Pharmacognostical and pharmacological antioxidant profiling of Martynia Annua Lin. Root extract

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Abstract:

Medicinally important plant-foods offer a balanced immune function, which is essential for protecting the body against antigenic invasion, mainly by microorganisms. The genus Martynia annua L. (Martyniaceae) is one of the restorative spices. Phytochemicals such as alkaloids, flavonoids, terpenoids, carbohydrates and polyphenols have been reported as responsible for the immunomodulatory effects of several medicinal plants. Martynia annua extracts have demonstrated antioxidant and antimicrobial activities, and to be safe. This proposed work compiled the medicinal properties of Martynia annua, their cultivation, chemical composition, and the potential uses of these plants as antioxidant agents in with repect to clinical context. The results showed that root possessed antioxidant properties including radical scavenging, xanthine oxidase inhibition and nitrites scavenging activities. The antioxidative activities were correlated with the total phenol. The metanolic root extract of Martynia annua linn was more effective than that of other parts. The root extract is a promising candidate for use as natural products based antioxidant for the health of human being.

Introduction:

Natural products medicines are the main contributors of the leads in the design and development of therapeutic agents. Several plant derived compounds have been identified over the years for their immunomodulatory characteristics. Numerous illnesses can be alternatively treated by immunomodulation using medicinal plants, instead of chemotherapy [1]. A few plant derived compounds, including polysaccharides, are extremely diversified in terms of molecular weight and structure; therefore, it is challenging to produce the similar quality in every batch. Accumulative requirement for these plant-derived products would motivate improvement to overwhelming these obstacles for reaching market [2]. Immunity is the body's natural defense system against various infectious diseases. The factors which trigger immunity include previous infection, immunization, and various external stimuli.

Besides, immunity is capable of discriminating among body's own proteins/cells and foreign entities. As soon as the foreign particle is identified, the collective and coordinated response of specific cells and mediators against strange substances constitutes the immune response. Based on the function, immune system has been categorized in two broad categories, i.e., innate immune system (non-specific immune system) and adaptive immune system (specific or acquired immune system [3]. Moreover, antibodies have the ability to opsonize different pathogens, immobilize bacteria, and trigger microorganisms' destruction by phagocytes via activation of complement proteins [4]. Various monoclonal antibodies and synthetically combined compounds are additionally being utilized as immunomodulators [5]. The plant-based intensifies that filled in as lead structures and additionally were artificially adjusted are dicoumarol (warfarin), artemisinin (artemether), camptothecin (topotecan and irinotecan), morphine (scores of subordinates), and salicylic acid (acetylsalicylic acid) [6]. Next to these mixtures, different phytochemicals including medicinal oils, steroids, terpenoids, phenolics, shades, flavonoids, and alkaloids, and so on have displayed worth focusing on immunomodulatory impact. Plant-determined intensifies showing promising potential as immunomodulatory specialists [7]. Different natural and pharmacological properties of curcumin has been accounted for including hostile to disease, cancer prevention agent, against angiogenic, hostile to proliferative, favorable to apoptotic [8-9]. Martynia annua L. (Martyniaceae) is one of the restorative spices involved by local individuals of Mexico since antiquated time for various helpful purposes. The Martyniaceae family has three genera and these genera have tacky, furry leaves, orchid-like blossoms and woody, nose molded cases. The compound investigation of Martynia annua L. plant uncovers the presence of glycosides, tannins, starches, phenols, flavonoids and anthocyanins. The leaves fundamentally have chlorogenic corrosive, p-hydroxy benzoic corrosive, snapic corrosive and unsaturated fats, for example, palmitic corrosive and stearic acid. The blossoms of this plant primarily contain cyanidin-3-galactoside and pelargonidin-3, 5diglucoside.Gentisic corrosive is available in natural products.

Material and Methods

The root of *Martynia annua* was collected from the field area of Bhopal district M.P. India. For identification and taxonomic authentication, plant material was submitted in Department of Botany, Saifia College, Bhopal, India. Collected plant materials were shade-dried and coarsely powdered.

