SUMMARY

Five popular herbs and spices of Iran/India, namely borage, valerian, dry ginger, dry lime and shallot were selected for study. All samples were evaluated for their *in vitro* and *in vivo* nutritional and antioxidant potential.
Chemical composition, antioxidant component and activity were determined using standard procedures.

1. CHEMICAL COMPOSITION

- Among all samples borage had highest protein content (8.10 g), soluble fiber (49.9 g), ash (9.2 g) and phosphorus (395 mg). Valerian was highest in insoluble fiber (79 g), total carotenoids (132.7 g), Fe (272 mg), Zn (4.8 mg), Cu (2.96mg), Mn (11.47 mg) and Cr (249 mg/100g dw). Lime showed highest Vitamin C content (88.10 mg/100 g dw). Shallot had highest carbohydrate (61.7 g/100gdw). Among all samples fat content was highest in ginger (4.37 g/100gdw).

2. *IN VITRO* ANTIOXIDANT POTENTIAL

- In borage, ginger and lime, all examined antioxidant components were found to be highest in 100°C aqueous extract and the least content were observed in acetonic extract whereas in valerian, the highest total polyphenol content was observed in 80% methanolic extract, total tannin in 100°C water extract and flavonoids in 30°C water extract. The least components were observed in acetonic extract. In shallot, also 100°C water extract had highest antioxidant components. Except total polyphenols which were not found in methanolic extract, other two components (tannin and flavonoids) showed least content in acetonic extract. The highest flavonoids content was seen in whole lime followed by borage and shallot showed the least flavonoids. In all different extracts, between five tested samples, tannin content of borage was more than other samples. It also showed the highest polyphenols compared to other samples.
The highest total antioxidant activity of borage and lime was observed in 100°C followed by 30°C aqueous extract, valerian in 80% methanolic and 100% methanolic extract, ginger in methanolic and ethanolic extract and shallot in 100°C aqueous extract followed by 80% methanolic extract. Except in shallot (ethanolic extract) all samples, showed least antioxidant activity in acetonic extract. Among all samples shallot followed by borage showed the highest total antioxidant activity. Borage showed highest reducing power properties and free radical scavenging activity in hot water and 30°C water extract. In valerian and ginger both showed highest reducing power properties in 80% methanolic and 80% ethanolic extract and highest FRSA in 80% methanolic extract. In lime, higher reducing power and FRSA was seen in 80% methanolic extract followed by 100°C aqueous extract. Shallot showed the highest activity in both method (FRSA and reducing power) in 100°C and 30°C aqueous extract. All samples except shallot, exhibited the least ability to reduce ferric to ferrous. Results showed that shallot, in alcoholic extract did not show reducing power but among extracts with reducing power ability, acetonic extract, exhibited the least power. The order of reducing power ability was seen as follows: Borage> valerian> ginger> lime> shallot. It was also observed that, similar to reducing power, all samples showed least activity in acetonic extract and shallot did not show FRSA in alcoholic extract. Except for valerian and ginger, all samples showed high correlation between antioxidant components and all three method of antioxidant activity in water extracts at two temperature ($R^2=1$). In valerian only flavonoids showed negative correlation with antioxidant activity and ginger showed positive correlation only between polyphenol and antioxidant activity.

In solvent extracts, except valerian and ginger, all samples showed correlation with DPPH and antioxidant components. Polyphenol and total tannin showed correlation with reducing power in all samples except shallot. In valerian only the tannin content had correlation with reducing power and total antioxidant activity.
3. **ANTIBACTERIAL ACTIVITY**

Antibacterial activity of all samples was evaluated with in both aqueous and methanolic extract against 3 gram positive and 4 gram negative pathogenic bacteria and zone of inhibition was expressed in mm. It was observed that:

