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The Study Of Phytochemical Analysis And Antioxidant Properties Of Leaf Extracts Of *Carica Papaya* And *Mangifera Indica* And Seed Extract Of *Momordica Charantia*

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Abstract:

The main objective of this study was to evaluate the phytochemical and antioxidant properties of leaves of *Carica papaya* and *Mangifera indica* and seeds of bitter gourd cultivated in the Bangalore region of Karnataka. The leaves of Karnataka *papaya* and *M. indica* and seeds of *M. charantia* were extracted with different solvents such as water, methanol, and ethyl acetate. The preliminary phytochemical screening was performed by standard methods as described by Harborne. The tests indicate the presence of alkaloids, tannins, flavonoids, and the absence of saponin and glycosides. The total phenolic content (TPC) was determined by the Folin-Ciocalteu method. In addition, leaf and seed extracts of methanol were analyzed by high-performance liquid chromatography to identify bioactive compounds. The results confirmed that extracts of *M. indica* and *C. papaya* and *M. charantia* accumulate large numbers of phytochemicals, but the higher percentage is related to phenolic compounds. These findings are helpful to the phytochemist and pharmacologists for the identification of new active principles in the future.

1. INTRODUCTION:

Plants are considered to be a good source for the exploration and discovery of new pharmaceutical compounds as well as medicines, which can be potential drugs for humans as they act as intermediates for the synthesis of useful drugs. Plants possess various phytochemicals with several bioactivities such as anti-inflammatory, antioxidant, and anticancer. Thus, among Papaya, mango leaves, and bitter gourd seeds, is widely used in the treatment of many Ayurveda as well as herbal and folk medicine.

C. Papaya is an evergreen shrub or small tree and a member of the Caricaceae family, represented by four genera and four species in India.It is commonly known as Papaya, Melon tree, Pawpaw, ‘Tree melon’ in Hindi, ‘papita’ in Manipuri, ‘Awathabi’ in Marathi, ‘Papaya’ in Tamil, ‘Pappali’ in Bengali, ‘Papaya’ in Kannada, ‘Parangi’ in Telugu. This plant originated in southern Mexico and Costa Rica and is distributed as

a plantation crop in India, Sri Lanka, Hawaii, Australia, and in tropical and subtropical regions. The herbaceous perennial plant. The entire plant possesses various phytonutrients and can be considered for commercial,pharmaceutical, and industrial applications.The papaya plant is best with a large variety of phytonutrients and antioxidant, antimicrobial, and anti-dengue properties.Some of the important phytochemicals found in *C. papaya* are Beta-carotene, Quercetin, Glycosides, Phenolic compounds, and tannins, proteins, Anthocyanins, Oxalate, Saponins, etc. Plants have a limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives (Rajendra Prasad et al..,2013).

Mangifera indica is a large evergreen tree belonging to the Anacardiaceae family. It is commonly known as ' Maram' in Tamil, 'Mango' in English, 'Aam' in Hindi, and 'Aamra' in Sanskrit.Different varieties of mango have been cultivated throughout the world and recently cultivated throughout the world and recently cultivated in Iraq (Rajan et al..,2011). Mango consists of about sixty genera and six hundred species, which are mainly tropical trees and shrubs. Its parts are commonly used in folk medicine for a wide variety of remedies. Many phenolic compounds have been detected in mango peels,bark,pulps, and seed kernels. Several pharmacological activities of mango extracts have been reported, including anti-inflammatory, antioxidant, anti-allergic, anthelmintic, and antiamoebic. Herbal drinks are very popular as they contain natural constituents, especially phenolic compounds (Abdelnaser Abdelghany et al.). The mango is a rich source of various polyphenolic compounds. The major polyphenols in the mango are mangiferin, catechins, quercetin, anthocyanins, alkaloids, flavonoids, tannins, steroids,saponins, and cardiac glycosides.

The amounts of the different polyphenolic compounds in the mango vary from part to part (pulp, peel,seed, bark, leaf, and flower) (Tabla et al.,2014). Polyphenols are secondary metabolites of plants and are widely distributed in beverages and plant-derived foods. Phenolic compounds have the capacity to quench lipid peroxidation, prevent DNA oxidative damage, scavenge free radicals, and prevent inhibition of cell communication, all of which are precursors to degenerative diseases.

Bitter gourd (*Momordica charantia L.*) has been regarded as a food and medicinal plant. It is a powerful nutrient-dense plant composed of a complex array of beneficial compounds. These include phytochemicals, vitamins, minerals, and antioxidants, which all contribute to its remarkable versatility in treating a wide range of illnesses. The medicinal value of bitter gourd has been attributed to its high antioxidant properties, including phenolic compounds. Vegetables prevent humans from several severe and chronic diseases. Consumption of vegetables prevents humans from cancer, cardiovascular disease, diabetes, hypertension, stroke, paralysis, urinary disorders, etc., since vegetables have antioxidant properties. Bitter gourd is a tropical and sub-tropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. It is commonly known as 'Chagkha' in Mizo, 'Karela' in Hindi, 'Bitter gourd' in English, 'Hagalakayi' in Kannada, and 'Kakarakaya' in Telugu. Its many varieties differ substantially in the shape and bitterness of the fruit. Many biologically active compounds have been identified in bitter gourd, including phenolics, steroidal glycosides, alkaloids, and conjugated linoleic acid isomers, organosulfur compounds. Certain common disease, HIV (Human Immunodeficiency Virus), cancer, and microbial infections have been investigated for treatment with phytochemical fractions and compounds isolated from the gourd family. Extraction involves the separation of medicinally active constituents in plant tissue from

