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EVALUATION OF ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOID CONTENTS OF *MOMORDICA CHARANTIA* LINN. FRUIT

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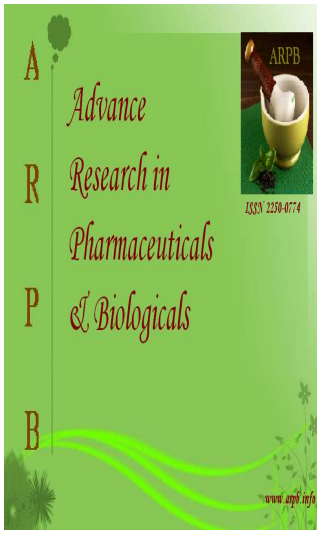

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ABSTRACT:

In present study, we carried out antioxidant activity, phenol and flavonoid contents of *momordica charantia* Fruit extracts. Total phenolic content in the alcoholic extract of *M. charantia* extract was found to be 5.61 ± 0.54 % w/w calculated in terms of Gallic acid. The total flavonoids content of aqueous extract of *M. charantia* Linn was found to be 1.77 ± 0.72 % w/w was expressed as equivalent to Rutin. The *Momordica Charantia* fruit were successively extracted using soxhlet apparatus with solvent petroleum ether (60-80 ^{0}C), chloroform, alcohol & water. These extracts were subjected to the DPPH radical scavenging & hydrogen peroxide radical scavenging activity was determined spectrophotometrically. The IC_{50} values of alcoholic extract in DPPH & Hydrogen Peroxide radical scavenging activity was found to be 120.07 ± 0.77µg/ml & 175.78 ± 0.63 µg/ml respectively. The highest radical scavenging effect was observed in alcoholic extract of *M. charantia* Linn. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by *M. charantia* Linn. extract.

KEYWORDS: Anti-oxidant, Flavonoids, Phenols, *Momordica Charantia*.

INTRODUCTION

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury[1]. Reactive oxygen species (ROS) include free radicals such as $^{\cdot}O_2^-$ (superoxide anion), $^{\cdot}OH$ (hydroxyl radical), H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen) can cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes[2, 3]. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in a high number of diseases[4]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, and AIDS[5]. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Oxidation process is one of the most important routes for producing free radicals in food, drugs, and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydro peroxides to non radical forms and functions as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different remedies, consuming antioxidants as free radical scavengers may be necessary[6,7,8]. Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinones and gallic acid esters have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity[9]. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds[10]. Flavonoids are a group of poly phenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action[11]. Some evidence suggests the biological actions of these compounds are related to their antioxidant activity. The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants[12]. *Momordica Charantia* Linn. Cucurbitaceae is a well known to possess antihyperglycemia, anticholesterol, immunosuppressive, antiulcerogenic, anti spermatogenic and androgenic activities, anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, anti-microbial and anti-tumor activities[13,14]. This study was designed to specifically investigate the

antioxidant efficacy of *Momordica charantia* fruit successive extract by *in-vitro* method with Total Phenolic content & Total Flavonoids content. In longer term, plant species identified as having high levels of antioxidant activity in vitro may be of value in the design of further studies to novel treatment strategies for disorders associated with free radicals-induced tissue damage.

MATERIAL AND METHODS

Plant materials:

The fruits of *Momordica charantia* Linn. were collected from local market of Anand Gujarat, India and authentified by Dr. G.C. Jadeja Professor & Head botany, Department of agricultural Botany, B. A. College of agriculture, Anand agricultural university. A herbarium specimen (skcop-2010-2) is deposited in the college herbal museum for future reference.

Preparation of extracts:

The shade dried fruits of *Momordica charantia* were reduced to fine powder (# 40 size mesh) and around 200 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether (40°-60°C), chloroform and alcohol. Finally the drug was macerated with chloroform water. Each time before extracting with the next solvent the powdered material was air dried. After the effective extraction, the solvents were distilled off and the extract was then concentrated on water bath, dried up to constant weight and the extract obtained with each solvent was weighed. Percentage of extract was calculated in terms of air dried weight of plant material. The colour and consistency of the extracts was noted. The obtained extracts were subjected to phytochemical investigation and pharmacological investigation.

