Evaluation of Phytochemical and Antioxidant Activity of Seeds Extract of *Momordica Charantia* and Leaf Extracts of *Carica Papaya* and *Mangifera Indica*.

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ABSTRACT

The purpose of the current study was to investigate 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 *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. 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.

Keywords: Carica papaya, *Mangifera indica Momordica Charantia*, Phytochemicals, Antioxidants.

INTRODUCTION

From the ancient times, people have used medicinal plants as a potential source of life for the convalescence from major and minor ailments (Hussain et al., 2018). Momordica charantia (MC) belongs to the family Cucurbitaceae. Carica Papaya is member of the Caricaceae family. Mangifera indica is belonging to the Anacardiaceae family (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. The amounts of the different polyphenolic compounds in the mango vary from part to part (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.

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. 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 research article 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.

3.MATERIALS AND METHODS

3.1 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.

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 (60°C) 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 (70°C) 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 (100°C) 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 (60°C) 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(70°C) 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(100°C) 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 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(60°C) 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(70°C) with 250ml of ethyl acetate in a Soxhlet apparatus.

The 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 (100^{0} C) 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.

3.2 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].

3.3 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.

3.4 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 ($4\mu g/ml$) using methanol. DPPH solution ($500\mu l$) 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 520 nm 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:

Absorbance of the sample at 517 nm.

Scavenging effect (%)= 1- ----- X100

Absorbance of control at 517 nm

3.5 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 seedmethanol extracts were injected [26].

4. RESULT

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
Carica 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

(B-T)

=----- X 100 =____%

(Control)

	Water	Methanol	Ethyl acetate
Mango	17.6mg	11.668mg	1.1209mg
Papaya	5.27mg	3.068mg	1587.3mg
Bitter gourd	3.09mg	6.11mg	157.96mg

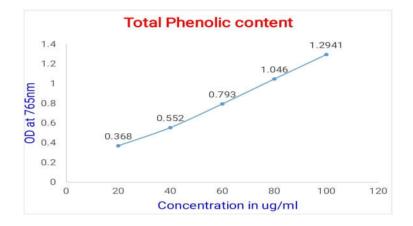
Table 4:- 50% of DPPH scavenging activity in different solvents extracts of leaves of

 C.papaya, M.indica and seed extract of *M.charantia*.

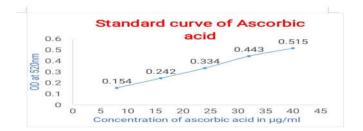
DISCUSSION

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. The plant-based medicines that have been used in treatment

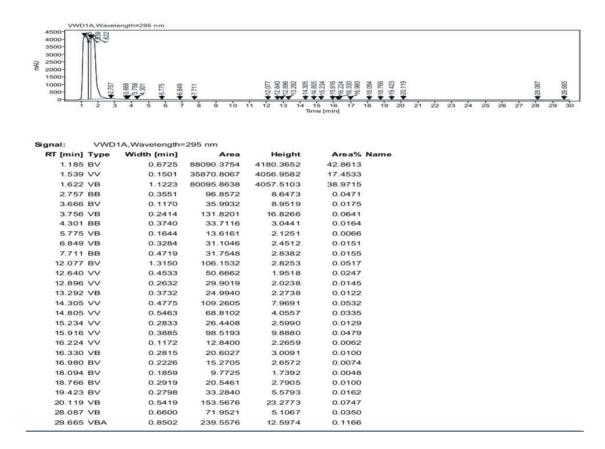
since ancient times reveal that in some cases, desirable effects were not achieved because the biological action of the herbal medicine or phytoconstituents may vary. Additionally, as well as the amount of phytoconstituents in a plant can vary according to the age of the plant, time of collection, and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



Graph 1: Standard Gallic acid calibration curve



Graph2: Standard ascorbic acid calibration curve.



Sum 205524.0425

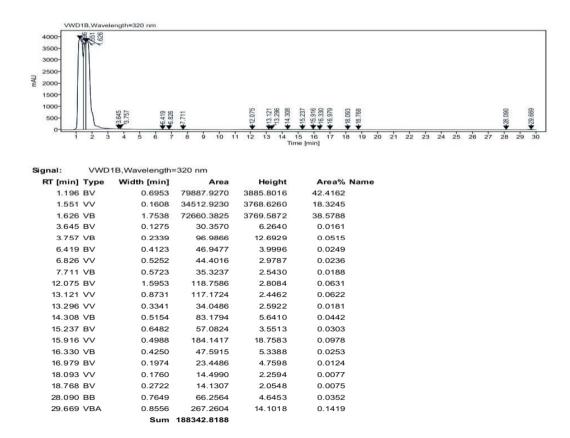
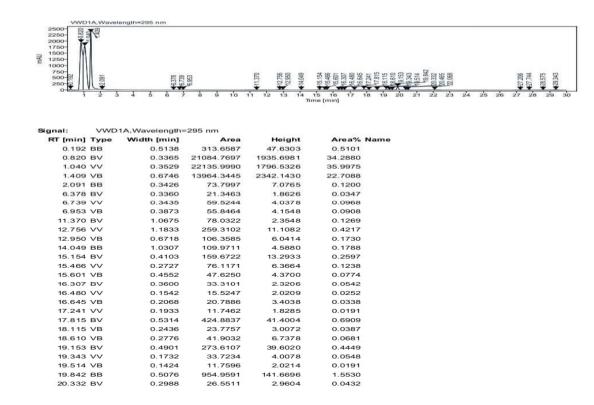


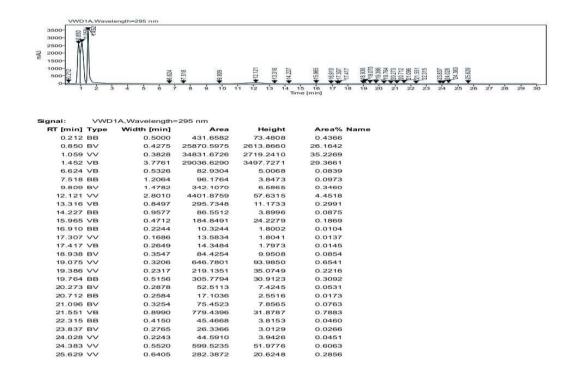
Fig 2: Methanolic mango leaf extract

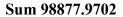


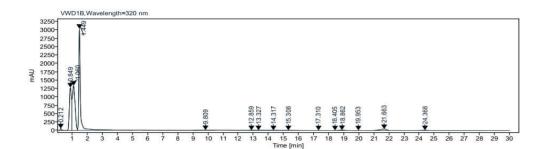
Sum:-61493.1257

	Sum	55399.4638		
29.347 VB	0.9945	378.8048	19.1844	0.6838
28.583 VV	0.7744	94.5997	4.3335	0.1708
27.747 BV	1.1310	105.1464	6.5235	0.1898
24.881 BB	1.3333	97.9910	2.4000	0.1769
22.082 VV	0.8833	583.2424	17.8843	1.0528
21.527 BV	0.4786	87.7549	7.9962	0.1584
20.325 VB	0.4377	21.6483	1.9819	0.0391
20.012 BV	0.2423	13.1236	1.7763	0.0237
19.694 VB	0.3039	56.0314	5.7819	0.1011
19.508 VV	0.1411	46.7611	6.5110	0.0844

Fig. No. 3: Methanolic papaya extract







Signal:	VWD	1B,Wavelength=	=320 nm		
RT [min]	Туре	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		

Fig no. 4: 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.

CONCLUSION

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 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. **REFERENCES**

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