CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2014 Vol. 3 (1) January-March, pp.18-25/ Prasad et al.
Research Article

PHYTOCHEMICAL SCREENING AND IN-VITRO EVALUATION OF ANTI-OXIDANT AND ANTI-BACTERIAL PROPERTIES OF MEDICINAL PLANTS

***Prasad M.P[1], Soumya M.[2] and Brinda[2]**
[1]*Sangenomics Research Lab, Domlur Layout, Bangalore 560071, India*
[2]*M.S. Ramaiah College for Arts, Science and Commerce, Bangalore*
** Author for Correspondence*

ABSTRACT
Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have been found to have medicinal properties. Antibacterial properties of various plant parts like root, stem, leaves, seeds, flowers have been well documented for some of the medicinal plants over the past decades. In present investigation some of traditionally used plants as food have been used to determine the antioxidant and antimicrobial properties (*Aegle marmelos* leaf, *Santalum album* leaf, *Allium cepa* leaf, *Citrus limon* leaf, *Piper nigrum* seed, *Syzygium aromaticum* bud, *Cuminum cyminum* seed, *Trigonella foenum-graecum* seed, *Trigonella foenum-graecum* stem, *Hordeum vulgare* seed). These plant extracts were tested for its antioxidant properties and also it antimicrobial activity against clinically isolated human pathogens (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Serratia sps*). It was found that all the plants were positive for presence of phenols, proteins, flavanoids and alkaloids and negative for anthraquinones. Antioxidant studies revealed maximum activity for *Syzygium aromaticum* bud, *Citrus limon* leaf, *Piper nigrum* seed, and *Cuminum cyminum* seed, and was found to be minimum for *Hordeum vulgare* seeds. Antimicrobial activity results showed maximum activity for *Aegle marmelos* leaf, *Citrus lemon* leaf, *Cuminum cyminum* seed and *Allium cepa* leaf extract against *Serratia* sps, *Santalum album* leaf extract against *Staphylococcus aureus.* The Maximum activity was seen for *Syzygium aromaticum* bud and *Piper nigrum* seed extract against all the test pathogens.

Keywords: *Phytochemical Analysis, Antioxidant, Antimicrobial, Medicinal Plants, Pathogens*

INTRODUCTION
Many of the spices and herbs used today have been valued for their antimicrobial effects and medicinal powers in addition to their flavor and fragrance qualities. Herbs and spices have been extensively used as food additives for natural antioxidants. Spices and aromatic herbs are considered to b essential in diets or medical therapies for delaying ageing and biological tissue deterioration (Frankel, 1996). The search for synthetic antioxidants as alternatives to naturally occurring antioxidants is of great interest both in industry as well as in scientific research (Lucia *et al.*, 2002). Plant secondary metabolites are excellent sources of phenolic phytochemicals, especially as antioxidants and antimicrobials. Crude extracts of some well known medicinal plants are used to control plant pathogens. Besides flavoring purposes, spices and herbs have been used also for their medical or antiseptic properties. The preservative effect of many spices and herbs suggest the presence of antioxidative and antimicrobial constituents. The proteins, tannins and reducing sugars are the primary metabolites of plants, necessary for cellular processes while phenol, flavonoid and alkaloids are the secondary metabolic compounds produced in response to stress, such as the case when acting as a deterrent against herbivores.
Antibacterial properties of various plants parts like root, stem, leaves, seeds, flowers have been well documented for some of the medicinal plants for past two decades. The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments. Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer.
The antioxidant property of the plant material is due to the presence of many active photochemical including vitamins, flavonoids, terpinoids, carotenoids, cumarins, curcumins, lignins, saponin, plant

steroids (Madsen *et al.*, 1996; Marja *et al.*, 1999 and Zheng *et al.*, 2001). Latest and previous studies have concluded to the beneficial aspects of the plant derived drugs or good source of antibiotics, antioxidants and anti-inflammatory agents (Matur *et al.*, 2010).

MATERIALS AND METHODS

Trigonella foenum graecum (seeds and stem), *Piper nigrum* (seeds), Syzygium aromaticum (buds), *Cuminum cyminum* (seeds), *Hordeum vulgare* (seeds) samples were collected from local market in Bangalore. *Aegle marmelos* (leaf), *Santalum album* (leaf), *Alium cepa* (leaf), *Citrus limon* (leaf) samples were collected in and around GKVK Agricultural Sciences, Bangalore. Samples were washed with water; shade dried and was powdered in grinder.