Estimation of Total Phenolic content:

Total phenolic contents in the *Momordica charantia* Linn. extract was determined using the Folin-Ciocalteu reagent. From the stock solution (1 mg/ml) of extract, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin-Ciocalteu Reagent. After 5 minutes 4 ml of 20% W/V sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The absorbance was recorded at 765 nm after 30 minutes. % of Total Phenolics was calculated from calibration curve of Gallic acid (5-200 µg) plotted by using the same procedure and Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference compound[15].

Estimation of Total Flavonoids content:

The probes were prepared by mixing: 5 ml of extract, 1 ml of distilled water and 2.5 ml of $AlCl_3$ solution (26.6 mg $AlCl_3.6H_2O$ and 80 mg CH_3COONa dissolved in 20 ml distilled water). A blank probe was prepared by replacing $AlCl_3$ solution with distilled water. The absorbance of probes and blank probe

were measured immediately at 430 nm. Total flavonoids content was calculated from calibration curve of Rutin (5-160 µg) plotted by using the same procedure and Total Flavonoid values expressed in terms of Rutin equivalent (mg g^{-1} of dry mass)[16].

***In-vitro* Anti oxidant activity:**

DPPH radical scavenging activity:

Plant extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80, 100,120,140, 180, & 200µg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations[17, 18].

Hydrogen peroxide radical scavenging activity:

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of plant extract and standard Ascorbic acid solution viz. 10, 20, 40, 60, 80, 100, 120, 140, 180, 200 µg/ml in methanol (1 ml) where added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard. The antioxidant activity of the extract was expressed as IC_{50}. All the tests were performed in triplicate and the graph was plotted with the average of three observations[17, 19].

RESULTS

Extraction of *M. charantia* Linn. fruit:

The powder of *M. charantia* Linn. fruit was successively extracted with Petroleum Ether, Chloroform and Alcohol by soxhlet apparatus & Maceration with Water. The results are described in Table no. 1. The Petroleum Ether *M. charantia* fruit extract was oily viscous, brown and the yield was 5.58% w/w, Chloroform extract was solid, dark brownish and the yield was 2.11% w/w, Alcohol extract was semi-solid, dark brown and the yield was 13.84% w/w and Water extract was solid, brown and the yield was 7.73% w/w.

Preliminary phytochemical screening:

Successive extract of *M. charantia* Linn. fruit was screened for various chemical

investigations of isolated successive extracts and the results are mentioned in Table no. 2. Qualitative chemical examinations of *M. charantia* fruit extract revealed the presence of Carbohydrates, Alkaloids, Steroids, Glycosides, Flavonoids, Amino acids, Saponins, Tannins and Phenolics.

Table No. 1 Extraction of *M. charantia* Linn. fruit

S. No.	Extract (200gm)	Colour in day light and Consistency	Weight (gm)	% yield
1	Petroleum Ether	Oily-Viscous Brown	11.16	5.58 %
2	Chloroform	Solid Dark Brownish	4.22	2.11 %
3	Alcohol	Semi-solid Dark Brown	27.69	13.84 %
4	Water	Solid Brown	15.46	7.73 %

Table No. 2. Qualitative chemical investigation of *M. charantia* Linn. Extract

Nature	Pet. Ether extract	Chloroform extract	Alcohol extract	Water extract
Alkaloids	-	+	+	-
Carbohydrates	-	+	+	+
Flavonoids	-	-	+	+
Amino acids	-	-	+	-
Steroids	+	+	+	-
Saponins	+	+	+	+
Glycosides	-	+	+	-
Tannins & Phenolics	-	-	+	+

Quantitative Determination of Successive Extract of *M. charantia Linn.*:

Total phenolics:

Total phenolic content in the alcoholic extract of *M. charantia* Linn. extract was found to be 5.61 ± 0.54 % w/w calculated in terms of Gallic acid. The phenolic content well complies with preliminary phytochemical investigation showing the presence of tannins in alcoholic extract of *M. charantia* Linn.