inactive/inert components by using selective solvents and the most appropriate extraction technologies. Solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity (Joona, Sowmia et al., 2013). Phytochemicals are natural, non--nutritive plant chemicals with defensive properties against cancer by protecting the cells from damage. Most of the phytochemicals possess biological antioxidant capacity that protects our cells against oxidative damage and reduces the risk of certain types of cancer. These phytochemicals tend to prevent the adhesion of pathogens to the human cell wall by physically binding to it. Antioxidants are substances that prevent oxidative damage to the target molecule. An antioxidant can scavenge the free radicals because of their singlet oxygen quenching and redox hydrogen donating features. In recent days, the usage of synthetic antioxidants has been taken over by natural antioxidants as they could be safer without any side effects. In recent decades, due to the various pharmacological actions of medicinal plants, many researchers are showing interest in studying the antioxidant phytochemicals such as phenols, flavonoids, and tannins, which have been recognized for their potential role in preventing human diseases. Free radicals cause depletion of the immune system, changes in gene expression, and induce abnormal proteins resulting in degenerative diseases and aging. Antioxidants have been found to be the solution to this problem as they interrupt these chain reactions to form radicals that can easily be removed from the human body, thereby generally improving health, assisting cell rejuvenation, preventing cancer, and cardiovascular diseases. Thus, it is important to investigate the antioxidant potential.

The objective of this paper is to provide a review of phytochemical studies that have addressed the extraction, measurement, and identification of bioactive compounds from plants. Therefore, in an attempt to explore plant-based alternative solutions in promoting health, as well as paving the way towards our future pre-clinical and clinical studies, we aimed to analyze the phytochemicals and antioxidant activities of different plant species under the same evaluation condition. Furthermore, the principal phenolic percentages were chromatographically characterized. Our results provide a basis for future studies on the identification and antioxidant assay of active compounds with potential applications in drug development.

2. MATERIALS AND METHODS

2.1 Plant materials

Carica papaya leaves were collected from Ramanagara Region, Karnataka, *Mangifera indica* leaves were collected from Malur, Kolar Region Karnataka, and *Momordica charantia* (Bitter gourd) seeds were collected from K.R Market, Bangalore Region, Karnataka.

2.2 Chemicals

Methanol, Ethanol, Ethyl acetate, Copper sulfate, Potassium hydroxide, Potassium iodide, Ferric chloride, Sulfuric acid, Sodium hydroxide, Glacial acetic acid, Ammonia, Sodium carbonate, Ninhydrin, 2,2,1-Diphenyl-1-picrylhydrazyl (DPPH), Gallic acid, Ascorbic acid.

2.3 Instruments

Soxhlet apparatus, UV visible spectrophotometer, Colorimeter, Hot air oven, pH meter, HPLC (High-performance liquid chromatography).

PREPARATION OF *C. papaya* LEAF EXTRACT

The collected plant materials were washed with running tap water to avoid surface contamination and shade dried for about 20 days. The dried leaves were cut into small pieces and macerated into a fine powder. The dried powder was soaked with different solvents such as methanol, ethyl acetate, and water, was subjected to solvent extraction using a Soxhlet apparatus.

Preparation of methanol extract of *C. papaya* leaves

50g of the powdered leaves were extracted exhaustively over a period of 6 hours using continuous hot extraction (60ºC) method with 350 ml of methanol in a Soxhlet apparatus.

Preparation of Ethyl acetate extract of *C. papaya* leaves

50g of the powdered leaves were extracted exhaustively over a period of 6 hours using continuous hot extraction (70ºC) method with 350ml of Ethyl acetate in a Soxhlet apparatus.

Preparation of Water extract of *C. papaya* leaves

50g of the powdered leaves were extracted exhaustively over a period of 6 hours using continuous hot extraction (100ºC) method with 350ml of water in a Soxhlet apparatus.

The extracted samples were kept in a hot air oven at a suitable temperature. The dried powder samples were stored at room temperature and used for further analysis.

Preparation of *M. indica* LEAF EXTRACT

The collected plant materials were washed with running tap water to avoid surface contamination and shade dried for about 20 days. The dried leaves were cut into small pieces and macerated into a fine powder. The dried powder was soaked with different solvents such as methanol, ethyl acetate, and water and was subjected to solvent extraction using a Soxhlet apparatus.

Preparation of methanol extract of mango leaves:- 50g of the powdered leaves were extracted exhaustively over a period of 6 hours using continuous hot extraction (60ºC) method with 350 ml of methanol in a Soxhlet apparatus.

Preparation of ethyl acetate extract of mango leaves:- 50g of the powdered leaves were extracted exhaustively over a period of 6 hours using continuous hot extraction(70ºC) method with 350ml of ethyl acetate in a Soxhlet apparatus.

Preparation of water extract of mango leaves:- 50g of the powdered leaves were extracted exhaustively over a period of 6 hours using continuous hot extraction(100ºC) method with 350ml of water in a Soxhlet apparatus.

The extracted samples were kept in a hot air oven at a suitable temperature. The dried powder samples were stored at room temperature and used for further analysis.

Preparation of *M. charantia* SEED EXTRACT

The bitter gourd seeds were washed thoroughly under running tap water to remove adhered dirt, dust, and other foreign debris. After washing, they were dried at room temperature for a few days (20 days). The dried material was ground further to fine powder using a small laboratory grinder. After preparation of the the powder was soaked with different organic solvents such as methanol, ethyl acetate, and water and was subjected to solvent extraction using the Soxhlet apparatus.

Preparation of methanol extract of bitter gourd seed extract:- 25g of powdered seeds were extracted exhaustively over a period of 6 hours using the hot extraction method(60ºC) with 250ml of methanol in an apparatus.

Preparation of the ethyl acetate extract of bitter gourd seed extract:- 25g of powdered seeds were extracted exhaustively over a period of 6 hours using the hot extraction method(70ºC) with 250ml of ethyl acetate in a Soxhlet apparatus.

Preparation of water extract of bitter gourd seed extract:- 25g of powdered seeds exhaustively over a period of 6 hours using the hot extraction method(100ºC) with 250ml of water in a Soxhlet apparatus.

