's Test:

The acid layer when mixed with Mayer's reagent (Potassium mercuric iodidesolution) gives creamy white precipitate.

Dragendroff's Test:

The acid layer with few drops of Dragendroff'sreagent (Potassiumbismuth iodide) gives reddish brown precipitate.

Wagner's Test:

The acid layer when mixed with few drops of Wagner's reagent(solution ofiodide in potassium iodide) gives brown to red precipitate.

Hager's Test:

The acid layer when mixed with few drops of Hager's reagent (Saturatedsolution of pricric acid) gives yellow coloured precipitate.

2.5.5) Tests for Carbohydrates:

Fehlings's Test:

The extract when heated with Fehling's A and B solutions gives an orangered precipitate showing the presence of reducing sugar.

Molischs's Test:

The extract is treated with Molisch's reagent and conc .sulphuric acid alongthe sides of the test tube, a reddish violet ring shows the presence of carbohydrate.

Benedict's test:

The extract on heating with Benedict's reagent, brown precipitate indicatesthe presence of sugar.

Barfoed's Test:

Barfoed's reagent is added and boiled on water bath for few minutes; reddishprecipitate is observed for the presence of carbohydrate.

2.5.6) Test for Flavonoids:

Shinoda Test:

The extract solution with few fragments of magnesium ribbon and concentrated hydrochloricacid produced magenta colour after few minutes.

Ferric chloride test:

Alcoholic solution of extract reacts with freshly prepared ferric chloride solution and givenblackfish green color.

Lead Acetate Test:

Alcoholic solution of extract reacts with 10% lead acetate solution and given yellowprecipitate.

2.5.7) Test for Glycosides:

Anthraquinone test:

Drug is powdered and extracted with either ammonia or caustic soda.The aqueous layershows pink color

Keller-killiani test:

This is for cardiac glycosides. The extract and 0.4 glacial acetic acid are mixed with ferrouschloride and 0.5 mi of concentrated sulphuric acid. The acetic acid layer shows blue color.

2.5.8) Test for Phenolic Compounds:-

Ferric chloride test:-

Treat the extract with ferric chloride solution then blue color appears ifhydrolysable tanninsare present and green color appears if condensed tannins are present.

Gelatin test: -

To the test solution add 1% gelatin solution containing 10% Nacl, and then pptis formed.

Test for chlorogenic acid:-

Treat the test solution with aqueous ammonia and expose to airgradually, green colour isdeveloped.

2.6) **QUANTITATIVE ANALYSIS**

2.6.1) **Determination of total flavonoid content**

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to a known method, 15 using quercetin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% $NaNO_2$ (0.03ml). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mMNaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm.

2.6.2) **Quantitative Estimation of Steroids:**

1ml of extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

2.6.3) **Quantitative Estimation of Phenolic compounds:**

The total phenolic content of the extract was determined by the Folin–Ciocalteu method. Briefly, 1 µL of crude extract was made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate and mixture was allowed to stand for a further 60 min in the dark. The final volume made up to 10 ml with distilled water and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results wereexpressed as mg of gallic acid equivalent per g dry weight.

2) Results & Discussions:

3.1) Extraction:

3.1.1) Chloroform Extaction: Sample was subjected to Chloroform extraction until the solvent was decolorizes in the soxhelet extractor. After complete extraction solvent was subjected to distillation to collect the solvent.

3.1.2) Methanol extraction: Sample was subjected to methanolic extraction until the solvent was decolorizes in the soxhelet extractor. After complete extraction solvent was subjected to distillation to collect the solvent.

3.2) PRELIMINARY PHYTOCHEMICAL SCREENING:

3.2.1) Results of steroids test:

Steroids have been identified in chloroform extracts. Samples were given red color when subjected to Salkowski Test and reddish brown ring is formed with Liebermann Burchard's Test confirms the present of steroids in the crudeextracts.

3.2.2) Results of Flavonoids test:

Flavanoids have been identified in Methanol extracts. Samples were blackish green color when subjected to ferric chloride test confirms the presence of flavanoids in the crude extracts.

