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# PHYTOCHEMICAL SCREENING, TOTAL FLAVONOID AND PHENOLIC CONTENT ASSAYS AND ANTIOXIDANT ACTIVITY OF *MOMORDICA CHARANTIA* L. LEAVES

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**Abstract**

The objective of this research was to conduct the preliminary phytochemical screening, total flavonoid and phenolic contents assays and antioxidant activity of various solvent extracts of leaves of *Momordica charantia*. Phytochemical screening was carried out according to the method of Trease and Evans, total flavonoid content was measured by the aluminium chloride colorimetric assay and total phenolic content was estimated spectrophotometrically by Folin-Ciocalteau method. Preliminary phytochemical screening reveals the presence of mucilage, proteins, tannins, saponin, flavonoids, anthraquinones and terpenoids in the extracts (petroleum ether extract and hydro-alcoholic extract). Leaves hydro-alcoholic extract has the richest content of both phenolics and flavonoids i.e. (14.28 mg GAE/g and 12.16 mg QE/g) respectively, and petroleum ether extract was the least i.e. (1.73 mg GAE/g and 1.16 mg QE/g). It can be hypothesised that the high contents of phenolic compounds of leaves of *Momordica charantia* indicated that these compounds contribute to the antioxidant activity and can be regarded as promising plant species for natural sources of radical scavenging activity with potential value for treatment of many life threatening diseases.

**Keywords:** *Momordica charantia*, antioxidant, total phenolic, flavonoids, phytochemical analysis.

## INTRODUCTION:

*Momordica charantia* L., also known as Jinlizhi, Laiputao, Laigua, Lianggua, etc., is a herbaceous climbing plant in the genus Momordica of family Cucurbitaceae, which is widely distributed in tropical, subtropical and temperate regions. *Momordica charantia* L. is bitter in taste and cold in nature, which is used in the treatment of fever with thirst, heat stroke, dysentery, dye redness & pain, carbuncles, erysipelas, malignant sores, etc.; [1] it is a common folk food and medicine. In recent years, research on *Momordica charantia* L. has been concentrated on fruits and seeds, and a variety of chemical constituents have been isolated and purified from fruits and seeds of *Momordica charantia* L. [2-4] Modern medical studies have found that *Momordica charantia L.* possesses hypoglycemic, anti-tumor and immunity enhancing actions. [5-7]

Free radicals cause oxidative damage to macromolecules in the body, such as lipids, proteins and nucleic acids. Antioxidants prevent such free radicals from oxidative damage to DNA, proteins, and cells by donating electrons to stabilize and neutralize the harmful effects of the free radicals. Plant-derived

antioxidants have received greater attention since they act as radical scavengers. Phenolics are regarded as the molecules with the highest potential to neutralize free radicals. These compounds act mainly as antioxidants due to their ability to scavenge free radicals and chelate metals in vitro and in vivo. [8]

Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer and age related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals. [9]

Therefore, the objective of this paper was to carry out the phytochemical screening, total contents of both phenolics and flavonoids and antioxidant activity of *Momordica charantia* L. leaves.

## MATERIALS AND METHODS

### Chemicals and reagents

Folin-Ciocalteu reagent, anhydrous sodium carbonate ($Na_2CO_3$), aluminium chloride, potassium acetate was obtained from Lobal Chemie (India). Methanol was bought from Sigma-Aldrich (Germany). Sodium hydroxide (NaOH), sodium nitrite (NaNO2), sodium phosphate (NaH2PO4) and ammonium molybdate were bought from from Qualikems (India). 2,2-Diphenyl-2-picrylhydrazyl (DPPH), potassium hexacyanoferrate ($K_3Fe(CN)_6$), trichloroacetic acid, gallic acid, ascorbic acid, quercetin, and $FeCl_3$ were purchased from Sigma chemicals. All chemicals used were of analytical grade.

### Plant material

The leaf of *Momordica charantia* were collected in month of January-February from local area of Sagar district after authentication by Dr. Pradeep Tiwari, Department of Botany, Dr. H. S. Gour University (Herbarium no. Bot./ 1723).

### *Drying*

The collected leaves were dried at room temperature under well ventilated shade by spreading them uniformly.

