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Original Article

EXPLORATION OF *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL STUDIES OF *MOMORDICA CYMBALARIA* FENZL LEAVES EXTRACTS

N. UMARANI, K. ILANGO*

Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM University, Kattankulathur 603203, Tamilnadu, India.
Email: ilangok67@gmail.com

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ABSTRACT

Objective: The target of this current research work sought to analyze the antioxidant and antimicrobial activities of ethyl acetate and hydro-alcoholic extracts of *Momordica cymbalaria* leaves through various *in vitro* assays.

Methods: The antioxidant capability of the extracts to quench free radical was explored spectrophotometrically against DPPH, nitric oxide, superoxide anion, hydroxyl radicals and ferric reducing power. Metal chelating effect was also carried out by measuring the ferrous ion chelating property. The total phenolic and flavonoid contents were assessed by Folin-Ciocalteau and aluminium chloride reagents. The antimicrobial activity of ethyl acetate and hydro-alcoholic extracts of *Momordica cymbalaria* leaves was screened by agar diffusion method using *Klebsiella pneumoniae* (Gram positive bacteria), *Escherichia coli* (Gram negative bacteria) and *Aspergillus niger* (Fungi) as test microbes.

Results: The ethyl acetate and hydro-alcoholic extracts yielded 0.5551±0.78µgmL^{-1}, 0.2035±0.35µgmL^{-1} gallic acid equivalent phenolic content and 0.6666±0.24µgmL^{-1},0.1154±0.62µgmL^{-1} quercetin equivalent flavonoid content per 1 mg of plant extracts respectively. Overall, both extracts showed better antioxidant activity in a concentration-dependent manner due to better oxidative stability. Amongst the extracts, ethyl acetate extract exhibited a higher percentage of free radical scavenging effects, increment of phenolic and flavonoid contents, maximum reducing power and metal chelating property. The quantitative determination of the detected phytoconstituents in the ethyl acetate extract also indicated the dominant presence of alkaloid, phenolic, flavonoid and steroid contents. The antimicrobial study reveals that the *Momordica cymbalaria* leaves extracts exhibited significant inhibitory activity against the test pathogens when compared with the standard.

Conclusion: The output of overall results evokes that ethyl acetate extract of *Momordica cymbalaria* leaves can be utilized as a potential source of antioxidants. Moreover, both the extracts can also be effectively used as an antimicrobial agent to fight against harmful pathogens.

Keywords: *Momordica cymbalaria*, Oxidative stability, Folin-Ciocalteau, DPPH, Antimicrobial study, Agar diffusion method.

INTRODUCTION

Oxidative metabolism is essential for the survival of cells. Imbalance of this defence mechanism generated excessive reactive oxygen species, the important initiators of lipid oxidation and free radicals which can overwhelm protective enzymes and causes destructive lethal cellular effects (e.g., apoptosis) by oxidizing membrane lipid cellular proteins, DNA and enzymes thus shutting down cellular respiration [1-2]. The pathological disruption of these radicals in the human cells could be prevented by quenching the upshot of catalytic activities [3-4]. Free radicals and reactive oxygen species generated in the human system have been increasingly recognized in the pathogenesis of many human diseases including cancer, cardiovascular diseases, asthma, hepatitis, atherosclerosis and immunodeficiency diseases [5]. Antioxidants are acquiring pivotal benefits as a panacea for a large number of degenerative diseases owing to our sedentary way of life, stressful existence, deleterious effects of pollution and exposure to harmful chemicals [6]. The quest for natural antioxidants is gaining importance day by day in pharmaceuticals to replace synthetic antioxidants which are being restricted due to their carcinogenicity. Hence, substantial attention has been focused towards credentials of plants with antioxidant ability. Resistance to antimicrobial agents has become the prime necessity and pressing global problem. In the overall population, nearly 70% acquire microbial infections [7]. Contrary to the synthetic drugs, antimicrobials of plant origin have sans side effects and possess enormous therapeutic potential to cure infectious diseases. Substantial investment and research in the field of anti-infectives are now desperately needed to meet the public health crisis [8].

Momordica cymbalaria Fenzl. belonging to the family Cucurbitaceae is a perennial herbaceous climber distributed over tropical parts of western peninsular, India and well known as athalakkai in Tamil. This plant possesses manifold folkloric claims [9]. Our research interest in this plant arose because of its myriad effects as substantiated in the legendary claims. Folkloric record reports its fruits as an antidiabetic, antihyperlipidemic and antiulcer agent [10-11], the root tubers are used by the natives of north interior Karnataka and Andhra Pradesh to treat gynecological ailments and to induce abortions [12]. Moreover, leaves are used to alleviate whooping cough in the legendary medicine [13]. The extracts of dried fruits of *Momordica cymbalaria* have been reported to possess hypoglycemic and hypolipidemic properties [14]. Ethanolic extract of root tubers are documented as anti-ovulatory, anti-implantation, abortifacient, cardioprotective and smooth muscle relaxant agents [15-17]. Thorough literature review indicated that, up till now the pharmacological property of the leaf has not yet been scientifically corroborated. Henceforth, our current study was attempted to investigate the *in vitro* antioxidant and antimicrobial ability of *Momordica cymbalaria* leaves extracts.