3.2.3) Results of Phenolic Compounds test:

Phenolic compounds have been identified in methanol extracts. Samples were given green color when subjected to ferric chloride test and confirms the presence of phenolic compoundsin the crude extracts.

3.3) Crude extract:

Methanol extract: 0.777 Grams of crude extract was gained.

Chloroform extract: 1.26 Grams of crude extract was gained

Results of phytochemical screening of plant extracts: Table:3.1

S.No	Compound	Methanol extract	Chloroform extract
1	Steroids	-	+
2	Terpinoids	-	-
3	Saponins	-	-
4	Steroids	-	-
5	Triterpinoidalsaponins	-	-
6	Alkaloids	-	-
7	Carbohydrates	-	-
8	Flavanoids	+	-
9	Glycocydes	-	-
10	Phenoilc compounds	+	+

Note: + (present), - (absent)

3.4) Quantitative estimation:

3.4.1) Determination of Total flavanoid content:

Total flavanoid content in the plant extract was estimated using spectrophotometer. Quercetin was used as standard flavanoid and results were expressed in quercetin equivalents. The water extract of *mimusops balata* have the positive results for flavanoids. The absorbance of the samples were substituted in y = 0.0236x - 0.0003 (standard equation) and the amount present in the samples was calculated. It was found that the 1gr of the extract contains 3.269of flavonoids in water extracts.

3.4.2) Quantitative Estimation of Phenoilc Compounds:

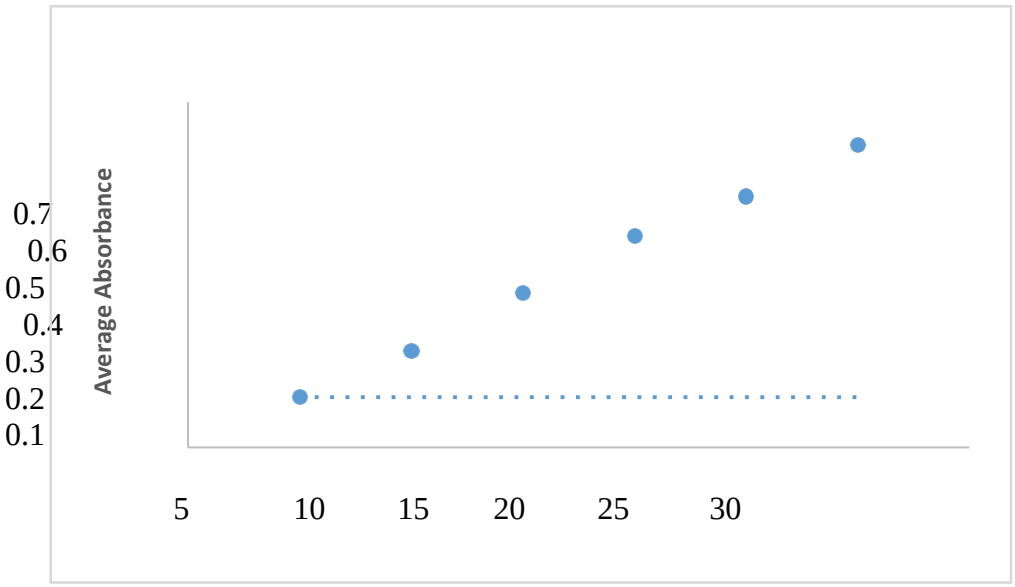
The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR). In the procedure, 1ml of extract was mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made up to 10 ml with distilled water and allowed to stand for 90 min at room temperat00ure. Absorbance of sample was measured against the blank at 765nm using a spectrophotometer. A calibration curve was constructed using Gallic acid solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of gallic acid per gram of dry weigh of *mimusops balata* extract.