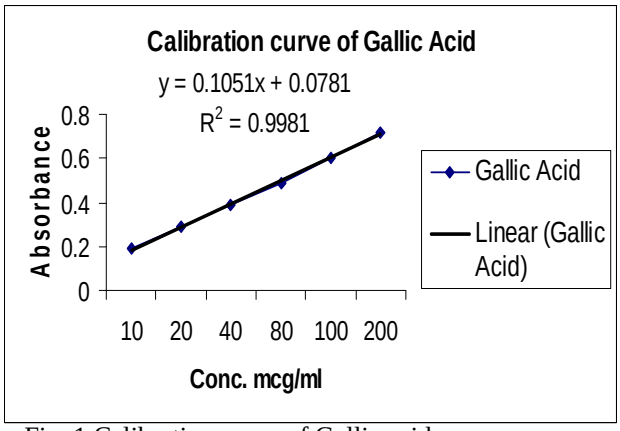
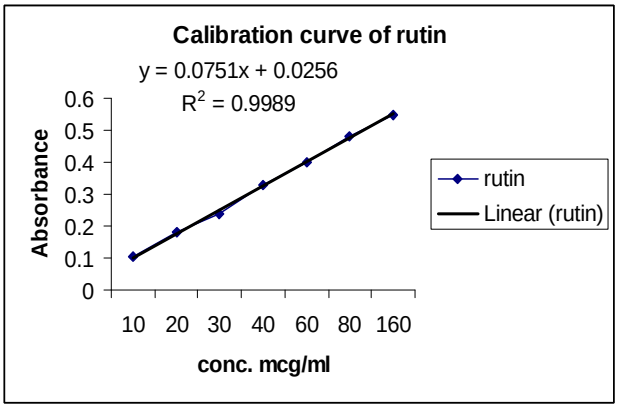


Fig: 1 Calibration curve of Gallic acid

Total flavonoids:

The total flavonoids content of aqueous extract of *M. charantia* Linn. was found to be 1.77 ± 0.72 % w/w expressed as equivalent to Rutin. It is well confirm with preliminary phytochemical screening.



***In-vitro* Antioxidant Activity of Successive Extract of *M. charantia* Linn.:**

Antioxidant activity of successive extract of *M. charantia* Linn. was carried out by *in vitro* antioxidant models. In the models tested the antioxidant activity of *M. charantia*

Linn. was studied in relation to Ascorbic acid, a known antioxidant.

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity:

Result illustrates a significant decrease in the concentration of DPPH radicals due to the scavenging ability of successive extract of *M. charantia* Linn. and Ascorbic acid. Maximum inhibition of alcohol, aqueous extract of *M. charantia* Linn. and Ascorbic acid was exhibited 69.729 ± 0.76 %, 55.783 ± 0.96 % and 94.021 ± 0.40 % inhibition respectively in 200µg/ml. The IC_{50} values in DPPH radical scavenging model were 45.54 ± 0.80 and 120.07± 0.77 , 182.74 ± 0.90 µg/ml for Ascorbic acid, Successive alcohol and aqueous extract respectively. This activity may be attributed to the phytoconstituents present in the respective extract. (Fig 3, Table No. 3)

Table No 3: Antioxidant activity of Alcohol and aqueous extract of *M. charantia* Linn and Ascorbic acid (DPPH)