### *Extraction*

The dried leaves were coarsely powdered, weighed and filled in Soxhlet's apparatus for extraction. Drug was defatted by extraction with the petroleum ether then remaining marc was reextracted by using ethanol: water (80:20). % yield was calculated for each extract and dried using rota vapors evaporator; calculate the percentage yield. [10]

The percentage yield of each extract was calculated by using following formula: -

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder drug taken}} \times 100$$

**Phytochemical analysis**

The phytochemical screening of the plant extracts was performed by thin layer chromatography (TLC) according to Harborne [10] and Roberts et al. [11]

**Determination of total phenolic content**

Total phenolic content was determined by the Folin-Ciocalteu method to Li et al. [12] Two hundred microliters of diluted sample were added to 1 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 mL of saturated sodium carbonate solution (75 g/L) was added. After 2 h of incubation at room temperature, protected from light, the absorbance at 765 nm was measured in triplicate. Gallic acid (0 - 500 mg/L) was used for calibration of standard curve. The results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of plant extract.

**Determination of flavonoids**

The determination of flavonoids follows the methodology proposed by Woisky and Salatino [13] to 0.5 mL of diluted samples, was added 0.5 mL of 2% $AlCl_3$ (w/v) solution prepared in methanol. After 30 minutes of incubation at room temperature, protected from light, the absorbance at 420 nm was measured in triplicate. The results were expressed as milligram quercetin equivalent (mg QE)/g dry weight of plant extract.

**DPPH radical scavenging assay**

The DPPH free radical scavenging activity of the extracts was performed according to Brand-Williams et al. [14] with some modifications. A methanolic DPPH stock solution (200 µM) was further diluted in methanol to obtain a UV-VIS absorbance between 0.6 - 0.7 at 517 nm, obtaining the DPPH working solution. Different concentrations of the extracts (40 µL) were mixed with DPPH solution (250 µL) and after 30 min incubation in darkness the absorbances were read at the same wavelength mentioned above. The measurements were triplicated and their scavenging activities were calculated based on the percentage of DPPH scavenged.

## RESULTS AND DISCUSSION

### Extraction of plant material

The percentage yield obtained after extraction of plant material by using Soxhlet's apparatus given below:-

**Table 1**: **Extraction of *Momordica charantia* L. leaf extract**

| S. No. | Extract | % Yield | Characteristic |
|---|---|---|---|
| 1. | Petroleum ether extract (PEE) | 1.82 | Semi Solid, slight yellow brown in color with characteristic odor |
| 2. | Hydro-alcoholic extract (DHE) (Ethanol : water 80:20) | 14.12 | Semi-solid, dark brown with yellowish shade in colour, characteristics odour |

### Qualitative chemical examination

Qualitative test shows presences of various phytochemicals are tabulated in table 2.

**Table 2: Results of qualitative chemical tests**

| S. No. | Experiment | Pet ether extract (PEE) | Hydro-alcoholic extract (DHE) |
|---|---|---|---|
| 1. | Test for carbohydrates | - | + |
| 2. | Test for gum and mucilage | + | - |
| 3. | Test for Proteins | - | + |
| 4. | Test for alkaloids | - | - |
| 5. | Test for glycosides | - | + |
| 6. | Test for steroids | + | - |
| 7. | Test for tannins | _ | + |
| 8. | Test for saponins | - | + |
| 9. | Test for flavonoids | - | + |
| 10. | Test for anthraquinones | - | + |
| 11. | Test for furanoids | - | - |
| 12. | Test for coumarin | - | - |
| 13. | Test for terpenoids | + | + |

+ Sign indicates presence whereas – indicates absence of constituents

## Estimation of total phenolic content (TPC)

The total phenolic content (TPC) was expressed as mg/gm of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y =0.007x+0.267 $R^2$ = 0.987, where x is the absorbance and y is the tannic acid equivalent (GAE).

