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In Vitro Evaluation of Antioxidant Activity of Bitter Melon (*Momordica charantia* L.)

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Abstract: Find out the scientifically evidence of Bitter Melon (*Momordica charantia* L.) for it's Antioxidant property. Preliminary phytochemical screening and in-vitro antioxidant activity of Bitter Melon (*Momordica charantia* L.) extract were investigated but the extraction was done at different temperature respectively (35ºC,60ºC, 100ºC) by decoction process. The antioxidant activity was studied in some in-vitro antioxidant models like DPPH radical scavenging activity, Superoxide radical scavenging activity, Ferric reducing power and Hydrogen peroxide scavenging activity. Total Antioxidant capacity was also determined. The Bitter Melon (*Momordica charantia* L.) extract showed antioxidant activity by inhibiting DPPH, scavenging superoxide and hydrogen peroxide. It also showed reducing power ability in ferric reducing model. Total antioxidant capacity was found to be 19.22 mg/gm expressed as L-Ascorbic acid. Significant antioxidant activity of Water extract of Bitter Melon (*Momordica charantia* L.) was found which might be due to the presence of Acidic compounds, Flavonoids, Phenols, Saponins, Tannins (Phenolic compounds) and Triterpenoids etc found in the preliminary Phytochemical screening.
Key words: Antioxidant, Bitter Melon (*Momordica charantia* L.), Ferric Reducing, Cucurbitaceae.

Introduction:

In every Living cells needs Oxygen, Oxygen is essential for the survival of all on this earth. Other than Oxygen survival of life is not possible, During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen as per study gets univalently reduced to oxygen derived free radicals like Superoxide, Hydrogen peroxide, Hydroxyl and Nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second [1].

Antioxidants are added as Red-ox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule [2]. It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are present in plants and are the main source of Antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species. The Natural antioxidants may have Free-radical scavengers, Reducing agents, Potential complexers of Pro-oxidant metals, Quenches of singlet oxygen etc [3]. The antioxidants can interfere with the oxidation process by reacting with free radicals [4]. Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to

replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [5]. Antioxidants principles from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance [6]. Food industry uses natural antioxidants as a replacement of conventional synthetic antioxidants [7].

Literature Review:

Momordica charantia (MC), a member of the Cucurbitaceae family, is known as Bitter Melon, bitter gourd, balsam pear, karela and pare. It grows in tropical areas of the Amazon, East Africa, Asia, India, South America, and the Caribbean and is used traditionally as both food and medicine. The plant is a climbing perennial with elongated fruit that resembles a warty gourd or cucumber. The unripe fruit is white or green in colour and has a bitter taste that becomes more pronounced as the fruit ripens.[8]

The seeds, fruit, leaves, and root of the plant have been used in traditional medicine for Microbial infections, Sluggish digestion and Intestinal gas, Menstrual stimulation, Wound healing, Inflammation, Fever reduction, Hypertension, and as a Laxative and Emetic.[9].Clinical conditions for which *M.charantia* extracts (primarily from the fruit) are currently being used include Diabetes, Dyslipidemia, Microbial infections, and Potentially as a Cytotoxic agent for certain types of Cancer[10-13].

Botanical Description of Bitter Melon (*Momordica charantia* L.):

Momordica charantia L. (Bitter melon or Bitter Gourd) (Fig.1) is a flowering vine in the family Cucurbitaceae. It is a tropical plant that is widely cultivated in Asia, India, East Africa and South America for its intensely bitter fruits that are commonly used in cooking and as a natural remedy for treating antioxidant , Diabetes like disorders [26]. It is a climbing perennial that useally groes upto 5meter and bears elongated fruits with a knobbly surface.It is a useful medicinal and vegetable plats for human health and one of the most promising plants for Anti-oxidants and Diabetes [27].