MATERIALS AND METHODS

Plant material collection and extraction

The plant material of *Momordica cymbalaria* was collected in mid-November 2012 from Aruppukottai, Virudhunagar District, Tamilnadu and authenticated by Botanical Survey of India, Coimbatore (Ref No. 1081). The voucher specimen has been preserved in the herbarium, Department of Pharmacognosy, SRM College of Pharmacy, SRM University for the future reference.

The leaves were separated from stems and shade dried at room temperature. The dried plant leaves were coarsely grounded using a blender and then comminuted in a hammer mill to attain 30-40 mesh size and subsequently packed in a high-density airtight polyethylene pouch until further use. The pulverized plant leaves 950 g was subjected to successive solvent extraction process using a wide range of polarity indices menstrums such as hexane (defatting purpose), ethyl acetate and hydroalcoholic azeotropic mixture (ethanol and water) in the ratio of 9:1 for 72 h by steady state

successive cold maceration process until the solvent became colorless. All the miscellas were concentrated by distilling off the solvent in a rotary flash evaporator followed by lyophilization. The extract was stored in a refrigerator. The extractive values of ethyl acetate and hydroalcoholic extracts were found to be 19.60% w/w and 12.41% w/w respectively with respect to the air-dried plant.

Chemical reagents

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical, α-tocopherol, sodium nitroprusside, sulfanilamide, naphthyl ethylenediamine dihydrochloride (NEDD), Curcumin, reduced nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), EDTA were purchased from Sigma-Aldrich. Dimethylsulfoxide, trichloroacetic acid, ammonium acetate, acetylacetone, mannitol, ferrozine, potassium ferricyanide, Folin Ciocalteau, gallic acid, aluminium chloride, quercetin were purchased from Rankem India Ltd. All other chemicals and solvents used were of analytical grade.

Microorganisms and media

Klebsiella pneumoniae (Gram-positive bacteria), *Escherichia coli* (Gram negative bacteria), and fungi *Aspergillus niger* were obtained from Department of Biotechnology, SRM University. The bacterial, fungal stock cultures were maintained on Muller Hinton agar and Martin Rose Bengal agar mediums and stored at 4°C.

Preliminary phytochemical analysis

The preliminary phytochemical screening of the ethyl acetate extract of *Momordica cymbalaria* (EAMC) and hydroalcoholic extract of *Momordica cymbalaria* (HAMC) was performed to detect the presence of primary metabolites carbohydrates, proteins as well as secondary metabolites alkaloids, phenolics, flavonoids, steroids, triterpenes, saponins and glycosides by the regular standard protocol [18]. Quantitative determination of detected phytoconstituents was also carried out for EAMC and HAMC extracts [19].

DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radical was determined by the slightly modified method [20]. A portion of sample solution (100µl) of different concentrations (10, 20, 40, 60, 80,100µg/ml) was mixed with 1 ml of 5.25 X 10^{-5} M DPPH radical in methanol. Decrease in absorbance of the tested mixture was monitored for every 1 min for 30 min at 516 nm using a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. DPPH solution was used as a blank and α-tocopherol was used as a positive probe. The DPPH radical solution was freshly prepared, stored in a flask covered with aluminium foil and kept in dark at 4°C between measurements. The radical scavenging capability of the tested samples expressed as a percentage inhibition of DPPH was calculated according to the formula.

$$\% \text{ Inhibition} = \frac{\text{Ao} - \text{A}}{\text{Ao}} * 100$$

Where A_0 is the absorbance of the control and A is the absorbance of the plant extracts.

Nitric oxide scavenging assay

Nitric oxide radicals are generated from aqueous sodium nitroprusside solution which on interaction with oxygen at physiological pH yields nitrite ions. The quantification of nitrite ions are performed by Griess Ilosvay reaction which involves diazotization followed by coupling reaction [21]. The components in the reaction mixture are 10 mM sodium nitroprusside (SNP), phosphate buffered saline (pH 7.4) and different concentrations (10, 20, 40, 60, 80,100µg/ml) of the plant extracts in a total volume of 3 ml and incubated for 150 min at 25°C. To 0.5 ml of the incubated solution, about 1 ml of sulfanilamide in the concentration of 0.33% in 20 % glacial acetic acid was mixed and left at room temperature for 5 min. To the above solution, 1 ml of naphthyl ethylene diamine dihydrochloride (NEDD) (0.1% w/v) was added and the reaction mixture was incubated for 30 min at 25°C where pink color develops whose absorbance at 540 nm was measured spectrophotometrically against blank sample. All tests were performed in triplicates. Curcumin was used as a reference.