3.4.3) Quantitative Estimation of Steroids:

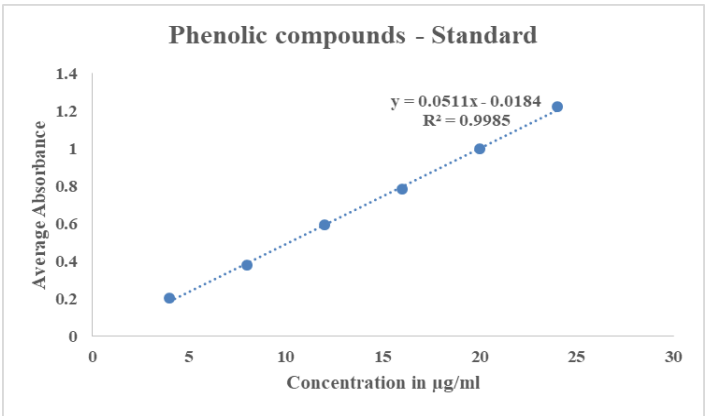
1ml of extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

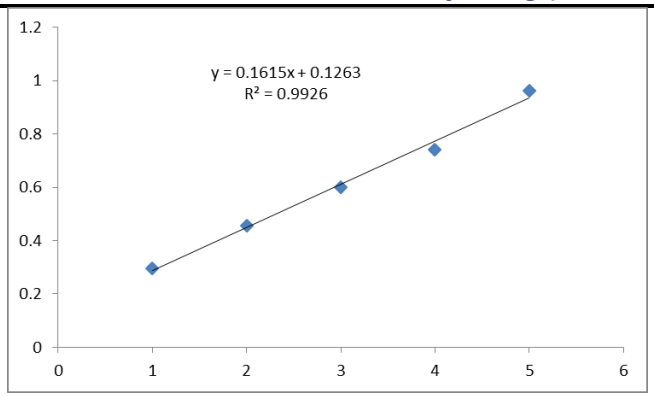
Table: Calibration results of flavonoid, Phenolic compound, steroid

S.No	Flavonoid		Phenolic compound		Steroid	
	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance
1	5	0.117	4	0.203	1	0.296
2	10	0.225	8	0.397	2	0.456
3	15	0.357	12	0.594	3	0.600
4	20	0.491	16	0.782	4	0.741
5	25	0.582	20	0.996	5	0.961
6	30	0.701	24	1.225	6	1.225



Graph: Calibration graph of flavonoid by quercetin





Graph: Calibration graph of steroids

Estimation results of steroid, Phenolic compound and Flavonoids

Name of the compound	Name of the Extract	Absorbance found	Amount found
Steroid	Chloroform	0.114 0.113 0.114	0.076 mg/gram
Phenolic compound	Methanol	0.179 0.179 0.179	0.191 mg/gram
Flavonoids	Methanol	0.242 0.243 0.243	0.549 mg/gram

REFERENCES:

1. Preethi R et al. Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants Against Food Borne Pathogens. Advances in Biological Research 2010; 4(2): 122-125.
2. Nair R, Chanda S. Activity of some medicinal plants against certain pathogenic bacteria strains. Indian Journal of Pharmacology 2006; 38:142-144.
3.Akinmoladun AC et.al. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Scientific Research and Essay 2007; 2:163-166.
4.Greathead W. Extraction and Antimicrobial Effect of Thai Herbs and Spices Extracts, Journal of Agricultural and Food Chemistry2003;43:361-371.
5.Gami B et.al. Ethnobotanical, phytochemical and pharmacological review of *Mimusops elengi* Linn. Asian Pacific Journal of Tropical Biomedicine 2012;2(9):743-748.
6.Baliga MS. Chemistry and medicinal properties of the Bakul (*Mimusops elengi* Linn):A review. Food Research International 2011; 44:1823-1829.
7. Khandelwal KR. Practical Pharmacognosy- Techniques and experiments. 19th ed. Pune: Nirali; 2008.
8.Evans W. Pharmacognosy, 16th ed. Singapore: Saunders Ltd; 1996.
9.Stankovic MS. Total phenolic content, flavonoid concentration and Antioxidant activity of *Marrubium peregrinum* L. Extracts. Kragujevac Journal of science. 2011;33:63-72.
10.Sen S et.al. Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of *Meyna spinosa* Roxb., an Indian medicinal plant. The Chinese Journal of Natural Medicines 2013; 11(2):149-157.
11.Ansari NM et. al. Antioxidant Activity of Five Vegetables Traditionally Consumed by South-Asian Migrants in Bradford, Yorkshire, UK. Phytotherapy Research 2005;19:907-911.