Physicochemical Evaluation

Ash values determination: The dried root powder and seed of herbs was used for determining ash values. Ash value determination is an important parameter to establish the quality and purity of herbal drugs

Extractive values determination: Extractive value determination gives an idea about the presence of chemical constituents in powdered crude drugs under study. The powder of plant material separately was subjected for determining following extractive values.

Loss on drying: Determination of Los on drying is one of the important parameter for quality control and identification of crude drugs. Presence of moisture in crude drugs may attract microorganisms to grow which in turn may spoil the crude drug material and can affect the phytochemical contents. About 1.5 G of powdered crude drugs was transferred separately to a weighed flat and thin porcelain dishes. The porcelain dishes were then dried in an oven at 100ºC till constant weight. The Loss on drying (LOD) was expressed in terms of percentage calculated in relation to initial weight taken of powdered crude drugs.

Extraction of plant material: The crude plant material was often subjected to the selective extraction comprised of treating the moderately coarse powder of plant material with non polar solvent in succession to extract various plant constituents according to their solubility. The powdered plant material (250 gm) were successively extracted in a soxhlet apparatus with petroleum ether (60-80°C), methanol and finally with water (by maceration process). After each extraction test was performed to see whether the drug had been completely exhausted or not. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent. After ethanol extraction the marc obtained was dried and macerated with chloroform water for 24 hrs repeatedly two to three times. The liquid extracts were collected in a tare conical flask. The solvent removed by distillation method. The last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w yield was calculated Successive solvent extracts (methanol and aqueous extract) were subjected for qualitative analysis.

Qualitative Analysis of Extracts: All extracts were subject to various qualitative analyses to detect the presence of plant constituents. The various phytovhemical analysis i.e. alkaloid, glycosides, saponins, carbohydrates, phytosterols, tannins and phenolic compounds (flavonoids), and proteins and free amino acids were performed.

***In-vitro* antioxidant assay:** Antioxidants may be defined as any substance, when present at low concentration compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate in a chain reaction. Antioxidant actions might be exerted by inhibiting generation of reactive oxygen species and reactive nitrogen species or by directly scavenging free radical or by raising the levels of endogenous antioxidant enzymes by up regulating expression of the genes encoding superoxide dismutase, catalase or glutathione peroxidase. For the assessment of free radical scavenging activity, the extracts of selected plants were dissolved in 5% DMSO. DPPH, Nitric oxide, hydroxyl radical, superoxide radical methods were carried in the present study [8-9].

Chemicals and plant extract: 1,1-diphenyl-2-picryl hydrazyl (DPPH), DMSO, Ascorbic acid Sodium nitro prusside, Griess reagent (1%w/v sulphanilamide, 2% w/v H3PO4 and N-(1-naphthyl) ethylene diamine di hydrochloride), Hydrogen peroxide (H2O2) Thiobarbituric acid Sodium phosphate buffer, 1mM ferric chloride Nitro blue tetrazolium Phenazene methosulphate, Nicotinamide adenine phosphate di nucleotide

MEAC: Methanol extract of *M. annua*

MEBC: Aqueous extract of *M. annua*

Few different assays were performed to determine the antioxidative power of *M. annua* extracts as described below. In each of these assays, ascorbic acid was used as a reference substrate. The ability of the extract to scavenge or inhibit free radicals was expressed as percentage inhibition and was calculated using the following formula:

Percentage inhibition=(A0-At)A0 ×100

Where, A_0 is absorbance of the control group (without plant extract) and A_t is absorbance of *M. annua* extracts. All determinations were carried out in triplicate with the readings taken in duplicate. These assays were performed to screen the best extracts in terms of its anti-oxidant potential. The percent inhibition of oxidation of different standard compounds was studied and the final selection was made [10].