Superoxide anion scavenging activity

Superoxide anion scavenging capacity of plant extracts was evaluated as per the following method [22]. In this assay the generation of superoxide radicals takes place by the addition of 1 ml of 16 mM, pH 8 Tris-HCl buffer containing 50µM NBT, 78µM NADH and several concentrations (10, 20, 40, 60, 80,100µg/ml) of extracts. The reaction was commenced by adding 10µM PMS solutions to the mixture, incubated at 25°C for 5 min, and finally the absorbance at 560 nm against the reagent blank was measured. Quercetin was used as a reference compound. All the tests were performed in triplicates, averaged and then converted into percentage inhibition of superoxide anion generation.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging ability was evaluated as per the protocol [23]. Different concentration of samples (10, 20, 40, 60, 80,100µg/ml) were taken in different test tubes. 1 ml of Fe-Ethylene diamine tetra acetic acid (Fe-EDTA) solution (0.1 %w/v Ferrous ammonium sulphate and 0.26%w/v EDTA), 0.5 ml EDTA (0.018%w/v), 1 ml DMSO in a concentration of 0.85 %v/v in 0.1 mol/L phosphate buffer of pH 7.4 were poured to these tubes. The reaction was commenced by adding 0.5 ml of 0.22%w/v ascorbic acid. Test tubes were tightly sealed and heated on a water bath at 90°C for 1 min. The reaction was stopped by the addition of 1 ml of cold trichloroacetic acid (TCA) (17.5%), 3 ml of Nash reagent (ammonium acetate 75 g, glacial acetic acid 3 ml and 2 ml of acetylacetone were amalgamated and raised to 1 L with methanol) was added to all of the test tubes and left at room temperature for 1 min for color development. The mixture was centrifuged at 5000 rpm for 5 min. The yellow color intensity was estimated spectrophotometrically at 412 nm against the blank. Mannitol was used as a reference in this case.

Metal chelating ability

The ability of the plant extracts to chelate the ferrous ions was estimated. Extracts of different concentrations ranging from (10, 20, 40, 60, 80,100µg/ml) were added to a solution of 0.05 ml, 1 mM $FeCl_2$. By the addition of 0.1 ml, 1 mM ferrozine the reaction was initiated on vigorous shaking. The resultant mixture was left aside for 10 min. The optical density of the same was then measured spectrophotometrically at 562 nm. EDTA was used as classical metal chelator. All analyzes were done in triplicates and averaged [24]. The percentage inhibition of ferrozine-Fe^{2+} complex formation was estimated by comparing with blank and test samples.

Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteau method [25], based on the reduction of a phosphor-wolframate, phosphomolybdate complex by phenolics to a blue coloured reaction product. 1 ml of sample solution (20-100µg/ml) was diluted with 100 ml of distilled water and then 5 ml Folin-Ciocalteau reagent, previously diluted 2 times, was mixed. After 5-10 min, a 15 ml of 20% solution of sodium carbonate was added and an obtained solution was diluted to 100 ml. The prepared sample solutions were allowed to stand for 2 h at room temperature and then the absorbance was measured at 765 nm. The data were calculated according to the standard curve of gallic acid (0.01-0.20 mg/ml) and they were expressed as gallic acid equivalent (GAE) per gram of dry extract.

Total flavonoid content

The total flavonoid content was assessed with aluminium chloride ($AlCl_3$) using standard quercetin [26]. To 1 ml of varying concentrations (20-100µg/ml) of the plant extracts, 0.3 ml of distilled water was added with subsequent addition of $NaNO_2$ (0.03 ml, 5%w/v). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 5%w/v) was mixed. Further after 5 min, the resultant content was treated with 0.2 ml of 1 mM NaOH. To the reaction mixture, 1 ml of water was added and then the absorbance was spectrophotometrically measured at 510

nm. All tests were done in triplicates. The flavonoid content was calculated from a quercetin standard curve.

Determination of reducing power

The ability of the extracts to reduce $FeCl_3$ solution was evaluated [27]. A 2.5 ml aliquot was mixed with 2.5 ml aliquot of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The resultant mixture was incubated at 50 °C for 20 min and then 10% of 2.5 ml trichloroacetic acid were added. This mixture was centrifuged for 10 min at 3000 rpm. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was estimated at 700 nm and ferric reducing power was calculated using ascorbic acid as the standard. Higher the absorbance of the reaction mixture indicates greater the reductive potential.

Antimicrobial activity

The extracts of *Momordica cymbalaria* leaves were screened for their *in vitro* antimicrobial activity by disc diffusion method [28]. Gram-positive bacteria *Klebsiella pneumoniae,* Gram-negative bacteria *Escherichia coli, Aspergillus niger* fungi were used as test organisms against standard antibiotics Ofloxacin and Griseofulvin. Muller Hinton agar and Martin Rose Bengal agar mediums were prepared, sterilized and poured into petriplates up to a depth of 3 mm. The microbes were suspended in saline, spread on those plates and wells were made using an 8 mm cork borer. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100µg/ml. To each well, 100µl of each extract was added, incubated at 37°C for 24 h for bacteria and at room temperature for 48 h for fungi. Finally, the results were recorded by measuring the diameter of a zone of inhibition surrounding the well. Standard antibiotic discs of 6 mm diameter such as Ofloxacin and Griseofulvin were used and the whole experiment was done in triplicates.

Statistical analysis

All measurements were run in triplicates. The experimental data were expressed as mean ± SEM. Two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test was carried out to determine significant differences ($p < 0.001$) between the means by using Graph Pad Prism version 5 software, San Diego, CA.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical reports of EAMC and HAMC extracts of *Momordica cymbalaria* leaves revealed the presence of secondary metabolites such as alkaloids, phenolics, flavonoids and steroid contents. The other remaining primary metabolites (proteins and carbohydrates) and secondary metabolites such as triterpenes, saponins and glycosides were least in both the extracts. The quantitative determination of detected phytoconstituents in the EAMC extract also indicated significantly dominant presence of alkaloid, phenolic, flavonoid and steroid contents ($p < 0.05$) compared to HAMC. Due to the polarity differences of the extracting solvents, slight quantity variation exists in the detected phytochemical constituents. Several phytochemical related literature reviews clearly highlights that phenolic compounds and flavonoids are potent inhibitors of free radicals and exhibits significant antioxidant activities [29]. The observed antioxidant property of EAMC and HAMC extracts are mainly due to the possession of phenolic and flavonoid contents. The results were compiled in table 1.