The extracted samples were kept in a hot air oven at a suitable temperature. The dried samples were stored at room temperature and used for further analysis.

METHODS

2.4 PHYTOCHEMICAL ANALYSIS

Phytochemical screening was performed for the presence of alkaloids, carbohydrates, amino acids, glycosides, protein, phenolic compounds, and tannins from respective solvents such as Methanol, Ethyl acetate, and Water, according to the standard procedure [11,12].

Stock preparation of *C. papaya* :- 200 mg of *C.papaya* extract in 10ml of each solvent, and 1ml of each solvent was used as a standard for various phytochemical analyses.

Stock preparation of *Mangifera indica* :- 200mg of *M.indica* extract in 10ml of each solvent, and 1ml of each solvent was used as a standard for various phytochemical analyses.

Stock preparation of *M. charantia* :- 200mg of *M.charantia* extract in 10ml of each solvent, and 1ml of each solvent was used as a standard for various phytochemical analyses.

ALKALOIDS

WAGNER'S TEST: For 1ml of the sample solution, a few drops of Wagner's reagent were added along the sides of the test tube. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

WAGNER REAGENT: Dissolve 2g of iodine and 6g of potassium iodide in 100ml of distilled water.

CARBOHYDRATES

BENEDICT'S TEST: For 1ml of the sample solution, a few drops of Benedict's reagent were added and heated for 2min. The appearance of a colored precipitate indicates the presence of carbohydrates.

BENEDICT'S REAGENT: Dissolve 17.3g of copper sulfate pentahydrate, 100g of sodium carbonate, and 173g of sodium citrate in 100mL of distilled water.

AMINO ACIDS

NINHYDRIN TEST: For 1 ml of the sample solution, two drops of ninhydrin reagents were added. The formation of a purple color indicates the presence of amino acids.

NINHYDRIN REAGENT: 2% solution of ninhydrin must be prepared by dissolving 0.2g of ninhydrin in 10ml of either ethanol or acetone.

GLYCOSIDES

KELLAR-KILLIANI TEST: For 1 ml of the sample solution, 1 ml of glacial acetic acid, a few drops of ferric chloride, and concentrated sulfuric acid should be added. The appearance of a reddish-brown ring at the junction of liquids indicates the presence of glycosides.

PHENOLIC COMPOUNDS AND TANNINS

FERRIC CHLORIDE TEST: For 1 ml of the extract, a few drops of neutral 5% ferric chloride should be added. The appearance of a dark green color indicates the presence of phenolic and tannin compounds.

FERRIC CHLORIDE TEST: 5% of ferric chloride should be dissolved in 90% alcohol.

PROTEIN

BIURET TEST: For 1 ml of the extract, one drop of 2% copper sulfate and 1 ml of ethanol and potassium hydroxide should be added. The presence of the pink color of the ethanolic layer indicates the presence of protein.

SAPONINS

FROTH TEST: For 1 ml of the extract, a few drops of distilled water should be added and shaken vigorously. The appearance of foam indicates the presence of saponins [13].

QUINONES:

For 1 ml of the extract, a few drops of concentrated hydrochloric acid should be added. The formation of a yellow precipitate indicates the presence of quinones [13].

OXALATE:

For 1 ml of the extract, a few drops of glacial acetic acid should be added. The appearance of a greenish-black coloration indicates the presence of oxalate.

ANTHOCYANINS:

For 1 ml of the extract, 2 ml of hydrochloric acid and 1 ml of ammonia should be added. The color changes from pink-red to blue-violet indicate the presence of anthocyanins.

DETERMINATION OF TOTAL PHENOLIC CONTENT(TPC)

The TPC of *C. papaya*, *M. indica* and *M. charantia was* determined spectrophotometrically according to the Folin-Ciocalteu method with slight modifications [11,12].

A standard solution of gallic acid was prepared using distilled water at a concentration of 1mg/ml. Different working standards were prepared to obtain the standard calibration curve, followed by dilution with distilled water with 3ml and 0.5ml of FC reagent (1:1) and incubated at room temperature for about 15 min, and then 2 ml of 7% sodium carbonate was added. Similar steps were followed for estimating phenolic content in the sample extract, and the absorbance was measured at 765nm against blank using a spectrophotometer. All experiments were made in triplicates, and the TPC was determined using the standard gallic acid calibration curve.

2.5 ANTIOXIDANT ASSAY:

2,2,1-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity DPPH.

The free-radical scavenging activity was determined by DPPH proposed by Zadeh et al., 2008, with slight modifications [14].

A standard solution of ascorbic acid was prepared at a concentration of 1mg/ml in methanol. The standard calibration curve was obtained using different working standards 4mg/100ml (4µg/ml) using methanol. DPPH solution (500µl) was then added and mixed vigorously. Similar steps were followed for the sample extract.

The reaction mixture was incubated for about 45 min in dark condition, and absorbance was measured at 520nm using a spectrophotometer. All determinations were made in triplicates, and the standard curve was obtained using ascorbic acid.

The % DPPH which was scavenged(% DPPH) was calculated using the formula:

$$\text{Scavenging effect (\%)} = 1 - \frac{\text{Absorbance of the sample at 517nm.}}{\text{Absorbance of control at 517nm}} \times 100$$

2.6 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY(HPLC) ANALYSIS

Fractionation of *C .papaya*, *M. indica* and *M. charantia* was performed by HPLC to identify active compounds. An isocratic HPLC, variable wavelength UV-Visible detector and a C18 phenomenex column was used. The mobile phase components, water and acetonitrile, were filtered through 0.22-micron membrane filters before use and pumped from the solvent reservoir at a flow rate of 1 ml/min, which resulted in column backup, max. pressure 25pk. The column was maintained at room temperature. One milliliter of papaya, mango leaf extract, and bitter gourd seed methanol extracts were injected [26].