S. No.	Conc. of extract(µg/ml)	% Inhibition *		
		Alcohol Extract	Water Extract	Ascorbic acid
1	10	25.945 ± 0.66	11.783 ± 0.51	35.783 ± 0.98
2	20	27.135 ± 0.37	16.324 ± 0.71	37.837 ± 0.46
3	40	30.594 ± 1.37	17.837 ± 0.45	48.913 ± 1.00
4	60	31.675 ± 1.0	18.702 ± 0.95	54.565 ± 0.55
5	80	34.918 ± 0.66	20.00 ± 0.78	60.869 ± 0.80
6	100	37.945 ± 0.83	20.864 ± 0.43	70.652 ± 1.19
7	120	54.486 ± 1.35	34.594 ± 0.25	81.729 ± 0.89
8	140	61.189 ± 1.15	45.189 ± 0.60	85.945 ± 0.64
9	180	64 ± 0.35	51.243 ± 1.37	90.326 ± 0.74
10	200	69.729 ± 0.76	55.783 ± 0.96	94.021 ± 0.40
11	IC_{50} value	120.07 ± 0.77	182.74 ± 0.90	45.54 ± 0.80

*Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

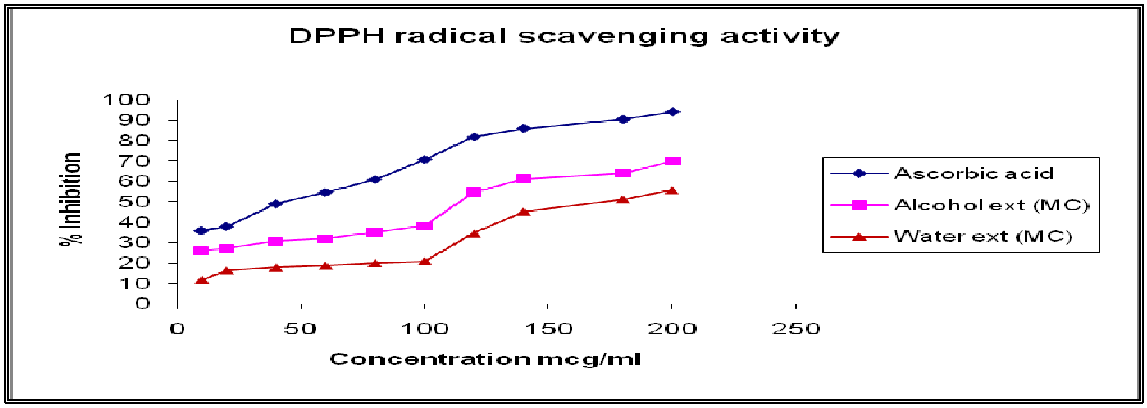


Fig 3: DPPH radical scavenging model of *M. charantia* Linn.extracts

Hydrogen peroxide radical scavenging activity:

Result shows that successive extract of *M. charantia* Linn. and Ascorbic acid demonstrated hydrogen peroxide scavenging activity was significant in a concentration dependant manner. Maximum inhibition of alcohol, water extract of *M. charantia* Linn. and Ascorbic acid was exhibited 63.447 ± 0.93 %, 0.18 % and 75.722 ± 0.66 % inhibition respectively in 200µg/ml. IC_{50} values in Hydrogen Peroxide scavenging model were found to be 128.81 ± 0.44 and 175.78 ± 0.63 , 130.26 ± 0.51 µg/ml for Ascorbic acid and successive extract of alcoholic, water respectively. (Fig 4, Table No. 4)

Table No 4: Antioxidant activity of Alcohol and Water extract of M. *charantia* Linn and Ascorbic acid (H_2O_2)