**Table 3: Total phenolic content absorbance at various concentrations**

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 25 | 0.375 |
| 2 | 50 | 0.688 |
| 3 | 75 | 0.910 |
| 4 | 100 | 1.105 |
| 5 | 150 | 1.468 |
| 6 | 200 | 1.798 |
| 7 | **Sample** | 0.287 |

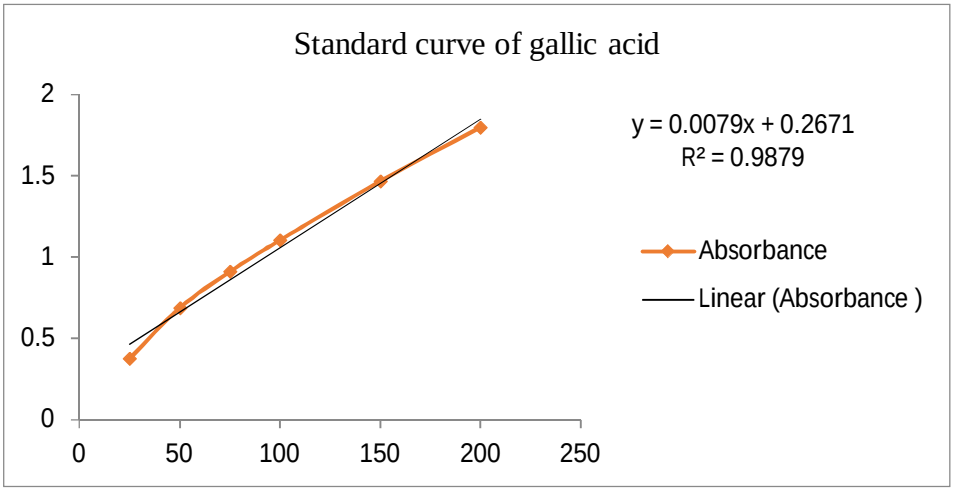


Figure 1: Standard curve of gallic acid

**Table 4: Total phenolic content (TPC) in *Momordica charantia* leaf extract**

| S. No | Extract | Absorbance | Total Phenol Content equivalent to gallic acid (mg/gm) of dried extract (GAE*) |
|---|---|---|---|
| 1 | PEE | 0.026 | 1.73 |
| 2 | DHE | 0.367 | 14.28 |

**Estimation of total flavonoids contents (TFC)**

Total flavonoid content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: Y=0.006x+0.164, $R^2$=0.968, where X is the absorbance and Y is the Quercetin equivalent (QE).

**Table 5: Total flavonoid content absorbance at various concentrations**

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 25 | 0.211 |
| 2 | 50 | 0.517 |
| 3 | 75 | 0.679 |
| 4 | 100 | 0.894 |
| 5 | 150 | 1.099 |
| 6 | 200 | 1.38 |
| 7 | **Sample** | 0.184 |

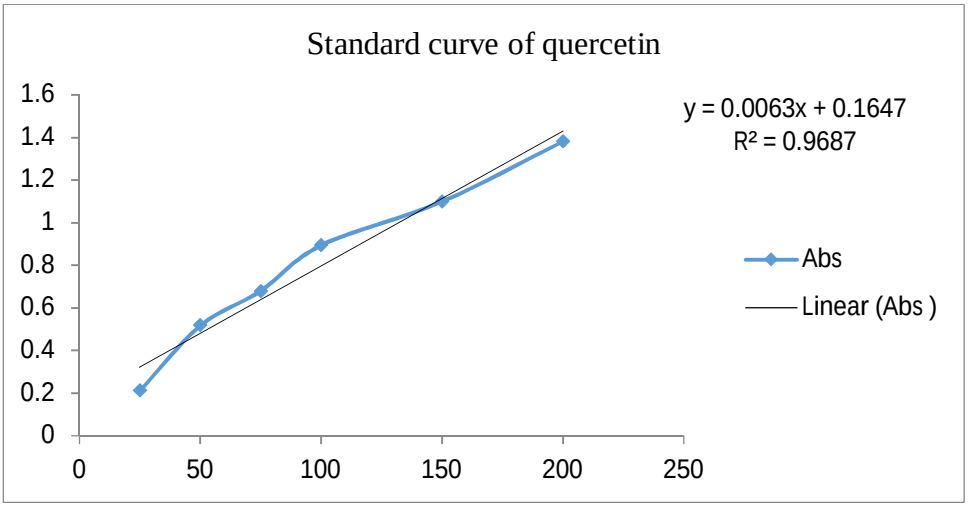


**Figure 2: Standard curve of quercetin**

**Table 6: Total flavonoids content (TFC) in *Momordica charantia* leaf extract**

| S. No. | Extract | Absorbance | TFC equivalent to quercetin (mg/gm) extract |
|---|---|---|---|
| 1. | PEE | 0.046 | 1.16 |
| 2. | DHE | 0.237 | 12.16 |