Sample preparation:

Gallic acid (standard sample) - 22mg of the sample was dissolved in 1ml of methanol.

Mango Leaf methanol extract (test sample) - 22mg of the sample was dissolved in 1ml of methanol.

Papaya Leaf methanol extract (test sample) - 22mg of the sample was dissolved in 1ml of methanol.

Bitter gourd seed methanol extract (test sample) - 22mg of the sample was dissolved in 1ml of methanol.

3 . RESULT

Percentage of Yield (Dry sample)

Formula:

1) W2-W1

Where,

W2=weight of petri plate with sample

W1=weight of empty petri plate

50 of leaf sample yields =_____g

$$\text{Therefore 100gm of leaf sample yields} = \frac{(W2 - W1)}{50} \times 100 = \_\_\_\_\_\%$$

Yield	Water	Methanol	Ethyl acetate
Mango	18.3%	8.4%	18.76%
Papaya	22.44%	10.86%	26.72%
Bitter Gourd	13.2%	11.44%	17.68%

Table1: Percentage of yield (dry sample)

Phytochemical Analysis

Qualitative analysis was conducted to evaluate the phytochemical profile of *C. papaya*, *M. indica* leaves extract and *M. charantia* seed extract.

Phytochemical screening of *C.papaya, M.indica* leaves, and *M.charantia* seed extract shows the presence of alkaloids, proteins, glycosides, phenols, tannins, saponins,quinine, oxalate, and anthocyanins. The presence of alkaloids, carbohydrates, glycosides, phenols, tannins, saponins, and oxalates shows the greater intensity of their presence in the methanolic extract (Table 2). In the methanolic extract, all the bioactive compounds such as alkaloids, glycosides, phenols, tannins, saponins, quinine, and oxalates are present except for proteins and anthocyanins.

The overall result shows that methanol extracts possess a greater number of bioactive compounds when compared to other solvents.

	Water	Methanol	Ethyl acetate
Alkaloid	-	+	+
Carbohydrate	-	+	-
Amino acid,	+	-	-
Glycoside	-	+	-
Phenols tannin	+	+	+
Protein	-	-	-
Saponin	+	+	-
Quinine	-	+	-
Oxalate	-	+	-
Anthocyanin	+	-	+

Table 2: Qualitative analysis of *C. papaya* leaves extract: Phytochemical screening

	Water	Methanol	Ethyl acetate
Alkaloid	+	+	+
Carbohydrate	+	+	-
Amino acid	-	-	-
Glycoside	+	-	+
Phenols tannin	+	+	+
Protein	-	-	-
Saponin	+	+	-
Quinine	+	+	-
Oxalate	-	-	-
Anthocyanin	+	+	+

Table 2.1 : Qualitative analysis of M.*indica* leaf extract

	Water	Methanol	Ethyl acetate
Alkaloid	+	+	+
Carbohydrate	-	-	-
Amino acid,	+	+	-
Glycoside	-	-	-
Phenols tannin	+	+	+
Protein	-	-	-
Saponin	+	-	-
Quinine	-	-	-
Oxalate	-	-	-
Anthocyanin	-	-	-

Table 2.2 : Qualitative analysis of *M. charantia* leaf extract

TPC

The total phenol content was determined by the Folin-Ciocalteu method and reported as gallic acid equivalents (GAE) concerning the standard curve. The standard taken was gallic acid in a concentration of 1mg/ml. The concentration of the sample extract was evaluated by comparing it to the Standard Graph 1. The concentration of TPC present in leaf extracts of *C. papaya* , *M. indica,* and *M. charantia* in respected solvents is mentioned in Table 3.

	Water	Methanol	Ethyl acetate
C. papaya	0.6390 g	9.965 g	0.231 g
M. indica	1.490 g	16.186 g	6.468 g
M. charantia	0.319 g	18.778 g	0.290 g

Table 3: **Quantification of TPC in different solvent extracts of leaves of *Carica papaya* and *M. indica* and seeds of *M. charantia*.**

Antioxidant activity of *C. papaya* , *M. indica and M. charantia:*

DPPH assay DPPH radical scavenging activity

IC50 (Inhibitory Concentration) of the sample extract was determined by comparing it to the standard value of ascorbic acid. The result of the antioxidant activity of different solvents extract by DPPH assay shows that the presence of free radicals is greater and directly proportional to the concentration of the sample. It means higher the concentration higher the percentage of free radicals in the ethyl acetate leaves extract of *C.papaya*, *M.indica* and ethyl acetate seed extract of *M. charantia.*

The concentration of the sample extract was evaluated and compared to the standard value. The results show that different solvent extracts analyzed for the DPPH scavenging activity showed which can be comparable to the Standard Ascorbic acid.

Formula:- Percentage of Inhibition

$$= \frac{(B-T)}{(control)} \times 100 = \_\_\_\_\_\%$$

	Water	Methanol	Ethylacetate
Mango	17.6mg	11.668mg	1.1209mg
Papaya	5.27mg	3.068mg	1587.3mg
Bittergourd	3.09mg	6.11mg	157.96mg

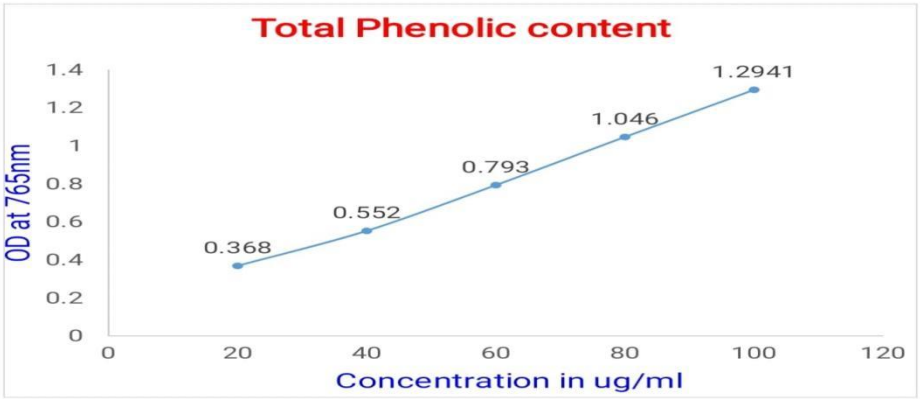
Table 4:- 50% of DPPH scavenging activity in different solvents extracts of leaves of *C.papaya, M.indica* and seed extract of *M.charantia.*