DPPH radical scavenging activity: DPPH radical scavenging activity of aqueous extract was determined according to the method. In brief, 0.5mL of 0.1mM DPPH solution was prepared in methanol just before use. 1.0 mL of aqueous extract was added at different concentrations (1-250µg/mL) to DPPH solution. Double distilled H_2O was used in the control group instead of samples, with the same procedures applied. The ability of the substrate to reduce the stable radical DPPH from deep purple to yellow coloured diphenylpicryl hydrazine indicates its antioxidative potential. The mixture was shaken vigorously and left to stand for 30min in the dark, and absorbance was measured at 517 nm using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). Lower absorbance at 517 nm represents higher DPPH scavenging activity [11].

Superoxide Radical Scavenging Activity: superoxide radicals were generated in 25 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 25 mL of NBT (50 mM) solution, 25 mL NADH (78 mM) solution and different concentrations (25, 50, 75, 100 and 200 µg/mL) of extracts (50 mL). The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 mM) to the mixture then incubated at 25º ˚C for 5 min. The absorbance measured at 560 nm. Ascorbic acid was used as a positive control. Decrease in absorbance of the reaction mixture incubated increases super oxide anion scavenging activity. All the test analysis were run in triplicate and averaged. L-ascorbic acid was used as a positive control [12].

The % inhibition of super oxide anion generated was calculated using the following equation

% inhibition = [(A0-AT)/A0]*100

Where A0 = Absorbance of the control, and

AT =Absorbance of test extract/ standard.

Determination of Nitric Oxide (NO) radical scavenging activity of *Martynia annua* extracts: Nitric oxide (NO) was generated from sodium nitro prusside (SNP) and was measured by the griess reagent (1% w/v sulfanilamide, 2%w/v H3PO3 and 0.1% w/v N-(1-Naphthyl) ethylene diamine dihydrochloride). SNP in aqueous solution at physiological PH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. SNP (1ml of mM) was mixed with 1ml of selected plants extracts in different concentrations in DMSO, incubated at 25ºC for 180 minutes. To 1ml of the incubated solution, 1ml of griess reagent was added.

The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with N-(1-naphtyl) ethylene diamine dihydrochloride was read at 546nm by UV-Visible spectrophotometer (Shimadzu UV-Vis1800). All the test analysis were run in triplicate and averaged. Lower absorbance of reaction mixture indicates higher free radical scavenging activity [13]. Ascorbic was used as a positive control. All the test analysis were run in triplicate and averaged. Lower absorbance of reaction mixture indicates higher free radical scavenging activity. Ascorbic was used as a positive control. The percentage inhibition was calculated by

Percentage of Nitric oxide scavenged = [(A0–A1)/A0] X 100,

Where A0 = Absorbance of the control, and

A1 =Absorbance of the extract/ standard.

Results and Discussion

Pharmacognostical parameters such as total Ash value, acid insoluble ash value, water soluble ash value, alcohol soluble extractive value, water soluble extractive value and loss on drying were determined for the herb.

Table 1: Physicochemical parameters of roots of *Martynia annua*

S. No.	Physicochemical parameter values (% w/w)	*Martynia annua* roots
1	Total ash	7.3
2	Water soluble ash	4.98
3	Acid insoluble ash	1.57
4	Loss on drying	7.21
5	Alcohol Soluble Extractive	17.2
6	Water Soluble Extractive	20.3
7	Foreign organic matter determination	1.4

Preliminary phytochemical screening of plant material: The extraction of drug signifies a solid from solid separation, as solid components must be extracted from a solid substance. This type of extraction is generally performed before any separation processing and should be differentiated from solid liquid extraction where the solid drug is extracted with a liquid medium. The liquid liquid extraction is one in which any of the two liquids that are not miscible includes the substance to be extracted.