- Among samples only shallot and lime exhibited antibacterial potential in both aqueous and methanolic extract.
- Antibacterial activity of aqueous extract of shallot and lime were found to be 26.5 and 19.0 mm against *E. coli* whereas in methanolic extract it was 25.0 mm for both shallot and lime.
- Aqueous extract of shallot and lime exhibited a zone of 33.5 and 23 mm and methanolic extract showed 25.0 mm inhibition in both shallot and lime against *staphylococcus aureus*.
- *Bacillus subtilis* was inhibited with aqueous extract of shallot and lime by 29.0 and 28.5 mm and methanolic extract by 32 and 31 mm respectively.
- *Ps. aeruginosa* inhibition was exhibited in aqueous extract of shallot and lime by 21.5 and 23.5 mm. In methanolic extract inhibition was 22.5 and 23.5 mm in shallot and lime respectively.
- Aqueous extract of shallot and lime showed a zone of 29.5 and 18.5 mm against *Kleb. pneumoniae* whereas methanolic extract had a zone of 24 and 25 mm respectively.
- *Bacillus cereus* was inhibited by aqueous extract of shallot and lime by 23.5 and 18.5 mm whereas methanolic extract exhibited a zone of 25 and 27 mm respectively.
- *Sal. typhi* was inhibited by 32.5 and 30.5 mm in aqueous extract of shallot and lime and 25 and 24 mm in methanolic extract of shallot and lime respectively.
- MIC of samples was found to be in the concentration of 5, 10, 25 and 50 µl/well.
4. Effect of borage supplementation on antioxidant status of Diabetic patients

Subjects with NIDDM without complications were selected for study and divided to two groups of experimental and control. Five hundred gram dry petals of borage and placebo were given to each group respectively. Serum TAC, total thiol and lipid peroxidation, food recall and blood parameters were determined.

- Supplementation of 500 mg of dry petals of borage in diabetic patients showed remarkable increase in antioxidant status of patients and significantly reduced lipid peroxidation level. In control group, which received placebo, no differences were observed after one month intake of placebo capsules.
- Serum total thiol molecule of diabetic subjects significantly increased after one month of borage supplementation but no difference was observed in control group.
- Dietary survey of diabetic patients showed that male subjects in both experimental and control group had inadequate intakes of energy, protein, iron, carotenoids, riboflavin and niacin in comparison with RDA values, whereas intake of fat and calcium were found to be more than RDA. Thiamin and vitamin C intakes were slightly lesser than recommendations. Female subjects were found to take lesser energy, iron and carotenoids than RDA for reference Indian woman. In both experimental and control group, protein, Vitamin C and thiamin were found to meet recommendations and niacin and riboflavin intakes were found to be very close to RDA value.
- Based on WHO’s recommendation (30g/day) of dietary fiber intake in both experimental and control group, dietary intake were lesser than recommended intake.
- Blood glucose determination of diabetic subjects showed that in both experimental and control group, there was no significant difference in FBG, PPBG and Hb\textsubscript{a1}.
- Lipid profile of subjects showed that a highly significant reduction was seen in cholesterol level, triglyceride and VLDL of study group patients after 30 days supplementation (P values: 0.001, 0.0008 and 0.002 respectively).
• No significant difference was observed in liver function test and renal test of both study and control group.

5. Effect of shallot supplementation on antioxidant status of cigarette smokers

For evaluation of antioxidant potential of shallot in cigarette smokers, 40 healthy subjects were selected for study. Subjects, who smoked minimum 5 cigarettes per day since last 3 years in the age group between 25-40 years, were selected for the study. Subjects were assigned in 2 groups of 20 (study group and control group). Control group received placebo (rice flour) whereas study group received 500 mg shallot capsules twice a day. Antioxidant activity, lipid peroxidation and total thiol was measured in serum of subjects before and after supplementation.

• A highly significant increase was observed in total thiol in smokers after a month of shallot supplementation (P=0.000) whereas there was no significant change in placebo group (P=0.439).

• Negative correlation was observed between No. of cigarettes per day and serum total thiol indicating that when the number of cigarettes smoked per day increases, serum total thiol decreases (R²=-0.130).