4. DISCUSSION

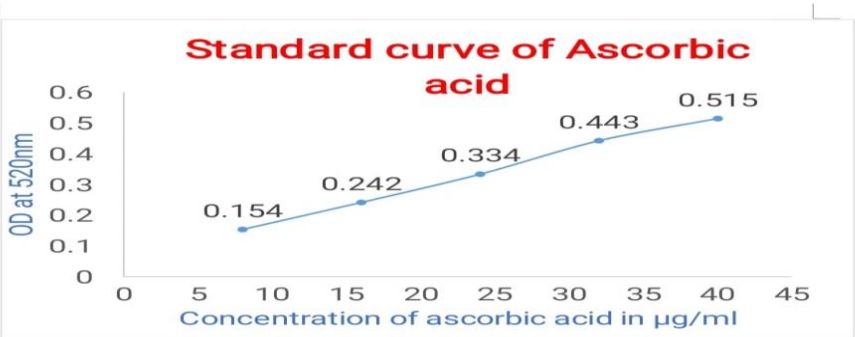
Medicinal plants constitute an important natural wealth of the country by playing a significant role in the primary health of mankind. They importantly serve as raw materials for manufacturing medicines as therapeutic drugs[15].

C. papaya, M. indica and M. charantia is used as a natural medicinal plant, recognized for its antimicrobial, anti-amoebic, antifungal, and hypolipidemic activity. Mainly, papaya is recognized as “King of medicine" due to the presence of a larger number of important phytonutrients loaded in it and also referred to as “Powerhouse of nutrients”.

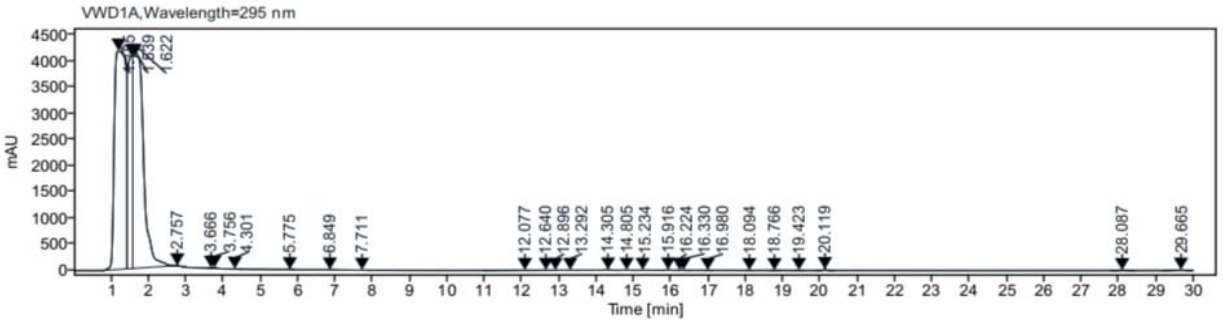
The plant-based medicines that have been used in treatment since ancient times reveal that in some cases, desirable effects were not achieved because the biological action of the herbal medicine or phytoconstituents may vary. Additionally, as well as the amount of phytoconstituents in a plant can vary according to the age of the plant, time of collection, and environmental conditions [18].



Graph 1: Standard Gallic acid calibration curve



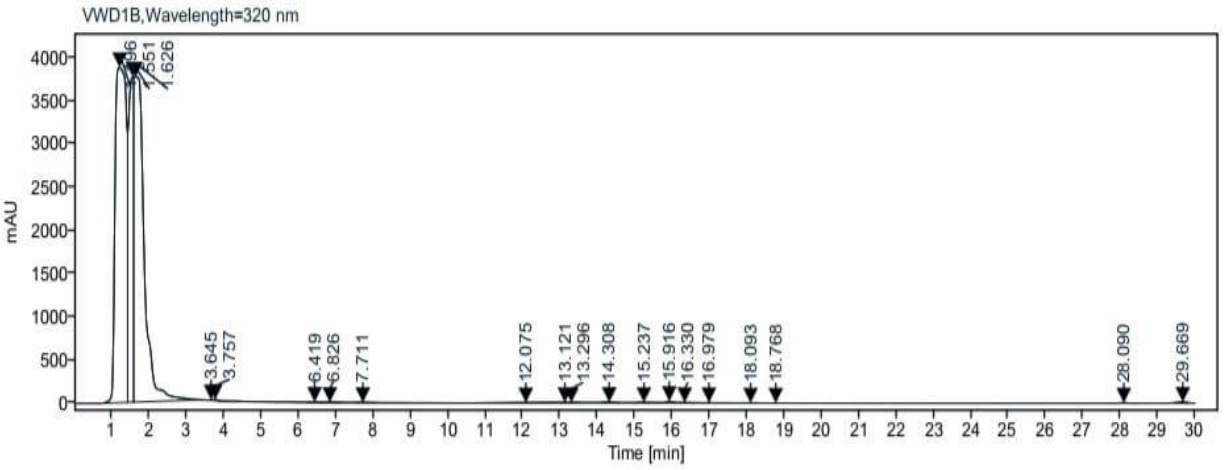
Graph 2: Standard Ascorbic acid Calibration curve