Fig.1 Bitter Melon (*Momordica charantia* L.)
***MC represent here *Momordica charantia* L.**

Phytoconstituents are present in Bitter Melon:

Main Phytochemicals:-Alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins,cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordicosides, momordin, momordolo, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecans, peptides, petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmarinic acid, rubixanthin, spinasterol, steroidal glycosides, stigmasta-diols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vacine, v-insulin, verbascoside, vicine, zeatin, zeatin riboside, zeaxanthin, zeinoxanthin

Active Constituents:

Although they have not been definitively determined, research indicates the primary constituents responsible for the hypoglycemic properties of Momordica are charantin, insulin-like peptide (plant (p)-insulin), Cucurbuta noids , momordicin, and oleanolic acids[14]. P-insulin is structurally and pharmacologically similar to bovine insulin and is composed of two polypeptide chains held together by disulfide bonds [15]. MC also has numerous other constituents including proteins (momordin, which may have anticancer properties), glycosides, saponins, and minerals[9]. It is also rich in vitamins A and C and beta-carotene, as well as the minerals iron, phosphorus, and potassium[8].

Documented Properties and Actions:

Anthelmintic, antibacterial, antibiotic, antidiabetic, antiinflammatory, antimicrobial, antimutagenic, antimycobacterial, antioxidant, antitumor, antiulcer, antiviral, aperitive, aphrodisiac, astringent, carminative, cytostatic, cytotoxic, depurative, hormonal, hypocholesterolemic, hypotensive, hypotriglyceridemic, hypoglycemic, immunostimulant, insecticidal, lactagogue, laxative, purgative, refrigerant, stomachic, styptic, tonic, vermifuge most of the all are allied disorders of Antioxidant Activity.

Mechanisms of Action

The most well researched MC mechanism is its blood sugar lowering effect. Research using a validated animal model of diabetes has demonstrated MC extracts increase glucose utilization by the liver[16], decrease gluconeogenesis via inhibition of two key enzymes (glucose-6-phosphatase and fructose-1,6-bisphosphatase), and improve glucose oxidation through the shunt pathway by activating glucose-6-phosphate dehydrogenase[17]. Extracts of MC also enhance cellular uptake of glucose, promote insulin release and potentiate its effect, [18,19] and increase the number of insulin producing beta cells in the pancreas of diabetic animals.[20] Bitter Melon extracts have been shown to inhibit growth and proliferation of various types of cancer cells in animals and *in vitro.* This may be attributed to the identification of a potent inhibitor of guanylate cyclase, an enzyme present in high amounts in many types of tumor cells [21,22]. Other research indicates MC extracts modify the immune response in cancer patients via decreased intestinal secretion of interleukin-7, reduced lymphocyte number, and increased T-helper and natural killer cell populations [23]. MC extracts have broad-spectrum antimicrobial activity, having been shown to prevent infection by numerous viruses, bacteria, parasitic organisms, and fungi. Although mechanisms have not been determined for all organisms, in the case of viral infection it is thought that certain Bitter Melon constituents prevent viral penetration of the cell wall. [15] The immune-stimulating properties of MC extracts may also contribute to decreased rates of microbial infection observed in animal studies. Animal studies demonstrate MC extracts, particularly the saponin fraction, have lipid-lowering effects resulting from inhibition of pancreatic lipase activity and subsequent decreased lipid absorption [11] Another study demonstrated MC juice has an inhibitory effect on membrane lipid peroxidation [13].

Experimental:

Material & Methods

Chemicals:-

L-Ascorbic acid, Rutin, Gallic acid, Hydrogen peroxide, Potassium ferricyanide, Trichloroacetic acid, Ferric chloride, Folin-ciocalteu reagent, Indigosulphonic acid, α-α diphenyl β picryl hydrazyl (DPPH), Riboflavin**,** Nitro Blue Tetrazolium (NBT) and Dimethyl Sulphoxide (DMSO) were all purchased from Merch chemicals, India, all other reagents used were of analytical grade.

Instruments:-

UV spectrophotometer (LABINDIA-Model-UV-3000^{+}), Laboratory Centrifuge Machine (LABY, Instrument Industry).

Plant Material:

The Fruit of Bitter Melon (*Momordica charantia* L.), were collected locally from the market of Nadaun, Dist;-Hamirpur, Himachal Pradesh(India) and were authentified by Central National Herbarium, Botanical Survey Of India, Botanical Garden,Howrah-711103, West Bengal.