• Serum lipid peroxides were found to be 4.9 and 4.79 at the beginning of the study in experimental and control group respectively. After 30 days supplementation, estimation was repeated again and found to be 4.3 and 4.71 in study and control group respectively. Changes were found to be highly significant in experimental group (P=0.000) whereas in control group it was not significant (P=0.05).

• A negative correlation was observed between total thiol and lipid peroxidation, which shows that, when oxidative stress is more, the total thiol molecule reduces due to the oxidation (R² = -0.369).

• Correlation between serum lipid peroxidation and No. of cigarette per day were found to be positive R² = (0.235).

• A significant increase was observed in TAC of serum in experimental group but changes in control group were not significant.
Slightly positive correlation was observed between serum total antioxidant capacity and total thiol molecule. A negative correlation was seen between serum total antioxidant capacity and No. of cigarettes smoked per day, whereas correlation between serum total antioxidant capacity and lipid peroxidation level and between TAC and total thiol molecule was found to be positive.

6. Influence of petals of borage on eight different mood states in adult human subjects

One hundred subjects aged between 25-35 years were asked to answer questionnaire at day 0 and 30. All subjects were asked to take one borage capsules containing borage petals (500 mg) per day for 30 days. Subjects with at least 5 mood states in high score were considered as experimental group and subject with at least 5 stated in average score, were considered in control group.

Experimental group showed a sten score of 7.55 for anxiety at the day 0. It was observed that the score was reduced to 6.75 at day 30. Sten score of stress was changed from 7.22 to 6.24 and 5.16 to 4.75 in experimental and control group respectively. The score of depression reduced in experimental group from 6.71 to 6.45 whereas very small difference was observed in control group (5.1 to 5.0). Score of regression initially was 6.43 which reduced to 6.22 after treatment. In control group it reduced from 5.68 to 5.52. Score of fatigue reduced from 5.37 to 5.10 in experimental group. In control group the score was found to be almost similar (5.45 to 5.43). Results showed that score of guilt significantly reduced in experimental group from 6.88 to 6.2. In control group, the scores were in the average range and did not change significantly after 30 days supplementation. Level of extraversion increased from 3.91 to 4.68 in experimental group and from 5.25 to 5.45 in control group. P value of 0.000 shows highly significant changes in experimental group whereas control group showed marginally significant difference in score of extraversion before and after the study. Score of arousal were improved from 4.33 to 4.73 in experimental group and in control group sten score was found to be very close to each other (5.31 to 5.25). In experimental
group score of arousal were found to be significantly different after 30 days supplementation (P=0.001) but significant difference was not observed in control group (P=0.284).

- Significant changes were observed in experimental group but in control group, difference was either not significant, or marginally significant.

7. Effect of valerian and Ginger supplementation on animal weight gain and food intake

To study the effect of valerian and ginger on weight gain, food intake and blood parameters, 40 adult wistar rat were selected and divided to 5 group of 8. Corn starch, 3.0 mg ginger, 6.0 mg ginger, 3.0 mg valerian and 6.0 mg valerian was administered to control and experimental group respectively. Food intake and weight gain was measured and at the end of the study, animals were sacrificed and blood and 4 vital organs were collected for further analysis.

- Significant weight reduction in both level of ginger group (3.0 and 6.0 mg) was observed compared to control group whereas in valerian group there was a significant weight gain after 30 days of treatment with both level of valerian (3.0 and 6.0 mg).

- Food intake of animals for duration of 30 days was; control group, 598 g, 3.0 mg and 6.0 mg ginger 3, 597 and 595 g and 3.0 and 6.0 mg valerian group 606 and 616 g respectively. Statistically there were no significant difference in food intake in control and ginger group whereas in valerian groups food intake increased significantly.

- The feed efficiency of the both valerian group, was higher than control and both level of ginger group whereas food intake in both ginger group was lesser than control group.