Table 2: Percentage yield of various extracts of *Martynia annua* roots

Solvents used for extraction	% yield (w/w)
Petroleum ether	3.1
Methanol (MEAC)	15.1
Water (MEBC)	17.3

Phytochemical screening: Phytochemical screening provides an idea about the preliminary chemical composition of the extracts. The chemical tests were performed as per the standard procedures. MEAC containing maximum number of secondary metabolites in comparison to other extracts. Various tests were performed to detect presence of various primary as well as secondary chemical constituents in the extract of *Martynia annua* roots

Table 3: Preliminary phytochemical screening *Martynia annua* rootsextracts

S. No.	Phytochemical Tests	MEAC	MEBC
1	Carbohydrates	+	+
2	Proteins And Amino Acids	-	-
3	Acidic Compounds	-	-
4	Mucilage	-	-
5	Fixed Oil	-	-
6	Alkaloids	+	+
7	Glycosides	+	-
8	Sterols And Steroids	+	-
9	Anthraquinones	-	-
10	Flavonoids	-	-
11	Phenolic Compounds	+	-
12	Terpenoids	+	+
13	Saponins	-	-

Anti-oxidant Activity: Analysis of the free radical scavenging activities of the *Martynia annua* extracts revealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, nitric oxide radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher.

DPPH radical scavenging activity of *Martynia annua* extracts: Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of l-Ascorbic acid, and plant extracts increased gradually with increase in concentration. Percentage inhibition of in vitro anti-oxidant result of various *Martynia annua* by DPPH Method and ascorbic acid as standard at various concentrations. Methanol extract were found to have greater reduction potential than aqueous extracts.

Table 4: Results of *Martynia annua* extracts on DPPH radical scavenging model

Concentration	200 µg/ml	400 µg/ml
Ascorbic acid	75.17	91.94
MEAC	72.87	81.87
MEBC	49.87	62.65

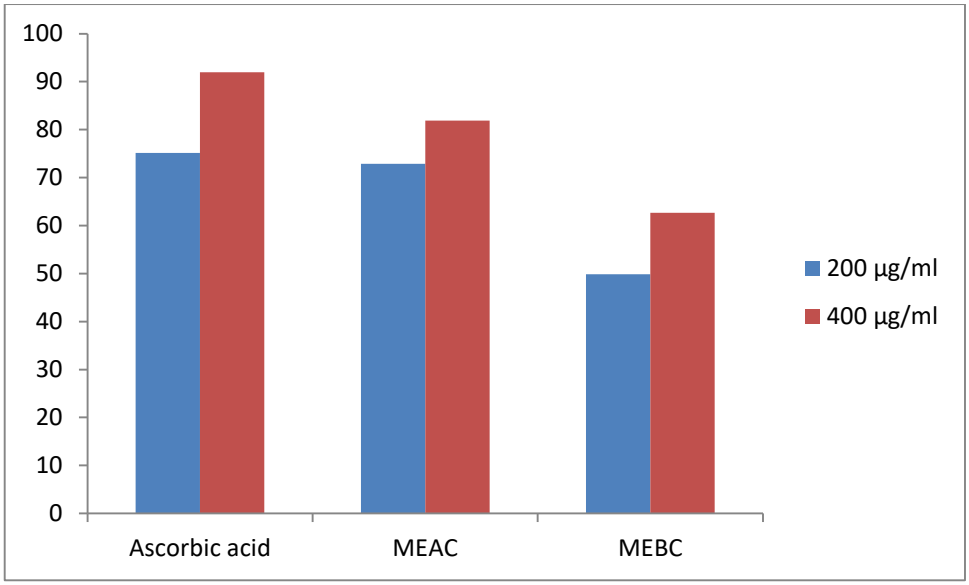

Figure 1: *Martynia annua* extracts on DPPH radical scavenging model

Table 5: *In vitro* 50% inhibition concentration (IC_{50}) of *Martynia annua* extracts on DPPH radical scavenging model

Extract /compound	50% inhibition concentration (IC_{50}) of DPPH model (µg/ml)
Ascorbic acid	65.5
MEAC	68.7
MEBC	77.8