**DPPH radical scavenging effect**

Absorbance of control: - 0.546

**Table 7: DPPH radical scavenging assay of the extract**

| Con. | ABS of Vit C | Vit- C % Inhibition | ABS of PEE | PEE % Inhibition | ABS of DHE | DHE % Inhibition |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0.319 | 43.43 | 0.536 | 1.83 | 0.478 | 15.2 |
| 20 | 0.186 | 67.02 | 0.519 | 4.94 | 0.422 | 25.17 |
| 40 | 0.096 | 82.97 | 0.497 | 8.97 | 0.401 | 28.9 |
| 60 | 0.079 | 85.99 | 0.486 | 10.98 | 0.398 | 29.43 |
| 80 | 0.042 | 92.55 | 0.446 | 18.31 | 0.326 | 42.19 |
| 100 | 0.033 | 94.14 | 0.423 | 22.52 | 0.286 | 54.78 |
| 150 | 0.021 | 96.27 | 0.379 | 30.58 | 0.255 | 54.78 |
| 200 | 0.018 | 96.8 | 0.311 | 43.0 | 0.224 | 60.28 |
| **IC 50** | **11.69** | | | **235.18** | | **130.34** |

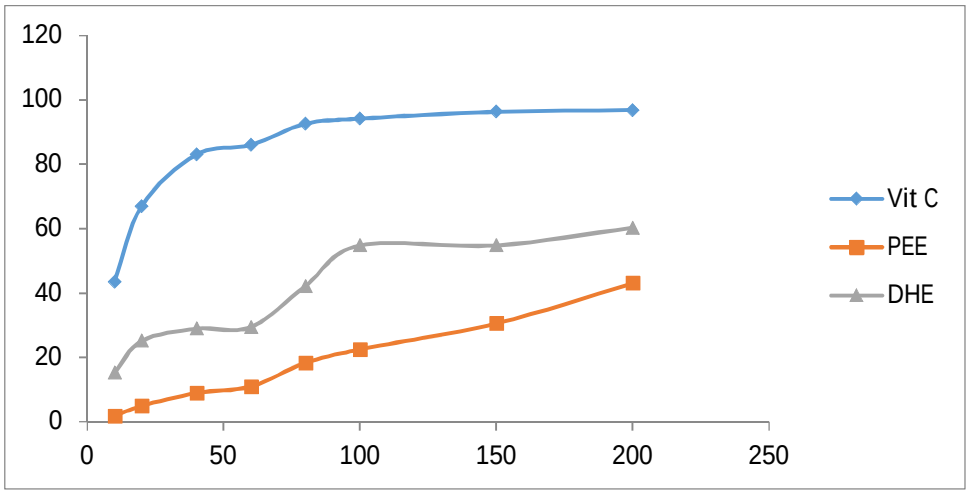


**Figure 3: DPPH radical scavenging activity (% inhibition Vs concentration)**

Since ancient times, many plants have been used for the treatment and prevention of many ailments and diseases and have shown a tremendous resource for the development of new drugs. Medicinal plants used in folk medicine are particularly interesting for investigation of their antioxidant effects. Some authors reported that the therapeutic benefit of medicinal plants is usually attributed to their antioxidant properties and oxidative stress is a prominent feature of these diseases. [15]

Extracts of *Momordica charantia* leaf was investigated for their phytoconstituents, antioxidant capacity, total phenolics and flavonoids content. The phytochemical screening was performed to identify the classes of chemical compounds present in the extracts. The phytochemical profile results showed that the plant extract has molecules with high technological potential for the development of new drugs with application in the treatment and prevention of various diseases. In the determination of total phenolics and flavonoids, the results showed that the hydroalcoholic solvent was better than petroleum ether solvent to extract phenolic compounds which may be explained by its good polarity and solubility for phenolic compounds extracted from plants. [16, 17]

The results obtained in this study indicate that petroleum ether extract and hydroalcoholic extracts of petroleum ether have a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species, reduce the oxidized intermediates and act as primary antioxidant substances. From the results presented above, it is evident that the extracts contained phenolic compounds at different levels in the following order: hydroalcoholic > petroleum ether.

