Discussion

5.1. Physico-chemical analysis of honey samples:-

Honey quality evaluation is an important applied research area with relevant impact on honey industry and consumer protection. Honey quality standards are defined by various national & international authorities like Codex Alimentarius, EU Honey Directive, ISO, NABL & FCI etc. Assessment of honey leads to authenticity concerning production of honey (aims at preventing falsification) and tracing out origin of honey (aims at preventing false labeling). The physico-chemical data of honey is also essential for its proper storage & for marketing purposes. In the present study, the honey samples collected from four different locations in and around Gwalior region have been analyzed and the data are discussed as follows:

Color

Color is an important optical property of honey. Color of honey may vary from virtually colorless to deep brown through the shades of amber, yellow & red etc. Results obtained during present studies revealed that all samples collected from beekeepers of Guna, Morena & Shivpuri district were having light amber or extra light amber color. Whereas, color of some of the Gwalior market samples is light amber and some are extra light amber.

The reason behind this might be the (1) Available bee flora, agro-climatic conditions, storage and processing (Phadke, 1967; White, 1975; Varju, 1970; Free et al., 1983; Feller-Demalsy et al., 1989; Salas et al., 1993; Terrab et al. 2003a,b,c, 2004; Khalil et al., 2012; Akhtar et al., 2014), (2) Temperature at the time of storage, processing and fructose/glucose ratio (Varju, 1970; Free et al., 1983; Feller-Demalsy et al., 1989; Salas et al., 1993; Terrab et al., 2003a,b,c, 2004; Khalil et al., 2012; Akhtar et al., 2014), (3) Optical density (Finola et al., 2007) and (4) Moisture & ash content (Schade et al., 1958 and Finola et al., 2007). Similar results on color of honey samples were reported by Fahim et al. (2014) & Krishna et al., 2015.

In the present studies, light color honey samples were found to have low moisture & ash content whereas samples having little darker color (amber) were having higher moisture
percentage & ash content (Schade et al., 1958; Finola et al., 2007). The color of Gwalior market samples was found darker as compared to others, reason being long storage, temperature at the time of storage, industrial processing method. It has already been reported by Faraji-wareni (1978), Crane (1984), Pereyra et al., (1999) and Terrab et al., (2004) that serious deterioration in colour of honey occurs during long storage or/ at temperature more than 50 °C. One more reason behind the dark color of honey samples can be industrial processing methods and temperature at the time of storage. Similar studies were conducted by Varju 1970; Free et al., 1983; Feller-Demalsy et al., 1989; Salas et al., 1993; Terrab et al., 2003a,b,c, 2004; Khalil et al., 2012; Akhtar et al., 2014 who reported that color of honey depends on industrial processing methods, and the temperature and/or time of storage.

pH

Honey is naturally acidic irrespective of its floral or geographical origin which may be due to the presence of organic acids that contribute to its stability against microbial spoilage. Analysis of pH of honey sample is important because it affects the texture as well as stability & shelf life of honey. In the present study, average pH of honey samples procured from different locations varied in the range of 3.5 to 4.2. Results obtained during the present study are in line with Lopez et al., 1996; Esti et al., 1997; Mateo and Bosch-reig, 1998; Anupama et al., 2003; Azeredo et al., 2003; Saxena et al., 2010; Agbagwa et al., 2011; Khalil et al., 2012 who reported pH of honey samples in the range of 3.62 - 4.5. Ouchemoukh et al., 2007 tested Algerian honey samples and concluded that pH was varying in the range of 3.49-4.43 & 3.29-4.37 respectively. Similar results were reported by Singh & Bath (1997) where pH of Indian honey of different floral sources was found to be 4.10-4.76. Results obtained were concurrent to the studies carried out by Habib et al. (2014), Fahim et al. (2014), Boussaid et al. (2014) Krishna et al. (2015), Oshomah et al. (2015) and Umarani et al. (2015).

None of the investigated sample collected from apiaries exceeded the allowed limit when compared with international standards Codex Alimentarious, which may be considered as an index of freshness of all honey samples. However, less pH was observed in samples
Discussion

procured from Gwalior market reason might be the long storage which leads to increase in HMF that ultimately decreases pH value leading to high acidic character.