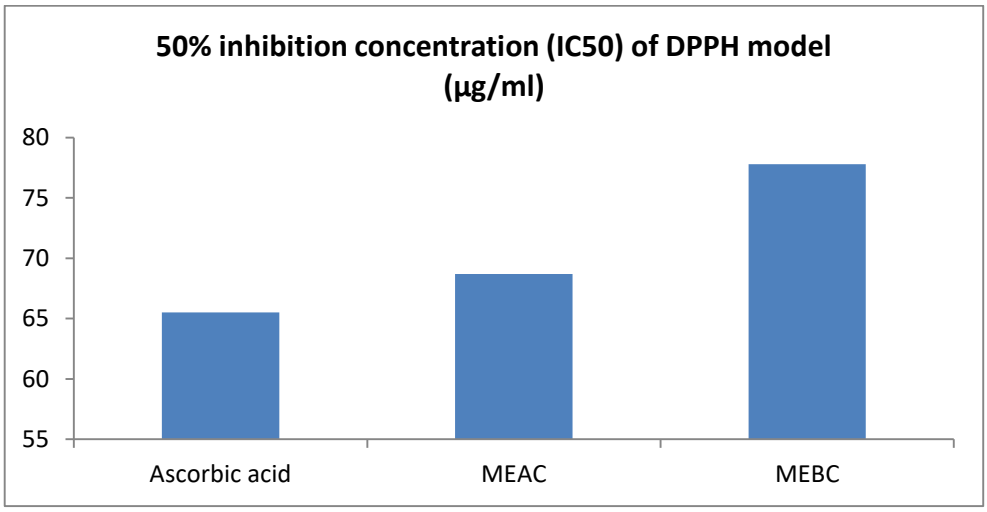

Figure 2: *In vitro* 50% inhibition concentration (IC_{50}) of *Martynia annua* extracts on DPPH radical scavenging model

Nitric Oxide (NO) radical scavenging activity of *Martynia annua* extract: Nitric oxide plays an important role in various types of inflammatory processes in the body. In the present study the *Martynia annua* extract checked for its inhibitory effect on Nitric oxide production. Nitric oxide radical generated for sodium nitroprusside at physiological pH was found to be inhibited by the extracts.

The methanol extract oat varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. Results revealed that all the tested extracts showed the percentage of inhibition in a dose dependent manner.

Table 6: *Martynia annua* extracts on Nitric oxide radical scavenging model

Concentration	200 µg/ml	400 µg/ml
Ascorbic acid	78.26	95.12
MEAC	61.14	71.52
MEBC	50.12	59.31

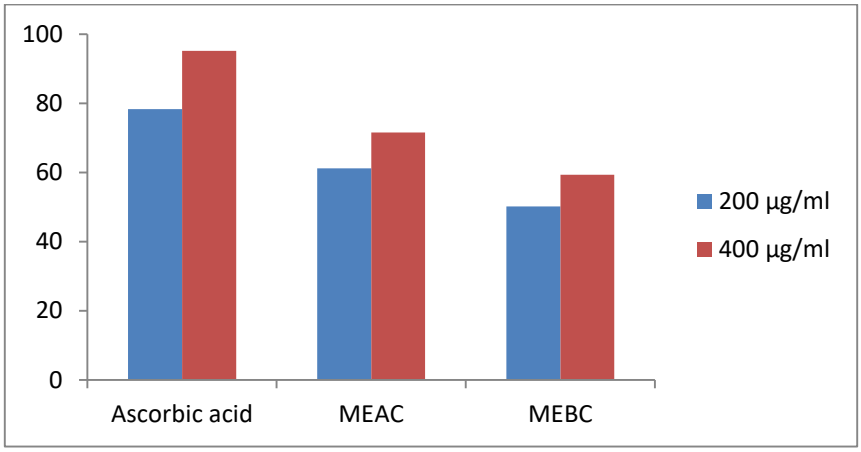

Figure 3: *Martynia annua* extracts on Nitric oxide radical scavenging model

Table 7: *In vitro* 50% inhibition concentration (IC_{50}) of *Martynia annua* extracts on Nitric oxide radical scavenging model