- Four vital organs (spleen, pancreas, liver and kidney) were measured. Liver weight was found to be 7.5, 7.3, 8.3, 7.3 and 7.7 g in control, group 2, 3, 4 and 5 respectively.
• Weight of spleen was found to be in the range of 0.42 to 0.48 g, pancreas 1.002-1.22 g and kidneys, 1.65-1.76 g. When statistically analyzed, there was no significant difference between control group and other groups, in any organ weight.

• Blood analysis namely; complete blood count, liver function test, renal function test, lipid profile and blood electrolytes were done and observed that there was no significant difference in any of the tested blood parameters between control and study groups.

• In histology study changes were not observed in tissue of liver, pancreas, spleen, and kidney compared to control group.
CONCLUSION

The present study evaluated the nutritional and antioxidant properties of five herbs and spices namely borage, valerian, ginger, shallot and lime.

Borage (*Echium amoenum*) is a popular Iranian herb used in the form of decoction to reduce anxiety and stress. Also, it is commonly used in common cold and viral fever. The analysis of proximate composition showed that borage contained the highest protein, soluble fiber and phosphorous compared to other selected herbs and spices. A very high antioxidant component as well as activity of borage petals in both *in vivo* and *in vitro* studies was also seen. Significant reduction was seen in cholesterol level, triglyceride and VLDL of study group patients after 30 days supplementation and P value was found to be 0.001, 0.0008 and 0.002 respectively. After 30 days borage supplementation there was no significant changes in renal and liver function test of diabetic patients. This can demonstrate the safety of selected dose.

We did not observe antibacterial potential against 7 pathogenic bacteria in both aqueous and methanolic extract of borage.

For the first time the effect of borage on mood state was evaluated on human subject and the results clearly confirm the traditional belief of anxiolytic potential of borage in improving mood states.

It can be concluded that borage can serve as nutraceuticals and can be used to enhance mood state in stress full life. And due to high antioxidant power, it can be used to reduce damage to cell wall due to free radicals in body. Also supplementation to diabetic patients can reduce complications of diabetes mellitus.

Valerian (*Valerian officinalis*) is over the counter medicinal plant which is used as sleep aid medicine worldwide since long ago. It was found that valerian is quite good source of minerals like iron, zinc, copper, manganese and chromium, compared to other selected samples. The present study reported relatively low antioxidant potential of valerian. It did not show antibacterial potential against selected (7) bacteria. Study on the effect of valerian on food intake and weight gain of wistar rat, stated that 15mg/kg and 30 mg/kg
of valerian can significantly increase weight gain of rats. It was observed that food intake of the animals were significantly increased. There were no changes in blood parameters like lipid profile, liver function test, blood electrolyte and complete blood count.

Ginger is widely used all over the world and is common and popular spice in Asian countries specially in India. In present study, it was observed that ginger fat content was higher than other samples. Relatively high antioxidant potential was observed in 7 different extract of ginger. Antibacterial activity of dry ginger in both aqueous and methanolic extract was not seen against tested bacteria though studies showed antibacterial potential in fresh ginger. The present study shows the anti-obesity potential of ginger. Ginger intake in laboratory wistar rat caused a weight reduction though the food intake was not reduced.

Whole lime is used in dry form in Iran for seasoning the food. Traditionally, it is known to be useful in infectious disease, anti-aging, anti cancer and to reduce blood pressure and blood cholesterol. We observed high antioxidant component as well as activity in whole dry lime in, in vitro determination, which is positively correlated with the traditional belief of protection against aging and cancer. It was observed that although it is in dry form, it still retained a high vitamin C content. We observed a high antibacterial potential of lime against all 7 selected bacteria in both aqueous and methanolic extract.

Shallot is used in Iran for seasoning and flavoring food. It is believed to have anticancer potential and useful in infectious disease. We observed high total antioxidant activity and high flavonoid content in, in vitro study. In vivo study of antioxidant potential of shallot showed a high antioxidant potential in cigarette smokers. Shallot also demonstrated antibacterial potential in all 7 tested bacteria which supports the traditional observation of protection against infections.

It can be stated that the herbs and spices investigated in present study have different degree of antioxidant potential, antibacterial activity and demonstrated nutraceutical properties.