The plant materials (10gms) were then taken in Erlenmeyer's flasks and 100ml of methanol, acetone and ethanol was added separately and was kept in shaker at room temperature for 2 days for extraction. The above extracts were further used for phytochemical screening, quantitative analysis, antioxidant activity. The quantitative assay for proteins, tannins, reducing sugars, phenols, flavonoids and alkaloids were followed as per the methodology of Sofowara (1993) Trease and Evans (1989) and Harborne (1973). The acetone and ethanolic extracts was used to check antibacterial activity.

RESULTS

The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves, stem and seeds were rich in alkaloids, flavonoids, tannins and saponins.

Qualitative Analysis

The qualitative assay of the ten medicinal herbs and spices samples were screened for their phytochemical constituents. The result obtained by the methanolic extract possessed all the active constituents except anthraquinones (Table 1).

Table 1: Qualitative analysis of Phytochemicals

Samples	Cardiac glycosides	Terpenoids	Saponins	Tannins	Phenol	Proteins	Anthra-quinones	lignin	Flavonoid	Alkaloids
***Aegle marmelos* leaf**	+	+	-	+	+	+	-	-	+	-
***Santalum album* leaf**	+	+	-	+	+	+	-	-	+	+
***Allium cepa* leaf**	+	+	+	+	+	+	-	-	+	+
***Citrus limon* leaf**	+	+	+	-	+	+	-	-	+	+
***Piper nigrum* seed**	+	+	+	+	+	+	-	-	+	+
***Syzygium aromaticum* bud**	+	+	+	+	+	+	-	-	+	-
***Cuminum cyminum* seed**	+	+	+	+	+	+	-	+	+	+
Trigonella foenum-graecum seeds	+	+	+	+	+	+	-	-	+	+
Trigonella foenum-graecum stem	+	+	-	+	+	+	-	+	+	+
Hordeum vulgare	+	+	+	-	-	+	-	+	+	+

Quantitative Analysis

Proteins was estimated as highest in the plant sample *Citrus limon* and least in barley, while tannin was moderate in pepper and highest in cumin and least in barley. Clove showed the highest total phenolic

content, while barley had the lowest content of phenol. From the present analysis, it can be concluded that most of these plants were moderate in flavonoid content. The highest content was found in *Santalum album* and lowest was obtained in *Cuminmum cyminum*. The alkaloid content from the study reveals that the *Piper nigrum* extract showed the highest content, was moderate in *Santalum album* and *Citrus limon* extracts and lowest in *Allium sepa* and *Aegle marmelos* (Figure 1- 6).

Antioxidant Activity

In the present study the antioxidant activity of the methanolic extracts was initially determined using three different methods DPPH, Reducing Power Ability and Thiobarbutric Acid. The obtained results are comparable with those of the total phenolics and flavanoids indicating that these herbs represent a reliable source of antioxidants (Figure 7-9).