Extract /compound	50% inhibition concentration (IC_{50}) NO model (µg/ml)
Ascorbic acid	68.2
MEAC	71.2
MEBC	75.2

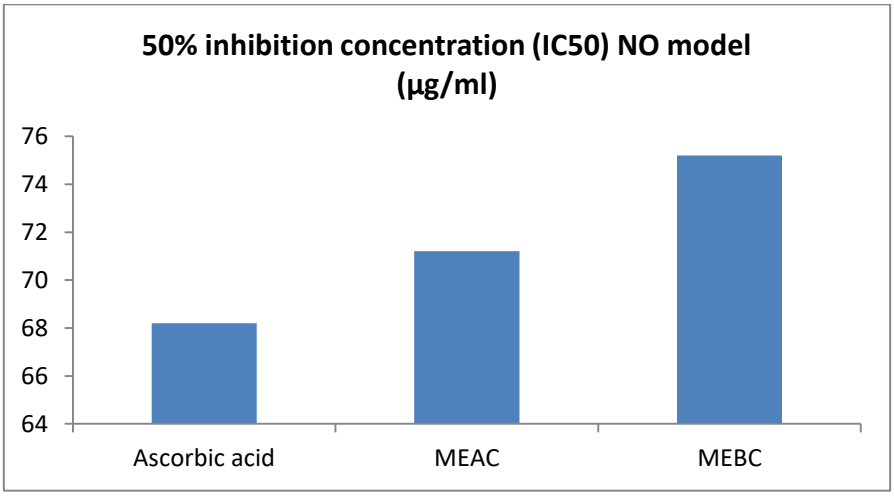

Figure 4: *In vitro* 50% inhibition concentration (IC_{50}) of *Martynia annua* extracts on Nitric oxide radical scavenging model

The inhibitory effect of *Martynia annua* extracts on Nitric oxide radical scavenging model were revealed that methanol extract of *Martynia annua* at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to aqueous extract.

Determination of super oxide radical scavenging activity of *Martynia annua* extracts: Percentage Inhibition of in vitro anti-oxidant result of various *Martynia annua* by super oxide radical scavenging activity and ascorbic acid as standard at various concentrations. Methanol extract were found to have greater reduction potential than aqueous extracts.

Table 8: *Martynia annua* extracts on Super oxide radical scavenging activity

Concentration	200 µg/ml	400 µg/ml
Ascorbic acid	67.26	94.23
MEAC	60.16	72.31
MEBC	51.12	60.31