Table 1: Quantitative determination of phytoconstituents in the EAMC and HAMC extracts

Phytochemicals	Concentration (%)
Alkaloids	3.18±0.08
Phenolics	2.38±0.06
Flavonoids	1.75±0.03
Steroids	1.46±0.07
Triterpenes	ND
Saponins	0.18±0.02
Glycosides	ND

N=3±SEM; ND-Not Detected. *Significant differences $p < 0.05$

DPPH radical scavenging activity

Free radicals cause autoxidation of unsaturated lipids in food whereas, antioxidants are believed to break up the free radical chain of oxidation and donate hydrogen thereby forming a stable end product, which does not propagate further lipids oxidation. DPPH is a relatively stable free radical which when encounters proton donors such as anti-oxidants, it gets quenched and the absorbance decreases. Antioxidant reacts with DPPH and converts it to α,α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the plant extracts. The DPPH radical scavenging abilities of EAMC and HAMC were increased in a concentration-dependent manner compared to positive probe α-tocopherol. Both the extracts were capable of neutralizing the DPPH free radicals via hydrogen donating ability and thereby inhibit the propagation phase of lipid oxidation. The output of the data foreshowed that, EAMC and HAMC exhibited strong percentage scavenging effect of 85.14 ± 0.22 and 65.43 ± 0.43 at a concentration of 100µg/ml respectively. It was further observed that, EAMC gave results on par with standard α-tocopherol. Results indicated higher scavenging activity of the EAMC followed by HAMC extract towards DPPH radicals in comparison with α- tocopherol. The phenolic compounds in the *Momordica cymbalaria* leaves extracts are directly proportional to its free radical scavenging ability since phenols act as antioxidants due to their hydrogen donating properties [30]. Presence of flavonoids also possesses higher antioxidant activity because of their structure and hydroxyl group arrangement. The phenolic contents and flavonoids presence could be responsible for the DPPH radical scavenging capability.

Nitric oxide radical scavenging activity

Sustained levels of production of nitric oxide radicals alter the structure and function of many cellular components leading to various pathological disorders. However, excess production of nitric oxide is associated with several diseases like adjuvant arthritis, cancer [31-32] etc. Nitric oxide (NO) free radical scavenging activity of the extracts was studied by using Griess reagent. The nitric oxide generated from aqueous sodium nitroprusside on reaction with oxygen produces nitrite ions. Antioxidants compete with oxygen leading to reduced production of nitric oxide and thereby inhibit nitrite formation [33]. The scavenging activity results revealed that, EAMC possessed potent ability by depleting nitric oxide radicals with percentage inhibition of 69.98 ± 0.24 whereas HAMC displayed considerable scavenging effect of 52.20 ± 0.29 compared with the standard curcumin. The *Momordica cymbalaria*leaves have potentials for counteract the effect of nitric oxide formation and thereby it helps to arrest the chain of reactions that are detrimental to human health.

Superoxide anion radical scavenging activity

The superoxide radical is a highly toxic species which is generated by numerous biological and photochemical reactions. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical. Hence, scavenging of superoxide radicals would be promising remedy for some oxidative damage related diseases. Superoxide radicals are generated from dissolved oxygen by the phenazine methosulfate-nicotinamide adenine dinucleotide a non-enzymatic system converts nitro blue tetrazolium (NBT) to a purple color formazan. Antioxidants are able to inhibit the formation of blue NBT [34]. The superoxide scavenging effects of EAMC, HAMC and reference quercetin exhibited concentration-dependent activity. It has been documented that antioxidant properties of some phenolic compounds are effective via scavenging of superoxide anion radical. The decrease in the absorbance of 560 nm with the extracts indicates the consumption of super oxide anion in the reaction mixture. Amongst the extracts, it was observed that EAMC showed superior scavenging percentage potential of 54.71 ± 0.28 than HAMC (46.70 ± 0.11).

Hydroxyl radical scavenging activity

In biological system, the hydroxyl radical is the most extremely reactive oxygen centered radicals and it has been implicated as a highly damaging species in free radical pathology, and also highly responsible for producing deleterious effects in the living cells which

in turn cause severe damage to the lipids and proteins. This radical has the ability to join with nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, this hydroxyl species is regarded to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids. Thus, removing hydroxyl radical is very important for the protection of living systems. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radical is formed by the oxidation reaction dimethyl sulfoxide (DMSO) to yield the formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent [35]. Since phenolic compounds are good electron donors, they may accelerate the conversion of H_2O_2 into H_2O [36]. In this assay system, EAMC and HAMC served as good antioxidants by eliminating the generated hydroxyl radicals. The output records illustrated that EAMC have a maximum percentage scavenging potency of 60.41 ± 0.04 but slightly less than the standard mannitol. The hydroxyl radical scavenging efficacy of the extracts in a concentration-dependent manner may be likely due to phenolics and neutralization of these radicals by abstracting hydrogen atoms thereby reduces to water [37-38]. The report reveals that both the extracts can be utilized as a good hydroxyl radical scavenger.