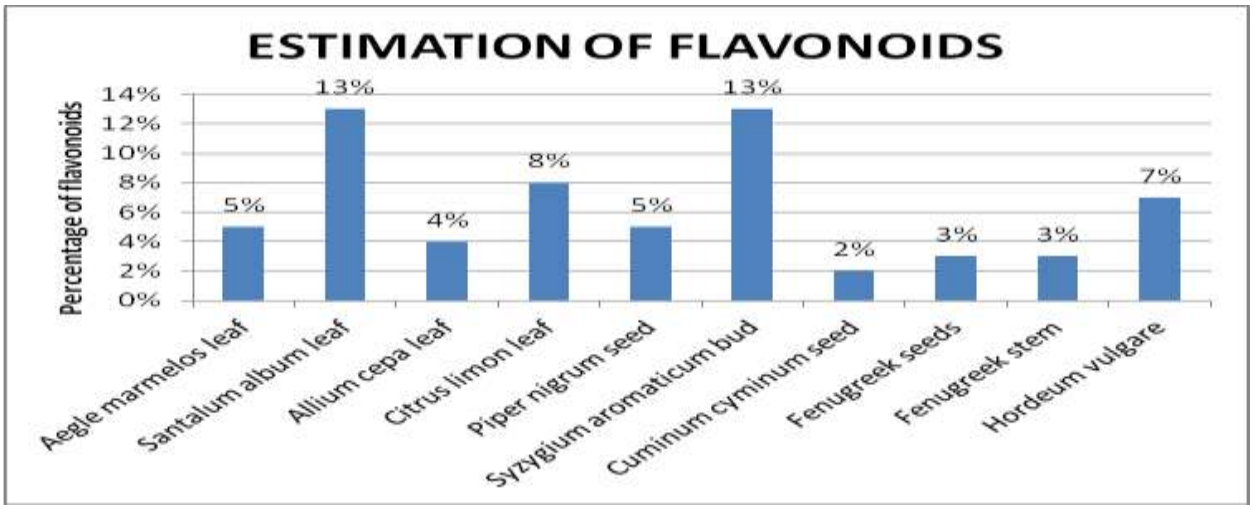

Figure 1

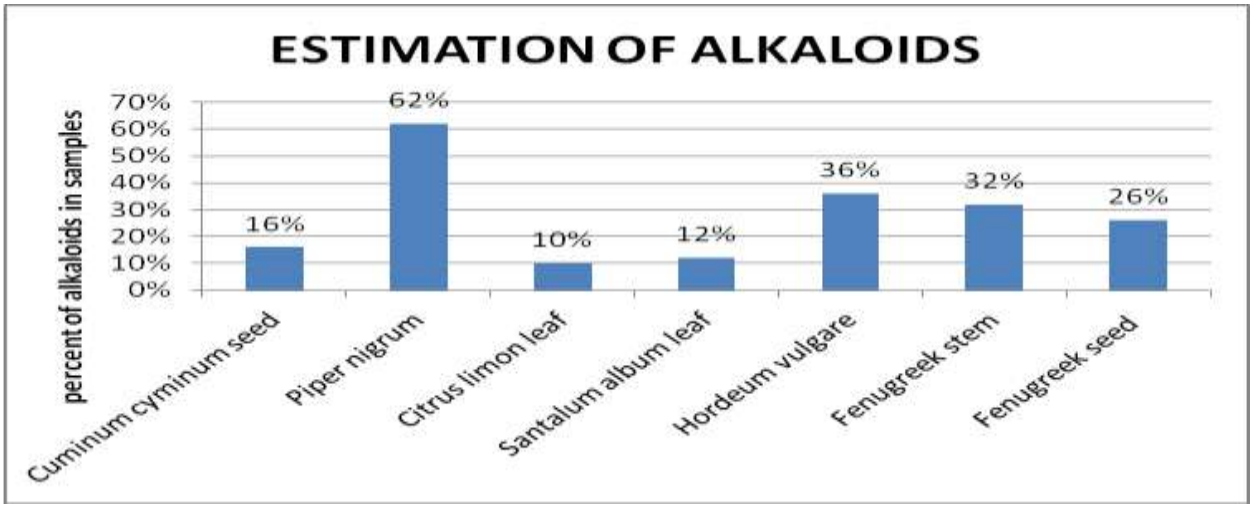

Figure 2

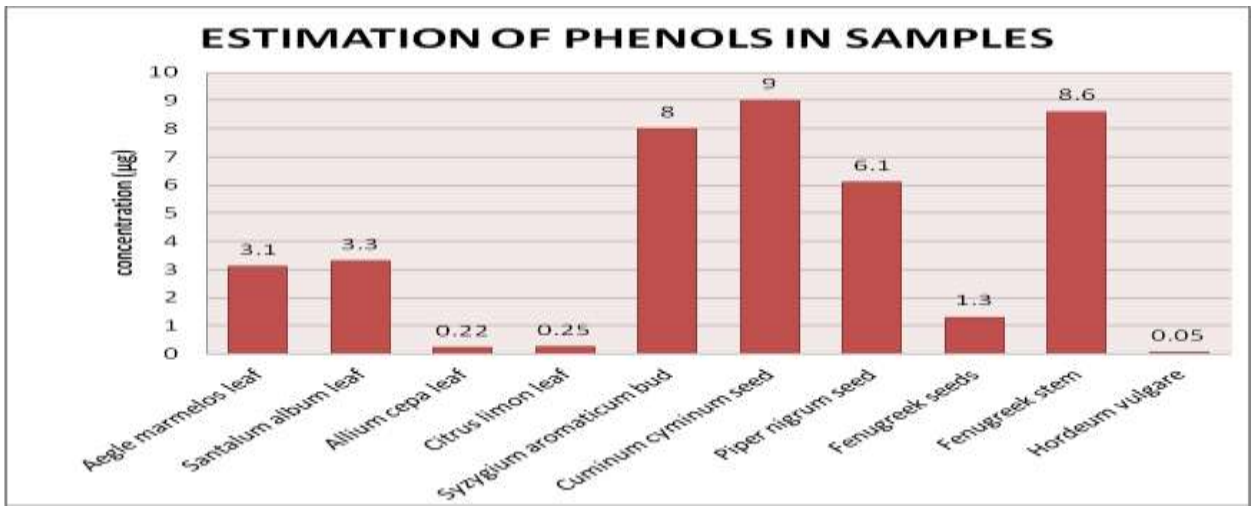

Figure 3

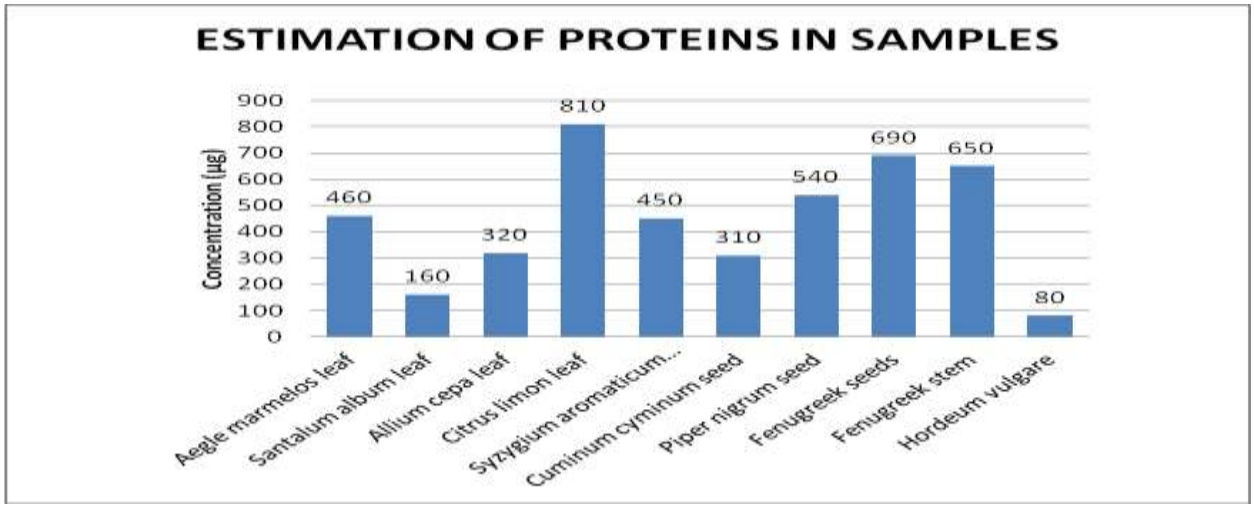

Figure 4

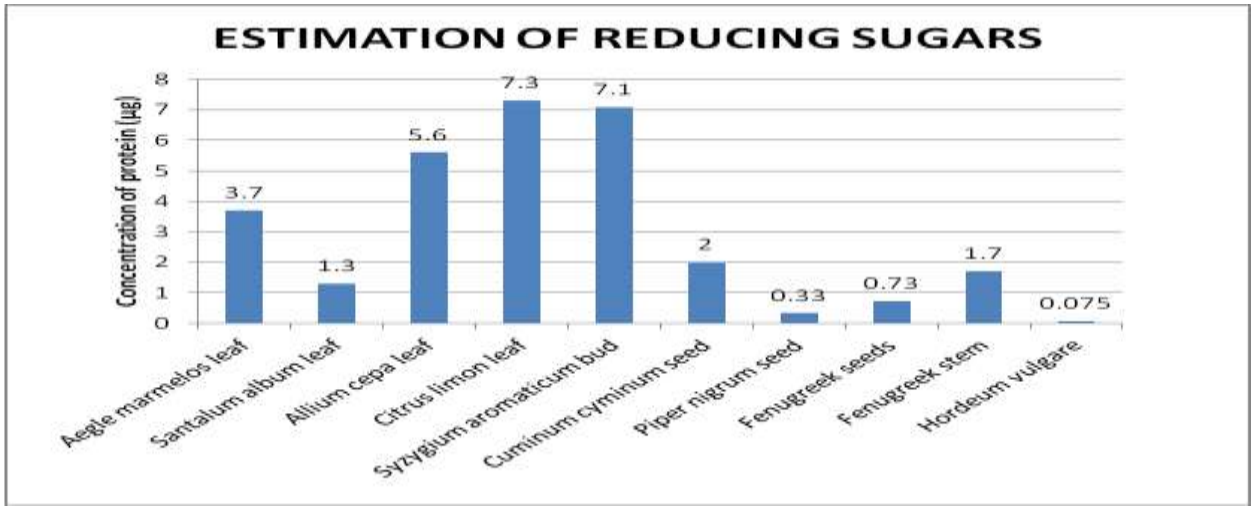

Figure 5

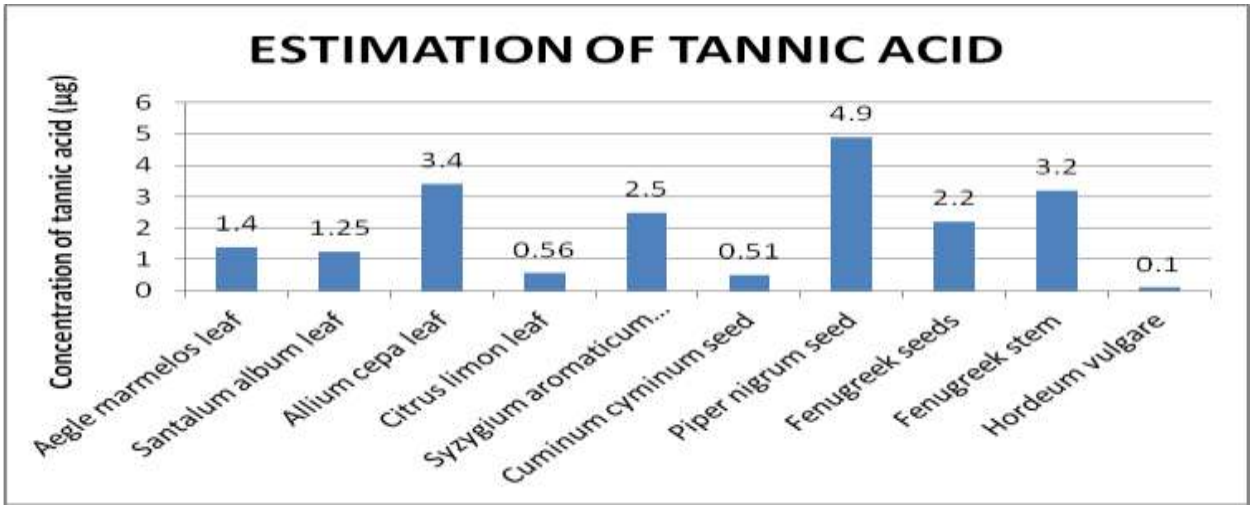

Figure 6