S. No.	Conc. of Extract µg/ml	% Inhibition *		
		Alcohol Extract	Water Extract	Ascorbic acid
1	10	1.624 ± 0.16	21.57 ± 0.57	0.722 ± 0.55
2	20	3.158 ± 0.40	23.375 ± 0.69	17.87 ± 0.68
3	40	12.184 ± 0.57	24.368 ± 0.50	20.126 ± 0.55
4	60	12.545 ± 0.55	25.09 ± 0.60	24.007 ± 1.01
5	80	16.064 ± 1.01	26.624 ± 0.18	26.263 ± 0.29
6	100	19.675 ± 0.46	28.429 ± 0.80	30.505 ± 0.92
7	120	27.978 ± 1.33	45.577 ± 0.30	46.028 ± 0.42
8	140	36.823 ± 1.04	57.581 ± 0.44	58.844 ± 0.51
9	180	53.79 ± 0.89	69.584 ± 0.63	72.924 ± 0.93
10	200	63.447 ± 0.93	73.555 ± 0.18	75.722 ± 0.66
11	IC_{50} value	175.78 ± 0.63	130.26 ± 0.51	128.81 ± 0.44

*Each value in the table was obtained by calculating the average of three experiments ± standard deviation

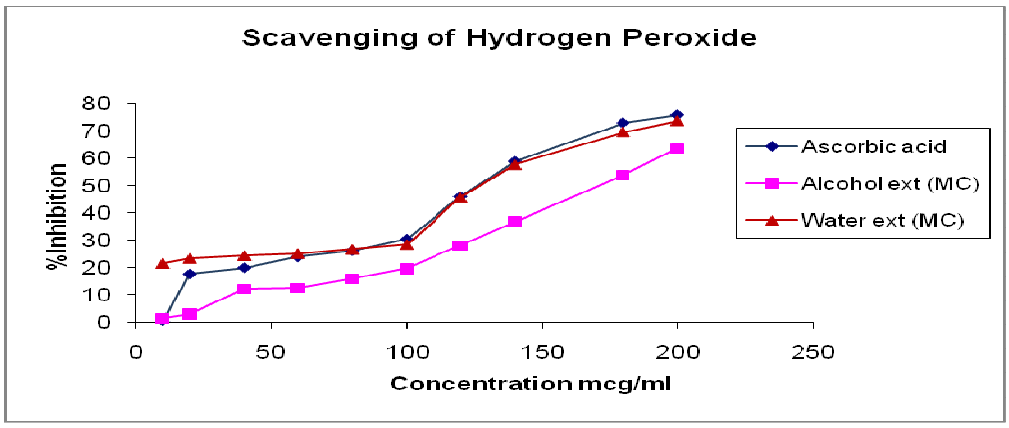


Fig 4: Hydrogen peroxide model of *M. charantia* Linn. extracts

DISCUSSION

Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases [20]. In all the models tested *M. charantia* Linn. extract showed its ability to scavenge the free radicals in a concentration dependent manner. The result suggests that the successive alcoholic and water extract showed Antioxidant activity in of *M. charantia* Linn. and absent in successive petroleum ether and chloroform extract. The result of the present study showed that the extract of successive alcohol extract of *M. charantia* Linn, which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. The high scavenging activity of *M. charantia* Linn. may be due to hydroxyl groups existing in the phenolic compounds that can provide the necessary component as a radical scavenger.

CONCLUSION

The data presented here indicate the marked antioxidant activity of *M. charantia* Linn. due to presence of flavonoids like flavones, flavanes, flavonols and phenolic compounds which may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction. The qualitative chemical tests also revealed the presence of flavonoids and phenolic compounds. Further total phenolic and flavonoids content of extract confirmed that *M. charantia* Linn. contains good amount of phenolics and flavonoids. There is good scope in examining the *M. charantia* Linn. for its antioxidant and free radical scavenging activity in *in vivo* models and for hepatoprotective activity and thus establish the evidence for using this plant in treatment of jaundice in folk medicine. Free radicals and reactive oxygen species are involved in a variety of pathological events such as aging, inflammation, cancer, atherosclerosis, diabetes. The *M. charantia* Linn. would be useful for the treatment of various diseases mediated by free radicals. Overall, the plant would be useful as an antioxidant and free radical scavenging agent and thus help in treatment of many diseases mediated by Reactive oxygen species.

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