Fig.2 Bitter Melon (Momordica charantia L.)

Extraction of Plant Material:

The Fruits of Bitter Melon(Momordica charantia L.) are graded, cleaned and disintegrated to required mesh (20-60). It is then weighed and equally divided into 3 parts, which were then extracted separately with distilled water at different temperature conditions by the method of Decoction viz. prevailing room temperature and more 35°C, 60°C and 100°C for 10-15 minutes and were kept overnight. Next day the extracts were filtered off and the resulting filtrates were used for the study.(Due to that processing of extraction at different temperature, different chemical constituents presents in different extracts or degrades some constituents at higher temperatures also possible by this study that will be establish also).

Preliminary Phytochemical Screening:

Preliminary Phytochemical screening of the Bitter Melon (Momordica charantia L.) Aqueous extract was carried out for the detection of the various constituents present in it. [24].

Preparation of Bitter Melon Stock Solutions:

Aqueous extracts (was done at three different temperature respectively 35º C,60º C,100º C for Ferric Reducing Power Determination for other activity use only aqueous extract was done at 35º C) of Bitter Melon was prepared at the concentration of 1,000 µg/ml in distilled water. From the stock solution different concentration viz. 10, 20, 40, 60, 80 and 100 µg/ml were prepared in same solvent and used for antioxidant studies.

Preparation of Standard Stock Solution of L- Ascorbic Acid:

L-Ascorbic acid used as standard for the study and its stock solution was prepared in the concentration of 1,000 µg/ml in distilled water. It was prepared freshly and used immediately for the study. From the stock solution different concentration viz. 10, 20, 40, 60, 80 and 100 µg/ml were prepared in distilled water and used for antioxidant studies.

Total Antioxidant Capacity:

For total antioxidant capacity assay, 0.3 ml of the Bitter Melon extract (at 35º C) (10 mg/ml) dissolved in water and mixed with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After 90 min, the mixture was cooled to room temperature; the absorbance was measured at 695 nm

against reagent blank. Methanol (0.3 ml) in the place of extract is used as the blank. L-Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of L-Ascorbic acid [5].

(For the purpose of determination of Total Antioxidant Capacity, using only aqueous extract of Bitter Melon which was done at 35º C, because in that extract most of the constituents are present and at that temperature no one constituents are degrades e.g. Flavonoids etc).

DPPH Radical Scavenging Activity:-

Bitter Melon extract (only for 35º C) and standard L-Ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80 and 100 µg/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 [(A0–A1)/A0]×100, where A0 is the absorbance of the control, and A1 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 [5].

Superoxide Radical Scavenging Activity:-

Each 3ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, and 12 mM EDTA and 0.1 mg NBT and 1ml of sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of Bitter Melon extract (only for 35º C) and standard ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml for 5min. Immediately after illumination, the absorbance was measured at 590 nm. Identical tubes with reaction mixture and 1ml of methanol were kept in the dark along and served as control. The percentage inhibition of superoxide anion generation was calculated from [(A0-A1)/A0]×100, where A0 is the absorbance of the control, and A1 is the absorbance of the 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 [5].

Scavenging Of Hydrogen Peroxide:-

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of Bitter Melon extract (only for 35º C) and standard L-Ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml in methanol (1 ml) where added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230nm was determined after 10 min 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 [(A0–A1)/A0]×100, where A0 is the absorbance of the control and A1 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 [5].

Ferric Reducing Power Determination:-

Different concentrations of Bitter Melon extract (which was done at three different temperature respectively 35º C, 60º C,100º C) and standard L-Ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M pH 6.6) and potassium Ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50° C for 20 min. A portion (2.5 ml) of Tricholoroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 g (rpm) for 10 min at room temperature. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride ($FeCl_3$) (0.5 ml, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700 nm. All the tests were performed in triplicate and the graph was plotted with the average of three observations [5] [25].