Signal: VWD1A,Wavelength=295 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
1.185	BV	0.6725	88090.3754	4180.3652	42.8613	
1.539	VV	0.1501	35870.8067	4056.9582	17.4533	
1.622	VB	1.1223	80095.8638	4057.5103	38.9715	
2.757	BB	0.3551	96.8572	8.6473	0.0471	
3.666	BV	0.1170	35.9932	8.9519	0.0175	
3.756	VB	0.2414	131.8201	16.8266	0.0641	
4.301	BB	0.3740	33.7116	3.0441	0.0164	
5.775	VB	0.1644	13.6161	2.1251	0.0066	
6.849	VB	0.3284	31.1046	2.4512	0.0151	
7.711	BB	0.4719	31.7548	2.8382	0.0155	
12.077	BV	1.3150	106.1532	2.8253	0.0517	
12.640	VV	0.4533	50.6662	1.9518	0.0247	
12.896	VV	0.2632	29.9019	2.0238	0.0145	
13.292	VB	0.3732	24.9940	2.2738	0.0122	
14.305	VV	0.4775	109.2605	7.9691	0.0532	
14.805	VV	0.5463	68.8102	4.0557	0.0335	
15.234	VV	0.2833	26.4408	2.5990	0.0129	
15.916	VV	0.3885	98.5193	9.8880	0.0479	
16.224	VV	0.1172	12.8400	2.2659	0.0062	
16.330	VB	0.2815	20.6027	3.0091	0.0100	
16.980	BV	0.2226	15.2705	2.6572	0.0074	
18.094	BV	0.1859	9.7725	1.7392	0.0048	
18.766	BV	0.2919	20.5461	2.7905	0.0100	
19.423	BV	0.2798	33.2840	5.5793	0.0162	
20.119	VB	0.5419	153.5676	23.2773	0.0747	
28.087	VB	0.6600	71.9521	5.1067	0.0350	
29.665	VBA	0.8502	239.5576	12.5974	0.1166	

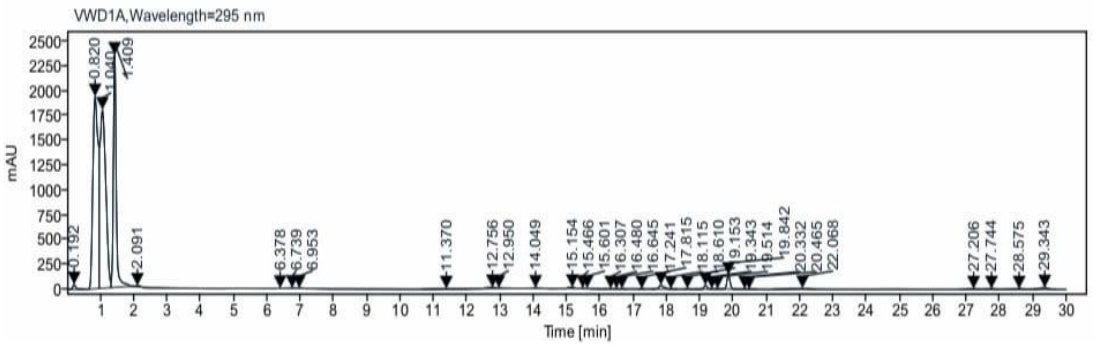
Sum 205524.0425



Signal: VWD1B,Wavelength=320 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
1.196	BV	0.6953	79887.9270	3885.8016	42.4162	
1.551	VV	0.1608	34512.9230	3768.6260	18.3245	
1.626	VB	1.7538	72660.3825	3769.5872	38.5788	
3.645	BV	0.1275	30.3570	6.2640	0.0161	
3.757	VB	0.2339	96.9866	12.6929	0.0515	
6.419	BV	0.4123	46.9477	3.9996	0.0249	
6.826	VV	0.5252	44.4016	2.9787	0.0236	
7.711	VB	0.5723	35.3237	2.5430	0.0188	
12.075	BV	1.5953	118.7586	2.8084	0.0631	
13.121	VV	0.8731	117.1724	2.4462	0.0622	
13.296	VV	0.3341	34.0486	2.5922	0.0181	
14.308	VB	0.5154	83.1794	5.6410	0.0442	
15.237	BV	0.6482	57.0824	3.5513	0.0303	
15.916	VV	0.4988	184.1417	18.7583	0.0978	
16.330	VB	0.4250	47.5915	5.3388	0.0253	
16.979	BV	0.1974	23.4486	4.7598	0.0124	
18.093	VV	0.1760	14.4990	2.2594	0.0077	
18.768	BV	0.2722	14.1307	2.0548	0.0075	
28.090	BB	0.7649	66.2564	4.6453	0.0352	
29.669	VBA	0.8556	267.2604	14.1018	0.1419	
		Sum	**188342.8188**			