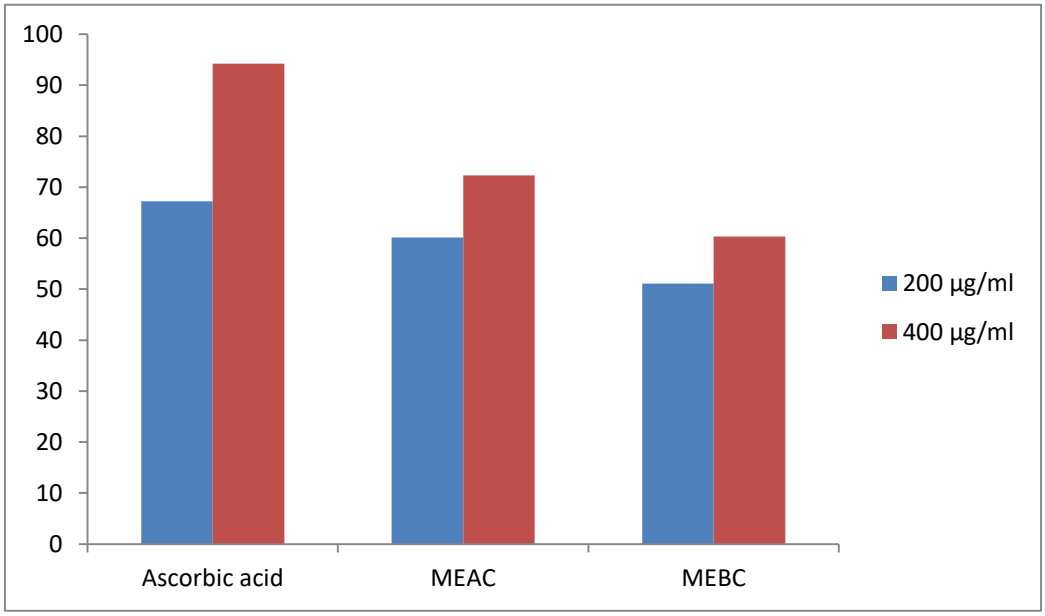

Figure 5: Results of *Martynia annua* extracts on Super oxide radical scavenging activity

Table 9: *In vitro* 50% inhibition concentration (IC_{50}) of *Martynia annua* extracts on Super oxide radical scavenging model

Extract /compound	50% inhibition concentration (IC_{50}) NO model (µg/ml)
Ascorbic acid	60.4
MEAC	66.3
MEBC	71.5

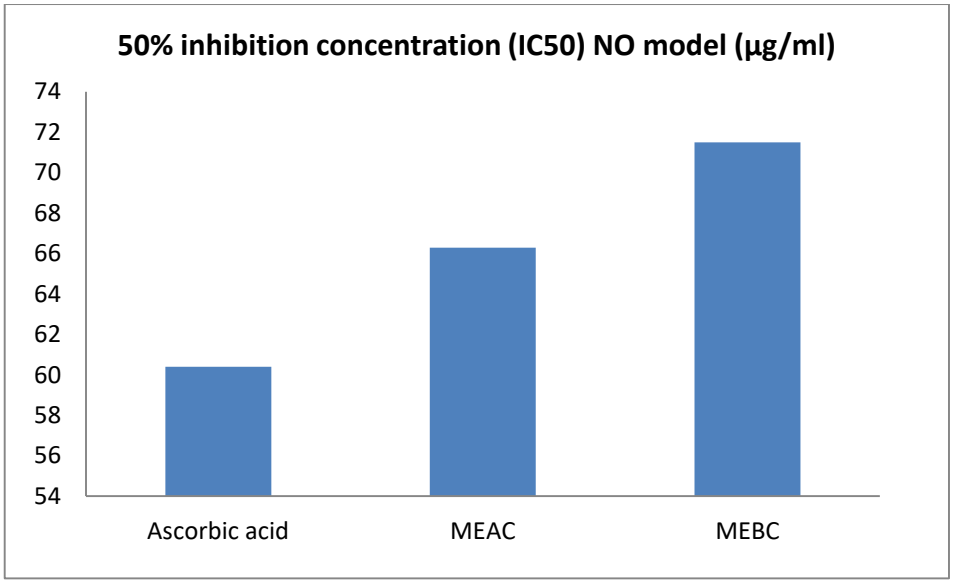

Figure 6: *In vitro* 50% inhibition concentration (IC_{50}) of *Martynia annua* extracts on Super oxide radical scavenging model

Summary and Conclusion:

Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. The antioxidant potential of Martynia annua Linn methanol and aqueos extracts was investigated in the search for new bioactive compounds from natural resources. It became clear that Martynia annua root aqueous present the valuable antioxidant activity compared with reference antioxidant Vitamin C for DPPH scavenging activity. Polyphenols was found in all the samples and in the following order: methanol extract than aqueous extract. The obtained results for DPPH are in agreement with the phenol contents determined for each sample. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups. Hence, we could conclude that these polyphenols are responsible for the observed antioxidant activity in this study. These findings show that the *Martynia annua* Linn extracts possesses antioxidant activity.

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