(For the purpose of other determinations except Ferric Reducing Power Determination using only aqueous extract of Bitter Melon which was done at 35º C, because in that extract most of the constituents are present and at that temperature no one constituents are degrades e.g. Flavonoids etc. But in Ferric Reducing Power Determination comparing with all Aqueous extract of Bitter Melon which was done at three different temperature respectively 35º C, 60º C, 100º C with a Standard).

Statistical Evaluation:

Experimental results were Mean±SEM of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value. Student's t-test was used for the comparison between two means for the possible significant interrelation. Data were considered statistically significant only when p value < 0.05.

Results and Discussion:-

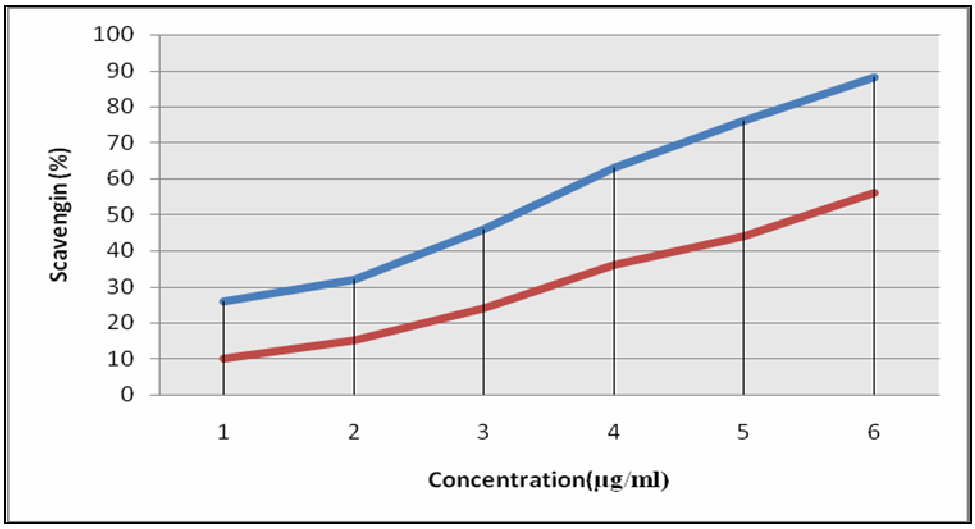
Table.1:- Preliminary Phytochemical Screening.

Name of Herb	Part used	Scientific name	Alkaloids	Acids	Carbohydrats	Fixed Oils	Flavonoids	Glycosides	Proteins	Resins	Saponins	Sterols	Tanins	Terpens
Bitter Melon	Fruits	*Momordica charantia* L.) Family:- Cucurbitaceae.	+	+	+		+	+	+		+	+		+

The phytochemical screening results showed the presence of Alkaloids, Carbohydrates, Proteins, Saponin glycosides and Triterpenoids ,Flavonoids in the Bitter Melon Aqueous extracts.

Total Antioxidant Capacity. The total antioxidant capacity in the Bitter Melon extract (at 35°C) measured spectrophotometrically was 19.22 mg/gm expressed as L-Ascorbic acid.