Table 2: Effects of EAMC and HAMC extracts on various free radical scavenging assays

Sample	Concentration (µg/ml)	Percentage of inhibition (%)			
		DPPH	Nitric oxide	Superoxide	Hydroxyl
EAMC	100	85.14 ± 0.22[a]	69.98 ± 0.24[a]	54.71 ± 0.28[a]	60.41 ± 0.04[a]
	80	81.98 ± 0.28[a]	64.17 ±0.19[a]	46.73 ± 0.07[a]	55.64 ± 0.09[a]
	60	78.25 ± 0.24[a]	56.82 ± 0.16[a]	42.16 ± 0.17[a]	50.37 ± 0.13[a]
	40	53.06 ± 0.38[b]	49.34 ± 0.27[b]	36.87 ± 0.04[b]	44.02 ± 0.10[b]
	20	43.41 ± 0.48[b]	41.44 ± 0.36[b]	31.31 ± 0.14[b]	36.91 ± 0.28[b]
	10	30.93 ± 0.48[c]	32.26 ± 0.22[c]	25.94 ± 0.47[b]	31.24 ± 0.07[b]
HAMC	100	65.43 ± 0.43[a]	52.20 ± 0.29[a]	46.70 ± 0.11[a]	39.43 ± 0.11[a]
	80	43.06 ± 0.45[b]	38.31 ± 0.23[c]	34.57 ± 0.21[b]	33.46 ± 0.12[a]
	60	30.97 ± 0.31[c]	28.72 ± 0.24[c]	20.58 ± 0.18[b]	27.56 ± 0.08[a]
	40	25.57 ± 0.54[c]	21.09 ± 0.34[c]	11.35 ± 0.40[c]	20.55 ± 0.08[b]
	20	17.45 ± 0.70[c]	16.37 ± 0.19[c]	7.44 ± 0.33[d]	16.73 ± 0.09[b]
	10	12.37 ± 0.67[c]	11.49 ± 0.28[c]	2.61 ± 0.30[d]	11.59 ± 0.10[b]
α-tocopherol	100	85.75 ± 0.12	-	-	-
Curcumin	100	-	85.25 ± 0.21	-	-
Quercetin	100	-	-	82.49 ± 0.12	
Mannitol	100	-	-	-	85.15 ± 0.18

Values are mean of three replicates ± SEM, values with a, b, c, d superscripts differ significantly ($p < 0.001$).

Metal chelation activity

Iron is an extremely reactive metal which will catalyze oxidative changes in lipid, protein and other cellular components. Moreover, liposome peroxidation and oxidative damage of protein model systems are induced by a Fenton reaction in which ferrous ions catalyze the conversion of hydrogen peroxide to hydroxyl radical with the production of ferric ion. Fe^{2+} also causes the production of oxyradicals and lipid peroxidation thereby minimizing its concentration in Fenton reactions thus affords protection against oxidative damage. Though metal chelating agents are not antioxidants, they are involved in the stabilization of fatty acids against rancidity. The reagent ferrozine forms a violet complex with Fe^{2+}. The existence of chelating agents in the antioxidant prevents the complex formation and thus reduces the complexity of violet color. The results summarized that the Ferrozine-Fe^{2+} complex formation is considerably disrupted in the presence of EAMC and HAMC extracts with the percentage inhibition of 66.04 ± 0.210 and 48.47 ± 0.147 respectively as compared with EDTA. Further the reports also suggested that both extracts showed strong iron chelating capacity. This metal chelation property is mainly due to the reduction in transition metal concentration that catalyzes lipid peroxidation (fig. 1). Iron chelators mobilize tissue iron by forming soluble stable complexes that are excreted in urine and faeces. Chelation therapy reduces iron-related complications in humans and thereby reduces dreadful diseases. The transition metal, iron is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease. In this assay, both the extracts and EDTA interfered with the Ferrozine-Fe^{2+} complex formation suggesting that the extracts have chelating activity. Chelating agents are effective as secondary antioxidants because they reduce the redox potential stabilizing the oxidized form of the metal ion. It was also reported that flavonoids can conjugate metals thus preventing the metal catalyzed free radical formation. Both metal chelating property and radical scavenging ability of flavonoids may functions as Fenton reaction inhibitors [39]. The higher total phenolic and flavonoid contents might lead to the higher reductive potential of the extract.

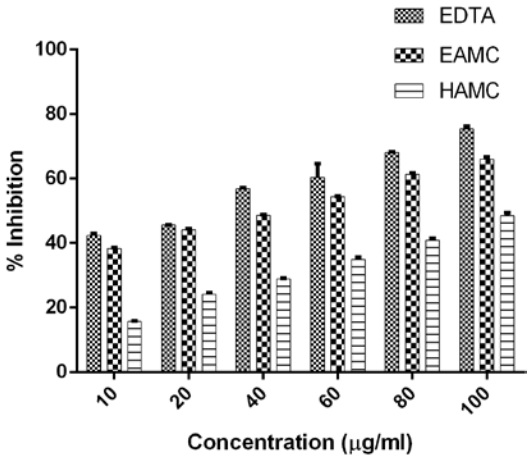

Fig. 1: Metal chelation activity. Effects of EAMC and HAMC plant extracts and the standard EDTA on ferrozine-Fe^{2+} complex formation

