ABSTRACT

A plant-based dietary health food is a rich source of multiple phytochemicals along with a high-class of natural antioxidant molecules. Consumption of natural antioxidants through foods and drinks prepared particularly with lofty herbs and spices not only promotes the quality of health and life, but also protects against chronic oxidative stress-related (OSR) diseases or disorders.Herbs and spices are included among the common food adjuncts which have been used as flavouring, seasoning and colouring agents, and sometimes as preservatives throughout the world for thousands of years. Use of herbs and spices in cooking is the oldest form of aromatherapy that stimulates gastric secretion and creates appetites, creates positive moods, stimulates the body, relieves cold symptoms and respiratory problems, and eases muscle pains. The active components in herbs and spices are considered as powerful means to create a state of wellness.

In the present study, twelve commonly used herbs and spices namely *Mentha piperita*, *Trigonella foenum-graecum*, *Coriandrum sativum*, *Murraya koenigii*, *Glinus oppositifolius*, *Foeniculum vulgare*, *Illicium verum*, *Myristica fragrans*, *Ceiba pentandra*, *Capsicum annuum*, *Parmelia perlata* and *Dregea volubilis* were selected and evaluated their biological activities. Plant samples were cleaned, dried and powdered and extracted with hot water and methanol, followed by lyophilization to obtain lyophilized aqueous and methanolic extracts respectively.

Preliminary phytochemical screening to detect the presence or absence of some significant phytochemicals viz. phenols, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, terpenes, steroid, etc. were performed according to standard protocols. Phytochemical analysis revealed that phenol, flavonoid, reducing sugar, free amino acids, tannins were detected in all plants tested; anthraquinone was absent in *I. verum*,*C. pentandra* and *P. perlata*; triterpenoid was not detected in *C. sativum*, *T. foenum-graecum* and *M. fragrans*; cardiac glycosides were detected in all samples except *T. foenum-graecum*, *I. verum* and *C. pentandra*; alkaloid was detected in all the test plants; saponin was absent in *C. sativum*; steriod was absent in *C. annuum*; phlobatannin was present only in *P. perlata* and *D. volubilis* and cardenolide was presen only in *D. volubilis*.

Samples were evaluated for the total soluble sugar content, reducing sugar content, soluble protein content and total lipid content, total phenol, flavonoid and flavonol content along with vitamin C and E. *G. oppositifolius* and *I. verum* had highest amount of total phenol, total flavonoid, total flavonol, total sugar and vitamin C. Highest amount of reducing sugar and total and vitamin E was present in *G. oppositifolius* and *I. verum*. Protein content was found to be highest in *M. koenigii*, lowest in *G. oppositifolius*.

Quantity of different plant pigments like total chlorophyll content and total carotenoid content was estimated and found highest in *G. oppositifolius* for both the pigments. Carotenoid content was lowest in *M. piperita*.

Extraction of the samples with methanol revealed higher yield than hot aqueous which may be due to the higher solubility potential of phytochemicals in methanol. Amongst the samples, *P. perlata* showed the highest yield.

Analysis of *in vitro* antioxidant activities of the extracts, DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, superoxide anion scavenging activity and nitric oxide radical scavenging activity were performed. It was found that, all herb and spice samples showed antioxidant activity at different levels. Among samples, *I. verum* and *G. oppositifolius* showed the highest antioxidant activity as well as phytochemical components. In all the cases, gradual rise in the activity with the increase in the concentration was observed but it was insignifcantly different to each other in case of majority of herb extracts.

Antimicrobial activity of methanolic extracts of different herbs and spices against both Gram positive bacteria (*Bacillus cereus* MTCC 10665 and *Bacillus pumilus* MTCC 1684) and Gram negative bacteria (*Serratia marcescens* NCBI GENBANK no.JN020963 and *Pseudomonas aeruginosa* MTCC 2453 by disc-agar diffusion method.*B. cereus* and *B. pumilus* were inhibited only by the *M. piperita* and *T. foenum-graecum* extracts at higher doses. No inhibition was observed by the other herbal extracts and against other test organisms. The MID value of *M. piperita* extract against *B. cereus* and *B. pumilus* was found to be 8.5 and 5.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID value of *T. foenum-graecum* extracts against *B. cereus* and *B. pumilus* was determined as 3.5 and 7.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID values of other herb extracts against respective organisms were found to be >10 mg lyophilized methanolic extract $disc^{-1}$. Among the spice extracts, extract of *I. verum* was found to be most potent showing

highest zone of inhibition against all test organisms, whereas *D. volubilis* did not show antibacterial activity against microorganisms except *B. cereus*. The MID value of *I. verum* extract against *B. cereus, B. pumilus, S. marcescens* and *P. aeruginosa* was found to be 1.25, 2.5, 3.5 and 1.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. *P. perlata* also found to be active against both Gram positive and Gram negative bacterial strains. The MID value of *P. perlata* extract against *B. cereus, B. pumilus, S. marcescens* and *P. aeruginosa* was found to be 2.0, 2.0, 3.0 and 2.0 mg lyophilized methanolic extract $disc^{-1}$ respectively. *M. fragrans* and *C. annuum* were also effective against all microorganisms at comparatively higher doses.