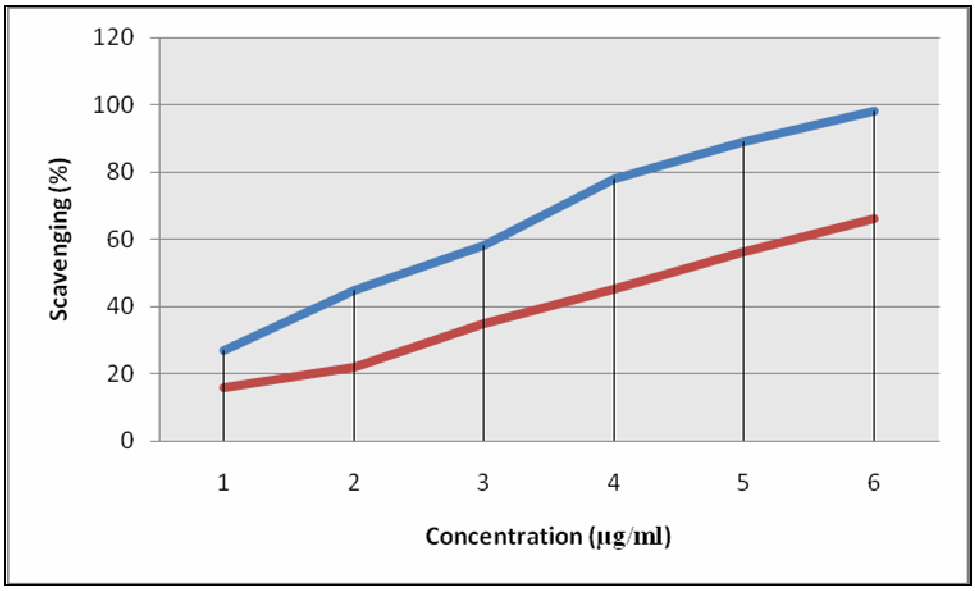
DPPH Radical Scavenging Activity:- Fig.3 illustrates a significant ($p < 0.05$) decrease in the concentration of DPPH radicals due to the scavenging ability of Bitter Melon.This activity was dose dependent. Maximum scavenging activity (56%) was observed at 100 µg/ml concentration and the IC_{50} value of Bitter Melon extract and L-Ascorbic acid were found to be 90 µg/ml and 45.60 µg/ml respectively.



- Blue line represents L-Ascorbic acid curve.
- Red line represents Aqueous extract of Bitter Melon curve.

Fig. 3. DPPH radical scavenging activity of Bitter Melon extract.

Super Oxide Radical Scavenging Activity: - Fig.4. Reveals that a significant ($p < 0.05$) dose response relationship is found in the superoxide free radical scavenging activity in Bitter Melon extract. Maximum scavenging activity (66 %) was observed at 100 µg/ml concentration of and the IC50 value of Bitter Melon extract and L-Ascorbic acid were found to be 71.40 µg/ml and 34.99 µg/ml respectively.

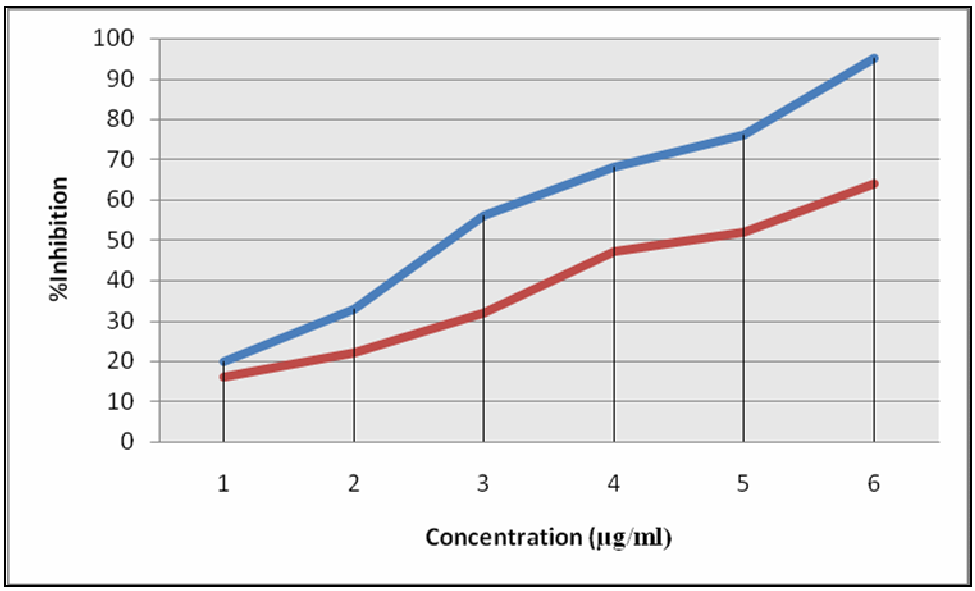


- Blue line represents L-Ascorbic acid curve.
- Red line represents Aqueous extract of Bitter Melon curve.

Fig. 4.Superoxide radical scavenging activity of Bitter Melon extract.