Determination of total phenolic and flavonoid content

The total phenolic content was 0.5551µg/ml and 0.2305µg/ml gallic acid equivalent per 1 mg of EAMC and HAMC extracts. The total flavonoid content of EAMC and HAMC extracts was 0.6666µg/ml and 0.1154µg/ml quercetin equivalent per 1 mg of plant extracts. This is clearly elicited in fig. 2. A significant variation in the phenolic and flavonoid contents exists between EAMC and HAMC extracts. Hence,

ethyl acetate solvent would be worthwhile for extracting phenolic and flavonoid contents. Phenolic compounds may contribute directly to antioxidant action because their hydroxyl groups confer free radical scavenging ability. Flavonoids, natural secondary metabolites of plants are regarded as potent antioxidants with numerous health promoting activities [40]. The mechanism of action of the flavonoid may be through free radical scavenging or chelation effect since flavonoid compounds showed oxygen scavenging as well as chelating activity. The antioxidant properties of phenolic compounds are mainly due to the redox properties which play a key role as free radical scavengers, reducing agents and complex of pro-oxidant metals [41].

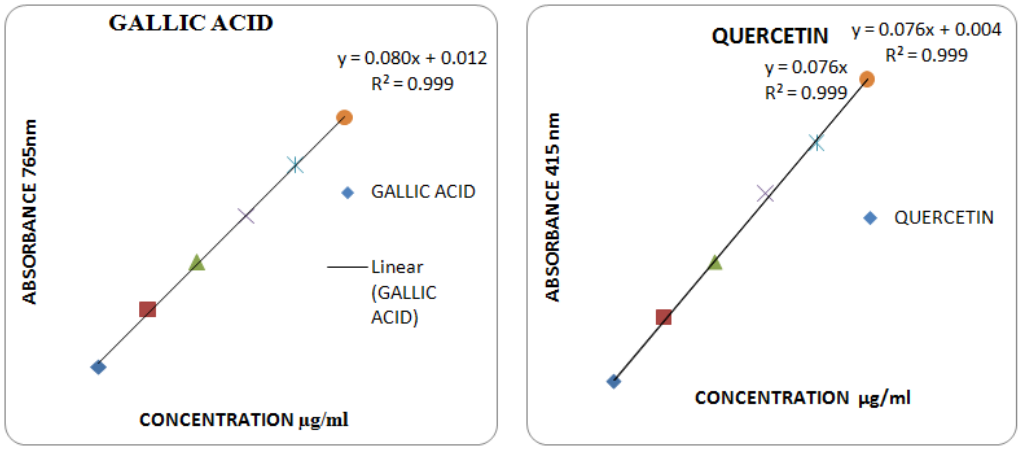

Fig. 2: Total phenolic and flavonoid contents of EAMC and HAMC extracts using calibration curve of gallic acid and quercetin as standards in triplicates

Reducing power assay

Measurement of reductive potential is an important parameter to measure the antioxidant activity of the plant extracts. The reducing property is associated with the presence of reductants. The antioxidant action of reductants depends on the breaking of free radical chain by donating hydrogen atoms. Reductants also react with certain precursors of peroxide, thus preventing peroxide formation. The mechanism of this assay involves the reduction of ferric to ferrous ions resulted in colour change in chromophore from yellow to bluish green. Moreover, the colour intensity of the chromophore relies on the redox potential of antioxidant compounds in the *Momordica cymbalaria* leaves extracts.

The reducing power of EAMC, HAMC and the reference compound ascorbic acid increased steadily with increasing concentration. It is an indicator of electron donating activity, considered to be an important mechanism of antioxidant activity [42]. The reducing powers of EAMC and HAMC were 2.83 and 2.02 at a dose of 1mg showing that they can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions. The superior reducing power, of EAMC than HAMC might be due to the high phenolic content (fig. 3). The results also showed a significant correlation between total phenolics and reducing power of the *Momordica cymbalaria* leaves extracts (EAMC r^2=0.9563 and HAMC r^2=0.8892). Transition metals are known to play key roles in lipid peroxidation in both biological and food system. In particular, reaction of ferrous iron with hydrogen peroxide generates the hydroxyl radicals, which are the most reactive and detrimental reactive oxygen. In this assay, the antioxidant activity is determined on the basis of the ability to reduce ferric (III) iron to ferrous (II) iron, this value reflects the reducing power. It was found that EAMC had maximum reductive potential as measured by ferric ion reduction but inferior to that of ascorbic acid.