This study revealed that the leaves of *Momordica charantia* contain appreciable amounts of polyphenolic compounds that are capable of eliciting potent antioxidant activities. The antioxidant profile of this plant can be harnessed to treat radicals related to pathological conditions.

**Conclusion**

It can be postulated that the contents of phenolic and flavonoid compounds of leaves of *Momordica charantia* contribute to the radical scavenging activity and can be regarded as promising plant species for natural sources of antioxidant with potential value for treatment of many life threatening diseases. The use of hydroalcoholic solvent was an efficient method of extraction of secondary metabolites with antioxidant activity compared to the use of petroleum ether. The antioxidant properties of the secondary metabolites of the leaves extract of this plant may represent a potential source of components that could improve the health, being applied as functional foods or incorporated biomolecules into pharmaceutical or nutraceutical preparations.

## References

1. Jiangsu New Medical College. Dictionary of Chinese Materia Medica. Shanghai: Shanghai Scientific and Technical Publishers. 1986: 123-124.
2. Ma L, Yu AH, Sun LL, Gao W, Zhang MM, Su YL, Liu H, Ji TF and Li DZ. Two new cucurbitane triterpenoids from the seeds of *Momordica charantia*. Journal of Asian Natural Products Research. 2014; 16: 476-482.
3. Zeng K, He YN, Yang D, Cao JQ, Xia XC, Zhang SJ, Bi XL and Zhao YQ. New compounds from acid hydrolyzed products of the fruits of *Momordica charantia* L. and their inhibitory activity against protein tyrosine phosphatas 1B. European journal of medicinal chemistry. 2014; 81: 176- 180.
4. Li QY, Chen HB, Liu ZM, Wang B and Zhao YY. Cucurbitane triterpenoids from *Momordica charantia*. Magnetic Resonance in Chemistry. 2007; 45: 451-456.
5. Nkambo W, Anyama NG and Onegi B. In vivo hypoglycemic effect of methanolic fruit extract of *Momordica charantia* L. African Health Sciences. 2013; 13: 933-939.
6. Hsiao PC, Liaw CC, Hwang SY, Cheng HL, Zhang LJ, Shen CC, Hsu FL and Kuo YH. Antiproliferative and hypoglycemic cucurbitane-type glycosides from the fruits of *Momordica charantia*. J Agric Food Chem 2013; 61: 2979-2986.
7. Fredulin SE, Cristina HFA, Castro PHC, Paula BRA, Aparecida CM and Luzía FE. Immunomodulatory effects of poly (ethylene glycol) microspheres adsorbed with nanofractions of *Momordica charantia* L. on diabetic human blood phagocytes. Science of Advanced Materials 2011; 3: 687-694.
8. Sahu N and Saxena, J. Total phenolic & total flavonoid content of *Bougainvillea Glabra* choisy and *Calforina gold* flower extracts. Int J Pharm Tech. 2013; 5: 5581-5585.
9. Dewick PM. Medicinal natural products. A biosynthetic approach, John Wiley & Sons England. 2001.
10. Harborne JB. Phytochemical methods. 3rd Edition, Chapman & Hall, Londres. 1998.

11. Roberts EAH, Cartwright RA and Oldschool M. Phenolic substances of manufactured tea. I. fractionation and paper chromatography of water-soluble substances. Science of Food and Agriculture. 1957; 8: 72-80.
12. Li AB, Wonga CC, Ka-Wing C and Chen F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. Swiss Society of Food Science and Technology. 2008; 41: 385-390.
13. Woisky RG and Salatino A. Analysis of propolis: Some parameters and procedures for chemical quality control. Journal of Apicultural Research. 1998; 37: 99-105.
14. Brand-WIliams W, Cuvelier ME and Berset C. Use of a free radical method to evaluate antioxidant activity. Food Science and Technology. 1995; 28: 25-30.
15. Javanmardi J, Stushnoff C, Locke E and Vivanco JM. Antioxidant activity and total phenolic content of *Iranian Ocimum* accessions. Food Chem. 2003; 83:547-550.
16. Siddhuraju P and Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleiferea* Lam.) leaves. Journal of Agricultural and Food Chemistry. 2003; 51: 2144-2155.
17. Roby MHH, Sarhan M, Selim KAH and Khalel IK. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), Sage (*Salvia officinalis* L.), and Marjoram (*Origanum majorana* L.) extracts. Industrial Crops and Products. 2013; 43: 827-831.