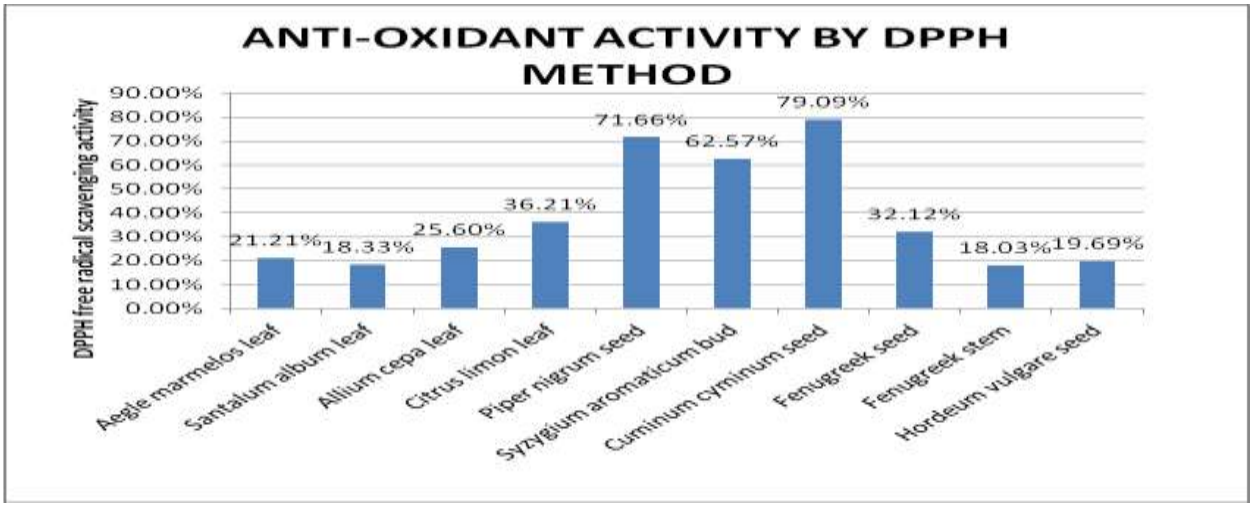

Figure 7

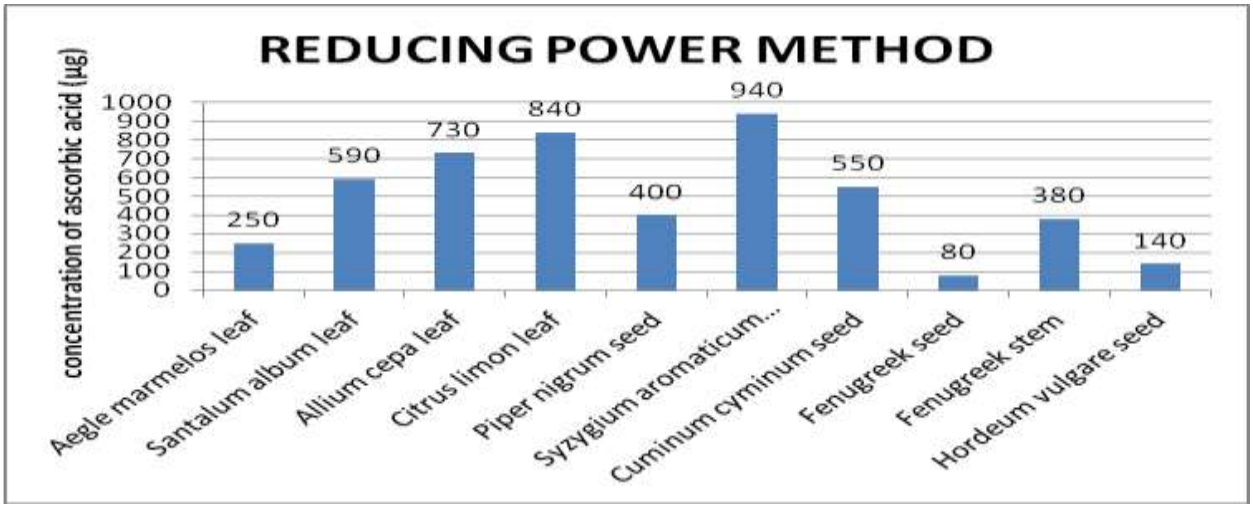

Figure 8

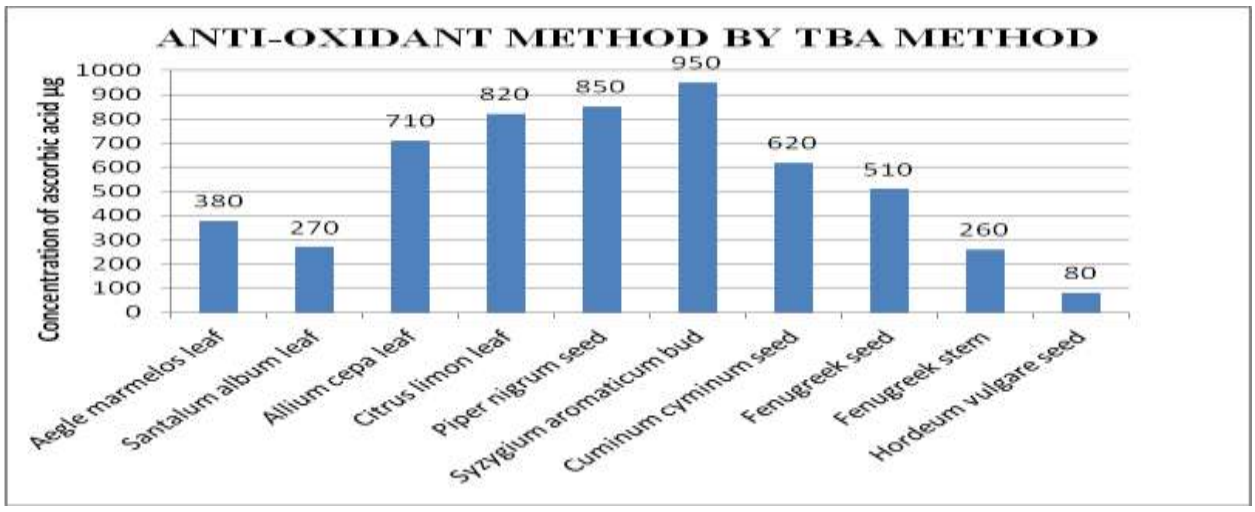

Figure 9

Antimicrobial Activity

According to the results obtained *Syzygium aromaticum* bud showed the maximum inhibition against all the test pathogens and *Piper nigrum* seed against *Staphyloccus aureus* and *E.coli*. *Cuminum cyminum* seed showed maximum activity against *Bacillus subtilis* and *Trigonella foenum-graecum* seeds showed good activity against *Serratia*. No zone formation was seen by *Aegle marmelos* for *Pseudomonas aeruginosa*, *Santalum album* for *E.coli*, *Cuminum cyminum* for *Pseudomonas aeroginosa*, *Piper nigrum* for *Pseudomonas aeroginosa* and *Serratia*, *Trigonella foenm graceum* seeds for *E.coli* and stem for *Pseudomonas aeroginosa*, *Horedeum vulgare* for *E.coli* in acetone extracts. Ethanolic extracts of all the plant samples used did not exhibit any activity (Figure 10).