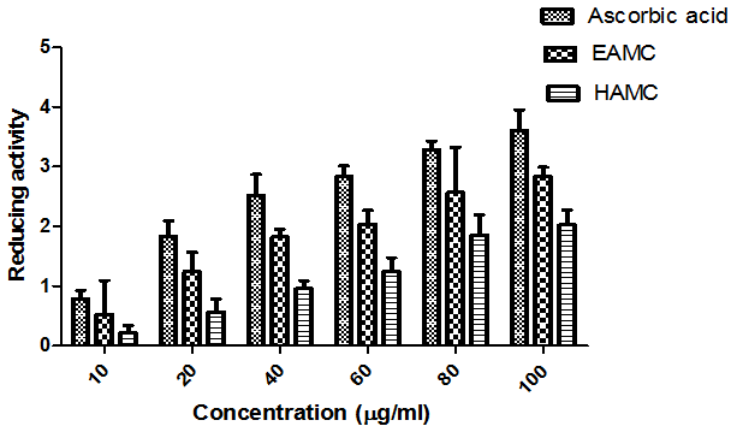

Fig. 3: The reductive abilities of EAMC and HAMC plant extracts and the standard ascorbic acid

The output of overall results evokes that comparatively EAMC extract possessed a superior scavenging potency than HAMC and hence ethyl acetate is regarded as the most propitious menstrum for separating extractable matter phenolics and flavonoids responsible for antioxidant activity. Phenolic compounds may contribute directly to antioxidant action because their hydroxyl groups confer free

radical scavenging ability and flavonoids through chelation effect. The antioxidant mechanism behind such criterion is ascribed partly to the possession of phenolic compounds, flavonoids, higher reducing power, electron transferring ability or radical trapping capacity.

Antimicrobial study

The results of antimicrobial profile were depicted in table 3. By visualizing the antimicrobial activity index, it was noticed that both the extracts have shown moderate to good inhibitory activity against all the tested organisms. EAMC and HAMC extracts showed the maximum zone of inhibition against gram-positive bacteria strain *K. pneumoniae.* Comparatively both the extracts exhibited stronger inhibitory activity against fungal strain *Aspergillus niger* than bacterial strains. Moreover, the inhibitory zone of extracts was equipotent to that of standard Griseofulvin. The antimicrobial mechanism of this plant extracts is yet to be identified.

Table 3: Antimicrobial effects of EAMC and HAMC extracts on tested pathogens

Plant extracts and standard	Antibacterial activity Zone of inhibition (mm)		Antifungal activity Zone of inhibition (mm)
	K.pneumonia	*E. coli*	*A. niger*
EAMC	19 ± 0.46	17 ± 0.73	34 ± 0.95
HAMC	18 ± 0.64	15 ± 0.38	30 ± 0.82
Ofloxacin	39 ± 1.04	38 ± 0.98	-
Griseofulvin	-	-	36 ± 0.79

Values are mean inhibition zone (mm) ± SEM of three replicates

CONCLUSION

The antioxidant effects of the plant extracts have been attributed by the multiple mechanisms due to the complex nature of the phytoconstituents and hence the antioxidant evaluation using any single analytical protocol seems to be rather impractical. Therefore, a systematic investigation of *Momordica cymbalaria* leaves extracts through various *in vitro* antioxidant models is necessary to make a valid judgment. The results in the current study have clearly revealed that EAMC and HAMC extracts could effectively scavenge various ROS and DPPH free radicals in a dose-dependent manner under *in vitro* condition. Most of the herbal antioxidant active compounds are phenolics and flavonoids. Plant phenolics mainly functions as chain breakers, free radical scavengers and electron donors capable of reducing the oxidative damage associated with cardiovascular disease, cancer and atherosclerosis. Moreover, flavonoids are well known as powerful antioxidants. Further the extraction with ethyl acetate showed higher antioxidant followed by hydro alcoholic solvent. Both extracts showed varying degree of antioxidant activity in each assay in a dose-dependent manner. The high phenolic, flavonoid content, free radical inhibition, ferric reducing power and metal chelating effects indicated stronger antioxidant activity of EAMC followed by HAMC extract. This might be the major factor responsible for the promising antioxidant activity of EAMC than HAMC extract. Therefore, it can be concluded that *Momordica cymbalaria* leaves extracts are the potential source of free radical scavengers and strong antioxidants. Hence further investigation, to elucidate the exact antioxidant phytoconstituents and to screen there *in vivo* antioxidant activity is underway in our laboratory. This revelation paved the way for exploration of natural antioxidants and this on clubbing with newly emerging metabolomics technology could help disease prevention using simple herbs. The antimicrobial activity data clearly expressed that *Momordica cymbalaria* leaves extracts have great potential as antimicrobial compounds against harmful pathogens. Thus, they could be utilized as an effective drug for the treatment of infectious diseases caused by resistant microbes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