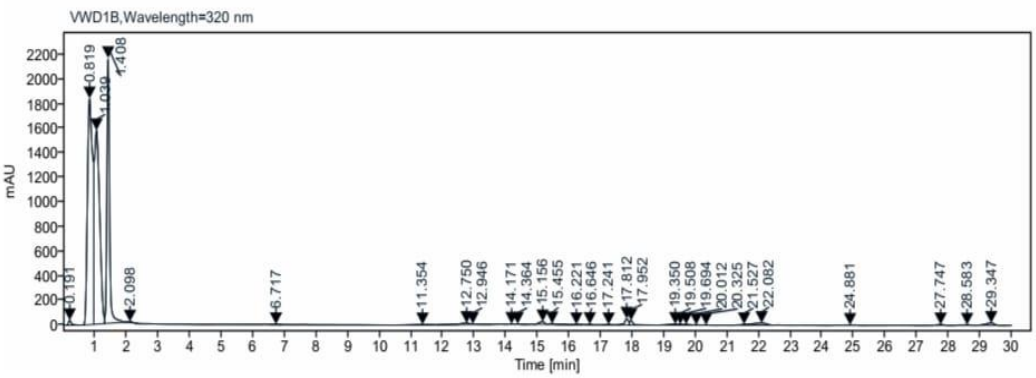
Figure 1: Methanolic mango leaf extract



Signal: VWD1A,Wavelength=295 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
0.192	BB	0.5138	313.6587	47.6303	0.5101	
0.820	BV	0.3365	21084.7697	1935.6981	34.2880	
1.040	VV	0.3529	22135.9990	1796.5326	35.9975	
1.409	VB	0.6746	13964.3445	2342.1430	22.7088	
2.091	BB	0.3426	73.7997	7.0765	0.1200	
6.378	BV	0.3360	21.3463	1.8626	0.0347	
6.739	VV	0.3435	59.5244	4.0378	0.0968	
6.953	VB	0.3873	55.8464	4.1548	0.0908	
11.370	BV	1.0675	78.0322	2.3548	0.1269	
12.756	VV	1.1833	259.3102	11.1082	0.4217	
12.950	VB	0.6718	106.3585	6.0414	0.1730	
14.049	BB	1.0307	109.9711	4.5880	0.1788	
15.154	BV	0.4103	159.6722	13.2933	0.2597	
15.466	VV	0.2727	76.1171	6.3664	0.1238	
15.601	VB	0.4552	47.6250	4.3700	0.0774	
16.307	BV	0.3600	33.3101	2.3206	0.0542	
16.480	VV	0.1542	15.5247	2.0209	0.0252	
16.645	VB	0.2068	20.7886	3.4038	0.0338	
17.241	VV	0.1933	11.7462	1.8285	0.0191	
17.815	BV	0.5314	424.8837	41.4004	0.6909	
18.115	VB	0.2436	23.7757	3.0072	0.0387	
18.610	VB	0.2776	41.9032	6.7378	0.0681	
19.153	BV	0.4901	273.6107	39.6020	0.4449	
19.343	VV	0.1732	33.7234	4.0078	0.0548	
19.514	VB	0.1424	11.7596	2.0214	0.0191	
19.842	BB	0.5076	954.9591	141.6696	1.5530	
20.332	BV	0.2988	26.5511	2.9604	0.0432	

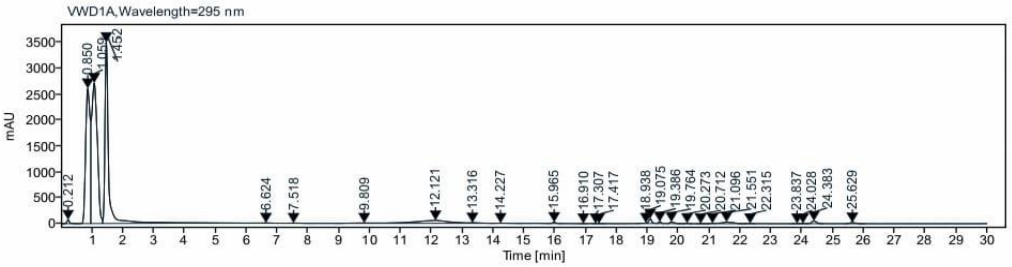
Sum:-61493.1257



Signal: VWD1B,Wavelength=320 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
0.191	BB	0.5113	254.8067	39.4793	0.4599	
0.819	BV	0.3417	20667.1994	1837.7402	37.3058	
1.039	VV	0.3421	18577.7757	1575.5512	33.5342	
1.408	VB	0.6743	12491.2345	2154.4871	22.5476	
2.098	BB	0.3686	67.9902	6.5147	0.1227	
6.717	VB	0.3724	20.6664	1.8759	0.0373	
11.354	BB	1.1090	73.1618	2.3270	0.1321	
12.750	BV	1.1084	258.4860	11.5520	0.4666	
12.946	VB	0.6206	78.8181	4.9787	0.1423	
14.171	VV	0.3674	57.2553	3.2240	0.1033	
14.364	VB	0.3789	38.2868	3.3600	0.0691	
15.156	BV	0.4137	272.9437	23.3888	0.4927	
15.455	VB	0.7214	125.5168	8.0120	0.2266	
16.221	BV	0.3544	32.2867	2.3260	0.0583	
16.646	BB	0.1981	25.6387	4.9672	0.0463	
17.241	BV	0.3332	23.2704	3.0999	0.0420	
17.812	VV	0.3791	424.7657	50.0820	0.7667	
17.952	VB	0.4167	251.6779	36.4964	0.4543	
19.350	BV	0.6875	172.5793	8.6686	0.3115	
19.508	VV	0.1411	46.7611	6.5110	0.0844	
19.694	VB	0.3039	56.0314	5.7819	0.1011	
20.012	BV	0.2423	13.1236	1.7763	0.0237	
20.325	VB	0.4377	21.6483	1.9819	0.0391	
21.527	BV	0.4786	87.7549	7.9962	0.1584	
22.082	VV	0.8833	583.2424	17.8843	1.0528	
24.881	BB	1.3333	97.9910	2.4000	0.1769	
27.747	BV	1.1310	105.1464	6.5235	0.1898	
28.583	VV	0.7744	94.5997	4.3335	0.1708	
29.347	VB	0.9945	378.8048	19.1844	0.6838	
		Sum	**55399.4638**			