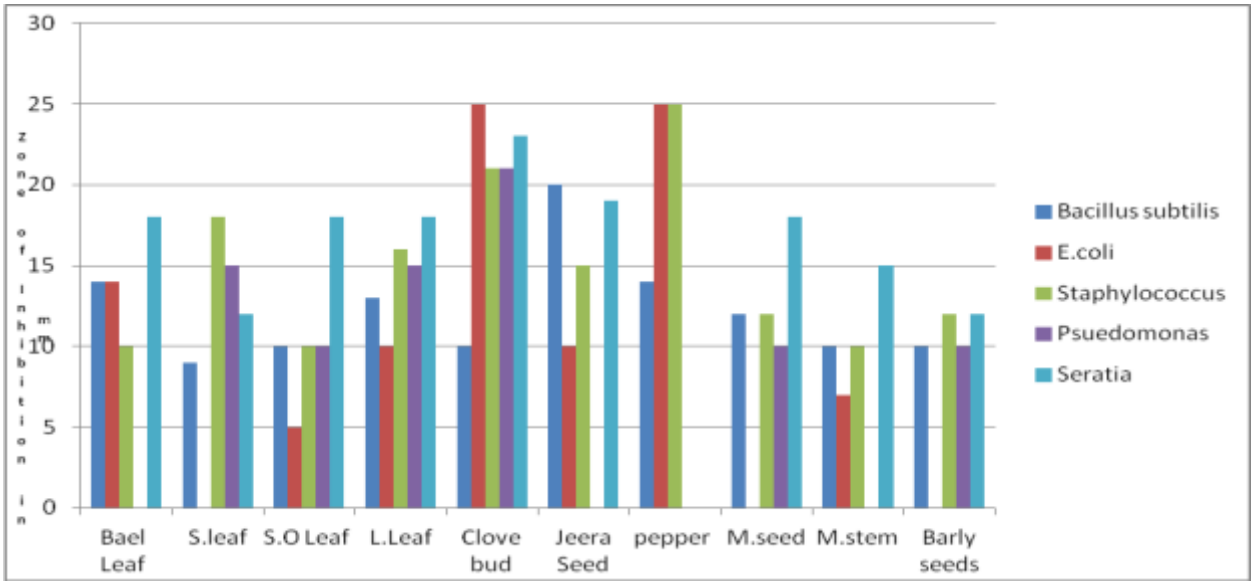

Figure 10: Antimicrobial Activity of Medicinal plant samples against Human Pathogens

DISCUSSION

The qualitative and quantitative assay and also the antioxidant and antimicrobial activity for the ten medicinal herbs and spices samples were screened as described in methodology for their phytochemical constituents.

Phenolics present in medicinal plants have received considerable attention because of their potential antioxidant activity. Similar results were obtained in the present study which indicates the abundance of phenolic compounds in these samples. Methanol and ethanol are proven as effective solvents to extract antioxidant phenolic compounds. In the present study clove showed the highest total phenolic content, while barley had the lowest content of phenol. Wu *et al.*, (2006) have shown that phenolic compounds found in plants, vegetables, spices and herbs possessed antioxidative and antimicrobial activities.

Flavonoids are one of the most diverse and wide spread groups of natural products which are probably the most important natural phenolics. Several flavonoids have been reported to quench active oxygen species and inhibit in vitro oxidation of low density lipoproteins. Kumar *et al.*, (2009) have reported that a variety of plant flavonoids and alkaloids are shown to be anti carcinogenic in several animal models. From the present analysis, it can be concluded that most of these plants were moderate in flavonoid content. The highest content was found in *Santalum album* which was in agreement with the previous study as stated by Dhanaprakash *et al.*, (2007). The lowest was obtained in *Cuminmum cyminum.*

The alkaloid content from the study reveals that the *Piper nigrum* extract showed the highest, and lowest in *Allium sepa* and *Aegle marmelos*. Roberts and Wink (1998) have proved the alkaloids as one of the most diverse groups of secondary metabolites found in living organisms with a wide array of biosynthetic pathway, structural types and even pharmacological activities (Penna *et al.*, 2001).