REFERENCES

1. Bauer V, Sotnikova R, Machova J, Matyas S, Pucovsky V, Stefek M. Reactive oxygen species induce smooth muscle responses in the intestine, vessels and airways and the effect of antioxidants. Life Sci 1999;65:1909-17.
2. Bae GU, Seo DW, Kwon HK, Hong S, Lee ZW, Ha KS, *et al*. Hydrogen peroxide activates p70(S6K) signaling pathway. J Biol Chem 1999;274:32596-602.
3. Oyedemi SO, Bradley G, Afolayan AJ. *In vitro* and *in vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr J Pharm Pharmacol 2010;4:70-8.
4. Ara N, Nur H. *In vitro* antioxidant activity of methanolic leaves and flowers extracts of *Lippa alba.* Res J Med Med Sci 2009;4:107-10.
5. Chen HX, Zhang M, Xie BJ. Components and antioxidant activity of polysaccharide conjugate from green tea. Food Chem 2005;90:17-21.
6. Sanja Cavar, Milka Maksimovic. Antioxidant activity of essential oil and aqueous extract of *Pelargonium graveolens*. Food Control 2012;23:263-7.
7. Tim Cushnie TP, Andrew Lamb J. Antimicrobial activity of flavonoids Review. Int J Antimicrob Agents 2005;26:343-56.
8. Marjorie MC. Plant product as antimicrobial agents. Clin Microbial Rev 1999;12:564-82.
9. Nadkarni KM. Nadkarni AK. Indian Materia Medica; 1994.
10. Kameswara Rao B, Kesavulu MM, Giri RR, Appa Rao C. Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. Fruit powder in alloxan-diabetic rats. J Ethnopharmacol 1999;67:103-9.
11. Bharathi Dhasan P, Jegadeesan M, Kavimani S. Cucurbitacians isolated from the fruits of *Momordica cymbalaria*. Pharmacogn Mag 2008;14:96-101.
12. Koneri R, Balaraman R, Saraswati CD. Antiovulatory and abortifacient potential of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl in rats. Indian J Pharmacol 2006;38:111-4.
13. Togunashi VS, Venkataram BS, Yoganarasimhan SN. Discussion on the origin and identification of Kuduhunchi-A hitherto unknown Ayurvedic Drug, introduced by Rajanarahari. J Res Indian Med Yoga Homoeopathy 1977;12:118-21.
14. Kameswara Rao B, Kesavulu MM, Apparao C. Evaluation of antidiabetic effect of *Momordica cymbalaria* fruit in alloxan-diabetic rats. Fitoterapia 2003;74:7-13.
15. Kameswara Rao B, Kesavulu MM, Apparao C. Antihyperglycemic activity of *Momordica cymbalaria* in alloxan-diabetic rats. J Ethnopharmacol 2001;78:67-71.
16. Koneri R, Balaraman R, Vinodh K, Hariprasad M. Cardio protective effect of *Momordica cymbalaria* Fenzl. In rats with Isoproterenol-induced myocardial injury. J Clin Diagn Res 2008;2:699-705.
17. Govinda Das D, Devasankaraiah G. Some pharmacological studies with *Momordica tuberosa*. Indian J Pharmacol 1981;13:94.
18. Khandelwal KR. Practical Pharmacognosy.19thed. Nirali Prakashan; 2009.
19. El-Olemy MM, Al-Muhtadi FJ, Afifi AA. Experimental phytochemistry. King Saud University press; 1994.
20. Braca A, De Tommasi N, DiBari L, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis.* J Nat Pro 2001;64:892-5.
21. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant activities of Iranian Corn silk. Turk J Biol 2008;32:43-9.
22. Fontana M, Mosca L, Rosei MA. Interactions of enkephalins with oxyradicals. Biochem Pharmacol 2001;61:1253-7.
23. Singh RP, Chidambara Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. J Agric Food Chem 2002;50:81-6.
24. Shymala BN, Gupta S, Lakshmi AJ, Prakash J. Leafy vegetable extracts-antioxidant activity and effect on storage stability of heated oils. Innov Food Sci Emerg 2005;6:239-45.

25. Singleton CL, Rossi JA. Colorimetry of total phenolics with phosphor molybdic-phospho tungstic acid reagents. Am J Enol Viticul 1965;16:144-58.
26. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64:555-9.
27. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the auto oxidation of soyabean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945-8.
28. Greenwood, Slack CB, Peutherer TT. Medical Microbiology, a guide to microbial infections, pathogenesis, immunity, laboratory diagnosis and control. Churchill Livingston; 2002.
29. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 1983;32:1141-8.
30. Tung YT, Wu JH, Huang YH, Ku Chang ST. Antioxidant activities and phytochemical characteristics of extracts from Acacia confuse bark. Bioresour Technol 2009;100:509-14.
31. Gibanananda Ray, Syed Akhtar Husain. Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol 2002;40:1213-32.
32. Ialenti A, Ianora A, Mancoda S. Modulation of acute inflammation by endogenous nitric oxide. Eur J Pharmacol 1993;211:177-82.
33. Garratt DC. The quantitative analysis of drugs. Chapman and Hall Ltd; 1964.
34. Koryeka Dahl M, Richardson M. Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and aminoacids. J Diary Sci 1978;1:400-7.
35. Singh RP, Chidambara Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. J Agric Food Chem 2002;50:81-6.
36. Spencer JPE, Jenner A, Aruoma OI, Kaur H, Dexter DT. Intense oxidative DNA damage promoted by L-Dopa and its metabolites, implications for neurodegenerative disease. FEBS Lett 1994;353:246-50.
37. Auroma OI. Free radicals and international nutrition. Asia Pacific J Clin Nut 1999;8:53-63.
38. Yen G, Du PJ. Scavenging effects of methanolic extracts of peanut hulls on free radical and active-oxygen species. Agric Food Chem 1994;42:629-32.
39. Gua Q, Zhao B, Li M, Shen S, Xin W. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. Biochem Biophys Acta 1996;1304:210-22.
40. Duthie GG, Dutie SJ, Kyle JAM. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev 2000;13:79-106.
41. Bhutkhup L, Samappito S. *In vitro* free radical scavenging and antimicrobial activity of selected thai medicinal plants. Res J Med Plant 2011;5:254-65.
42. Yildrim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumexcrispus* L extracts. J Agric Food Chem 2001;49:4083-9.