Anti-quorum sensing activities of different herbs and spices evaluated through preliminary screening for inhibition of violacein synthesis by whole plant parts. Among the plant tested *I. verum* was found to be most potent in inhibiting the violacein production in *C. violaceum*, followed by *P. perlata*. Methanolic extract of *I. verum* was also able in reducing the virulance phenotypes such as pyocyanin synthesis, ptotease production, swarming motility and biofilm formation in *Ps. aeruginosa*.

Evaluation of *in vivo* anti-diabetic activity was performed in Streptozotocin-induced rats using *I. verum* and *G. oppositifolius* methanolic extracts as they were traditionally reported as antidiabetics. The methanolic extracts were reconstituted in sterile distilled water and used to determine the toxicity and pharmacological effects on rats. Before performing *in vivo* assay, the crude extracts were tested for their acute toxicity at a concentration of 2000 mg kg^{-1} BW and analyzed the lethal and safer doses of extracts. For anti-diabetic assay 500 mg kg^{-1} BW and 250 mg kg^{-1} BW doses were selected as safer and non-toxic. Streptozotocin-induced diabetic rats treated orally with both the sample extracts and Metformin were able to reverse the diabetic conditions to near normal. Various biological markers such as fasting blood sugar level, cholesterol, triglycerides liver enzymes (SGPT and SGOT), serum urea and creatinine were reduced to nearly normal level while significant increase in body weight and HDL-cholesterol level was observed in compared to the diabetic controls. Among the plant extracts,*I. verum* extract (*Iv*ME)showed comparatively better *in vivo* antidiabetic activity than *G. oppositifolius* extract(*Go*ME). This may due to various antidiabetic compounds present in the extract.

Further, characterization of bioactive compounds present in the fractions of *I. verum* (*Iv*ME) and *G. oppositifolius* (*Go*ME) were performed by GC-MS analysis. GC-

MS profiling revealed the presence of myriad of chemical compounds including volatile compounds, phenolics, terpenoids, fatty acids, phytosterol etc. Many of them are reported to have antidiabetic and antimicrobial properties. Some of the compounds identified through GC-MS analysis of methanolic fraction of *I. verum* were Linalool, Estragole, n-Hexadecanoic acid; Benzaldehyde, 4-methoxy-; Benzene, 1-methoxy-4-(1-propenyl)-; cis-Vaccenic acid; 2-Propanone, 1-(4-methoxyphenyl)-; Benzhydrazide, 4-methoxy-N2-(2-trifluoroacetylcyclohepten-1-yl)-; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene; Octadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-. The chemical compounds identified through GC-MS analysis of hexane fraction of *I. verum* were Benzaldehyde, 4-methoxy-; Anethole; Anisaldehyde dimethyl acetal; 2-Propanone, 1-(4-methoxyphenyl)-; 4-(p-Methoxyphenyl)-1-butanol; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; n-Hexadecanoic acid; cis-Vaccenic acid; (2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-;4-Methoxy-benzoic acid N'-[2-(4-methoxy-phenyl)-acetyl]-hydrazide and Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)-. Some of the compounds identified through GC-MS analysis of ethyl acetate fraction of *I. verum* were Linalool; Estragole; Benzene, 1-methoxy-4-(1-propenyl)-; 2-Propanone, 1-(4-methoxyphenyl)-; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene; n-Hexadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel- and cis-Vaccenic acid.GC-MS analysis of different fractions of *G. oppositifolius* also revealed different types of chemical compounds. Compounds identified through GC-MS analysis of methanolic fraction of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; 1-Deutero-2,2,5,5-tetramethylcyclopentanol; n-Hexadecanoic acid; Phytol; 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydro cyclopropa [d]-naphthalene;(4aS,8S,8aR)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-exahydronaphthalen-2-yl and Retinol, acetate. Compounds identified through GC-MS analysis of hexane fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; Mome inositol; Hexadecanoic acid, methyl ester, n-Hexadecanoic acid; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Phytol; Linoelaidic acid; Octadecanoic acid; Squalene and Chondrillasterol. Chemical compounds identified through GC-MS analysis oethyl acetate fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; DL-Proline, 5-oxo-, methyl ester; Mome inositol; n-Hexadecanoic acid; Phytol; Lanosterol; Lup-20(29)-en-28-ol; Beta.-copaen-

4 .alpha.-ol and Retinol, acetate. Many of the identified compounds are reported to have versatile bioactivities. Compounds such as cis-1,2-Dihydrocatechol, trans linalool oxide, estragole, benzaldeyde, 3-methoxy and several others have antimicrobial, antioxidant and antidiabetic activities.

Hence, all the herb and spice samples tested showed nutritional and antioxidant potential confirming their nutraceutical and medicinal properties. But among them *I.verum* and *G. oppositifolius* were found to possess highest scavenging activities, good amount of phytochemicals, thus should be explored for nutraceutical and pharmacological applications.