Medicinal plants are considered to be potential antimicrobial drugs as well as a source of novel compounds such as flavonoids and alkaloids with antimicrobial activity (Parekh *et al.*, 2005). The extracts of the medicinal herbs against the microorganisms examined was assessed by the presence or absence of inhibition zones. According to the results obtained *Syzygium aromaticum* showed the maximum activity against all the test pathogens where as *Piper nigrum* seed against *Staphyloccus aureus* and *E.coli*. *Cuminum cyminum* seed exhibited maximum activity against *Bacillus subtilis* and *Trigonella foenum-graecum* seeds showed good activity against *Serratia*. This is similar to the experiments conducted by Ethan *et al.*, 2003), confirmed that some naturally occurring plant ingredients can kill antibiotic resistant strains of bacteria such as *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus.*

In the present study the antioxidant activity of the methanolic extracts was initially determined using three different methods DPPH, Reducing Power ability and Thiobarbutric Acid. All the three methods mentioned responded with good results. The obtained results are comparable with those of the total phenolics and flavanoids and indicating that these herbs represent reliable source of antioxidants. The results confirm that there is a direct correlation between total phenolic content and antioxidant capacity of the medicinal plants (Compos *et al.*, 2003).

Therefore in comparison to chemical or synthetic additives, herbal additives are preferred as these are safer, act as flavor enhancers and also are without any side effects (Brull and Coote, 1999). In view of this, further study is required from these herbs and spices for their complete spectrum of efficacy. These ethno medical spices and herbal resources or their combinations open the prospect of finding new clinically efficient antimicrobial compounds.

REFERENCES

Brull S and Coote P (1999). Preservative agents in foods: mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology* **50** 1-17

Compos KE, Diniz YS, Cataneo AC, Faine LA, Alves MJ and Nobeli EL (2003). Hypoglcemic and antioxidant effect of onion, Allium cepa: Diatary onion addition, antioxidant activity and hypoglycic effect on diabetic rats. *International Journal of Food Sciences and Nutrition* **54** 241 -6.

Dhanaprakash BN and Garima U (2007). Antioxidant and free radical scavenging activities of phenols from onion. *Food Chemistry* **102** 1389 – 1393.

Ethan B, Grace K, Michael S *et al.*, (2003). Therapeutic applications of Fenugreek. *Alternative Medicine Review* **8**(1) 20-27.

Frankel EN (1996). Antioxidants in lipid foods and their impact on food quality. *Food Chemistry* **57** 51-55.

Harborne J B (1973). *Phytochemical Methods* (London. Chapman and Hall Ltd.) 49-188.
Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N and Krishna MRV (2009). Phytochemical investigation on a tropical plant. *Pakistan Journal of Nutrition* **8**(1) 83-85.
Lucia C, Calogero P, Maurinio Z, Antonella C, Silvia G, Franco S, Sabrina T and Luciana G (2002). Effects of γ-lirradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *Journal of Agricultural and Food Chemistry* **51** 927-934.
Madsen HL, Nielsea BR, Bertelsen G and Skisbstad LH (1996). Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. *Food Chemistry* **57** 331-337
Marja PK, Anu IH, Heikki JV, Jussi-Pekka R, Kalevi P, Tytti SK and Marina H (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* **47** 3954–62.
Mathur A, Prasad GBKS and Dua VK (2010). Anti-inflammatory activity of leaves extracts of Murraya Koenigii. *International Journal of Pharma and Bio Sciences* **2**(1) 541-544.
Parekh Jigna and Chaada V Sumitra (2007). Invitro antimicrobial activity and phytochemical analysis of Indian medicinal plants. *Turkish Journal of Biology* **31** 53-58.
Penna C, Marino S, Vivot E, Cruanes MC and Munog JD (2001). Antimicrobial activity of argentine plants used in the treatment of infectious diseases isolation of active compounds from Sebastiania brasiliensis. *Journal of Ethnopharmacology* **77** 37-40.
Roberts MF and Wink M (1998). *Alkaloids: Biochemistry, Ecology and Medical Applications,* 1st edition (Plenum Press, New York) 87 – 107. ISBN: 030645-4653.
Sofowara A (1993). *Medicinal Plants and Traditional Medicine in Africa* (Spectrum Boots Ltd, Ibadan, Nigeria) 289.
Trease GE and Evans WC (1989). *Pharmacognosy,* 11th edition (Brailliar Tiridel Can, MacMillan publishers).
Wu C, Chen F, Wang X, Kim HJ *et al.*, (2006). Antioxidant constituents in Tanacetum Parthenium extract and their chromatographic quantification. *Food Chemistry* **96** 220 – 227.
Zheng W and Wang SY (2001). Antioxidant Activity and Phenolic Compounds in Selected Herbs. *Journal of Agricultural and Food Chemistry* **49**(11) 5165-5170