Scavenging of Hydrogen Peroxide:

Fig. 5 reveals that a significant ($p < 0.05$) dose dependent response was found in the hydrogen peroxide scavenging activity in Bitter Melon extract. Maximum scavenging activity (64%) was observed at 100 µg/ml concentration and the IC50 value of Bitter Melon extract and L-Ascorbic acid were found to be 74.60 µg/ml and 38.84 µg/ml respectively.

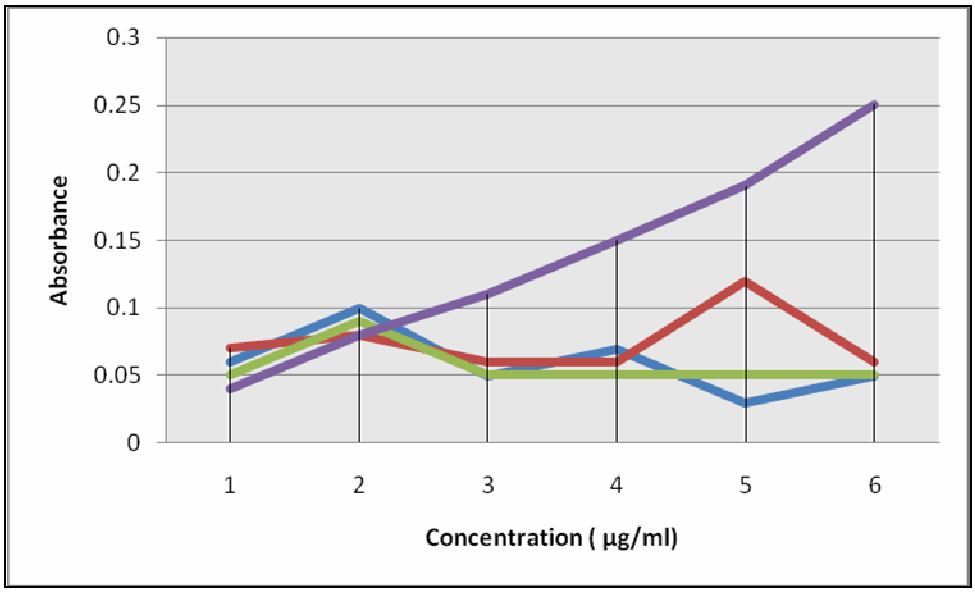


- Blue line represents L-Ascorbic acid curve.
- Red line represents Aqueous extract of Bitter Melon curve.

Fig. 5. Hydrogen peroxide radical scavenging activity of Bitter Melon extract.

Ferric Reducing Power Determination:

Fig. 6 reveals that reducing power of Bitter Melon extract was statistically significant ($p < 0.05$). The result clearly indicates that the reducing power of the Bitter Melon extract increased with increasing the concentration and is comparable with the standard L-Ascorbic acid, hence it is having the antioxidant activity.



- Blue line represent Bitter Melon Extract at 35°C
- Red line Represent Bitter Melon Extract at 60°C
- Green line represent Bitter Melon Extract at 100°C
- Violet line represent Standard curve for L-Ascorbic Acid.

Fig. 6. Reducing power of Bitter Melon extract (Represents a Comperison Study of Antioxidant Activity, between different extraction was done at different temparatures 35°C,60°C,100°C respectively of Bitter Melon)

Conclusion:

The Bitter Melon Aqueous extract showed antioxidant activity by inhibiting DPPH, scavenging super oxide as well as hydrogen peroxide and reducing power ability which may be due to presence of Alkaloids, proteins and saponin, glycosides, Triterpenoids, Flavonoids etc found in the preliminary phytochemical screening.

Thus, the radical scavenging, reducing power activity suggests that Aqueous extract of Bitter Melon *In-vitro* antioxidant activities. Further studies are needed to evaluate the *in-vivo* antioxidant potential of Bitter Melon extract in various animal models and preparing Aurvedic different types of Formulations and identify the major active potent Anti-oxidant component(s) standardize them but using marker compound with the help of Analytical procedure (HPLC,GC-MS,LC-MS etc) no needs to Isolate the active ingredient (s)because this is time consuming and costly process.

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