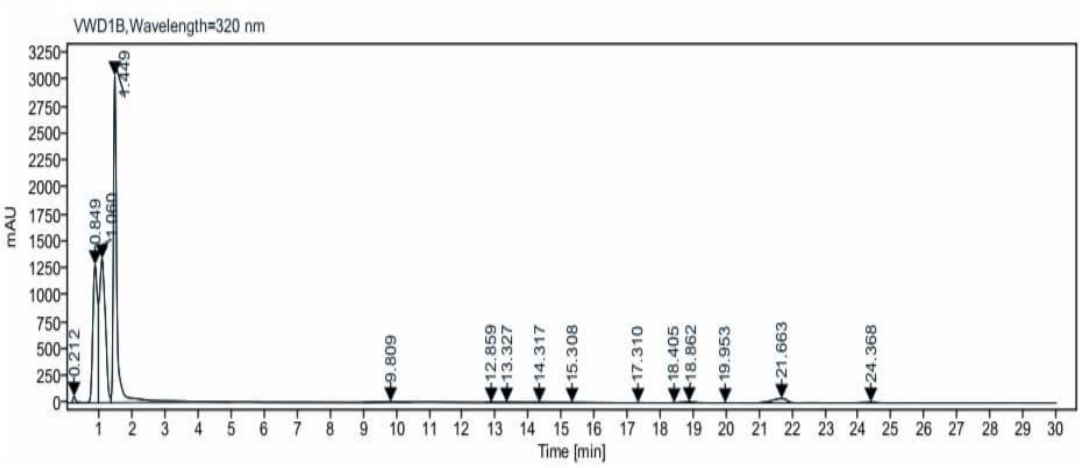
Figure 2: Methanolic papaya extract



Signal: VWD1A,Wavelength=295 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
0.212	BB	0.5000	431.6582	73.4808	0.4366	
0.850	BV	0.4275	25870.5975	2613.8660	26.1642	
1.059	VV	0.3828	34831.6726	2719.2410	35.2269	
1.452	VB	3.7761	29036.6290	3497.7271	29.3661	
6.624	VB	0.5326	82.9304	5.0068	0.0839	
7.518	BB	1.2064	96.1764	3.8473	0.0973	
9.809	BV	1.4782	342.1070	6.5865	0.3460	
12.121	VV	2.8010	4401.8759	57.6315	4.4518	
13.316	VB	0.8497	295.7348	11.1733	0.2991	
14.227	BB	0.9577	86.5512	3.8996	0.0875	
15.965	VB	0.4712	184.8491	24.2279	0.1869	
16.910	BB	0.2244	10.3244	1.8002	0.0104	
17.307	VV	0.1686	13.5834	1.8041	0.0137	
17.417	VB	0.2649	14.3484	1.7973	0.0145	
18.938	BV	0.3547	84.4254	9.9508	0.0854	
19.075	VV	0.3206	646.7801	93.9850	0.6541	
19.386	VV	0.2317	219.1351	35.0749	0.2216	
19.764	BB	0.5156	305.7794	30.9123	0.3092	
20.273	BV	0.2878	52.5113	7.4245	0.0531	
20.712	BB	0.2584	17.1036	2.5516	0.0173	
21.096	BV	0.3254	75.4523	7.8565	0.0763	
21.551	VB	0.8990	779.4396	31.8787	0.7883	
22.315	BB	0.4150	45.4668	3.8153	0.0460	
23.837	BV	0.2765	26.3366	3.0129	0.0266	
24.028	VV	0.2243	44.5910	3.9426	0.0451	
24.383	VV	0.5520	599.5235	51.9776	0.6063	
25.629	VV	0.6405	282.3872	20.6248	0.2856	

Sum 98877.9702



Signal: VWD1B,Wavelength=320 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
0.212	BB	0.4796	346.3388	59.1641	0.6252	
0.849	BV	0.4262	12539.3138	1280.7679	22.6373	
1.060	VV	0.3784	16874.2368	1336.2007	30.4632	
1.449	VB	3.6350	23235.9436	3035.6533	41.9480	
9.809	BB	1.4552	174.4624	3.9751	0.3150	
12.859	BV	0.5570	66.2349	3.0632	0.1196	
13.327	VV	0.7761	87.5756	2.2744	0.1581	
14.317	VV	1.1010	136.2809	2.4746	0.2460	
15.308	VV	0.8016	90.6179	2.4311	0.1636	
17.310	BV	0.3504	15.3544	2.1625	0.0277	
18.405	BV	0.4450	17.7589	1.9267	0.0321	
18.862	VB	0.8598	263.0617	10.5906	0.4749	
19.953	BV	0.4306	17.5131	1.8895	0.0316	
21.663	BB	1.6024	1326.5984	40.9188	2.3949	
24.368	BB	1.2933	200.9867	6.9506	0.3628	
		Sum	**55392.2779**			

Figure 3: Methanolic bitter gourd seed extract

An attempt to study in vitro antioxidant of leaves extract of *C.papaya, M.indica* and seed extract of *M.charantia* with methanolic fraction revealed the TPC in the methanolic fraction was high compared to other solvent extracts, the antioxidant activity showed the radical scavenging activity of ethyl acetate and methanolic extracts at different concentration with 50% of scavenging activity, a comparison of ability of the various extract have proved their limited DPPH scavenging activity .

5. CONCLUSION

Bioactive compounds can be studied by extraction and isolation, also with defining their structure and by analyzing it in laboratory models as in vitro and in vivo studies and importance was given for identification and characterization of the specific phytochemical which is primarily responsible for biological activity.

The current study which was aimed at investigating the presence of biologically active phytochemicals and antioxidant properties of *C.papaya, M.indica leaves* extract and seed extract of *M.charantia* reveals that samples with various solvents such as water, ethyl acetate and methanol have shown presence of phytochemicals constituents such as alkaloid, carbohydrates, and amino acids.

Among the used solvent extracts, the *C.papaya*, *M.indica* leaves and *M.charantia* seeds with methanolic fraction showed the presence of more phytochemicals and have effective phenolic, flavonoid content, and exhibited strongest antioxidant properties which can effectively scavenge reactive oxygen species.

Hence, *C.papaya, M.indica and M.charantia* can be considered as an important and potential natural source for various pharmaceuticals and medicinal applications. Interestingly, the broad pectrum of phytochemicals and antioxidants presents in them can be regraded as the reservoir of naturally occuring diverse bioactive molecules and papaya ,mango and bitter gourd as herbal medicine can be furnished for the quantitative extraction for exploring the new promising biomolecules for pharmaceutical application.

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