Moisture

Moisture content is one of the important characteristic of honey, having a profound effect on quality and consumption. Average maximum moisture content (21.96) was observed in Gwalior market samples followed by Guna samples (19.66), Shivpuri samples (19.17) & lowest in Morena samples (18.95). The maximum moisture content in Gwalior market samples might be due to the prolonged storage or degree of ripeness. Similar results were obtained by Anupama et al., 2003 who reported moisture content more than 20% in commercial honey samples. The moisture content investigated for all other locations was within the limit (20%) recommended by the international quality regulations (Codex Alimentarious, 2001 and CDEU, 2002).

In the present study, differences in moisture content of honey samples were found non-significant. The reason behind this can be different floral origin, degree of ripeness, weather conditions and method of extraction by the beekeeper. Similar type of studies were carried by Mahajan (1984) and Gupta (1992) who could not find any significant difference in the moisture content of honey samples in Shimla Hills & J & K respectively. Results were find concurrent with the findings of Hanson, 1951; Phadke, 1967; Perez and Rodriguez, 1970; Mahajan, 1984; Kassaye and Gasegaba, 1989; Gupta, 1992; Ghoshdastidar and Chakrabarti, 1992; Esti et al., 1997; Mateo and Bosch-reig,1998; Makhloufi et al., 2007; Finola et al., 2007; Cantarelli et al., 2008; Omafuvbe and Akanbi, 2009; Amir et al., 2010; Buba et al., 2013, Habib et al., 2014 & Krishna et al. (2015).

Specific Gravity

Specific gravity is an important physical characteristic of honey. As per US standards, Standard honey should have specific gravity more than 1.406. Also, the British National Mark scheme has covered honey under the best quality if Specific gravity of honey is at least 1.315.

In the current study, average value of specific gravity was found in the range of 1.40 to 1.44 and was almost similar in samples of all the four regions. So none of the analyzed
samples exceeded the standards set by US & British authorities. The results obtained are also concurrent to that of Deans (1953); Ibrahim et al. (1877) and Hussein (1989) and Krishna et al. (2015) who reported specific gravity as 1.433 in Natural honey and 1.427 in Industrial honey.

**Electrical Conductivity**

Electrical conductivity can prove an important characteristic for the authentication of honey. EC value largely depends on the amount of pollen content in honey. Electrical Conductivity was observed maximum (1.98) in Morena samples followed by Shivpuri (1.93), Guna (1.89) whereas, minimum EC (1.87) was analyzed in Gwalior samples.

Minor variations (non-significant) in EC values might be due to the presence of varying amount of pollen grains in honey. The reason behind low EC in Gwalior market samples might be the proper processing of honey in industry that removed most of the pollen grains. Results are similar to findings previously reported by Saxena et al., 2010; Alvarez-Suarez et al., 2010; Iftikhar et al., 2011; Agbagwa et al., 2011 and Khalil et al., 2012, Habib et al. (2014), Oshomah et al. (2015) and Krishna et al. (2015).

**Optical Density**

Optical density of honey samples is an important parameter for assessing its color & freshness.

Optical density was analyzed in honey samples collected from different locations. The results obtained were statistically similar. Maximum average optical density was observed in Gwalior samples whereas minimum value was observed in Morena samples.

The reason for minor variation in optical density might be due to light color of honey sample. The possible reason behind high optical density can be fermentation of honey due to prolonged storage which ultimately changes the color of honey to dark. Results are comparable with the reports of Wakhle, 1997 and Balasubramanyam, 1999 who reported optical density for honey samples to be 0.38 to 0.77 Balasubramanyam (2011) found that optical density of honey samples collected from Apis cerana bees was lower than that of honey obtained from Apis dorsata bees as the color of honey obtained from Apis dorsata
is little darker in color. Krishna et al. (2015) reported of optical density in Natural honey 1.061 and Industrial honey 1.056.

**Total solid**

Total solids indicate the cleanness of the honey. The results of the analysis of all the forty eight honey samples showed that the TS varied between 78.04 and 81.05 percent. Maximum TS percentage was calculated in Morena samples (81.05). Reason behind high TS percentage in Morena samples might be due to the presence of low moisture content as TS is inversely proportional to moisture content. The results are comparable with the findings of Agbawba et al., 2011; Iftikhar et al., 2011 and Khalil et al., 2012 who reported TS of honey samples in the range of 75-80%.

**Total carbohydrate**

Honey is mainly a solution of glucose and fructose with other minor constituents. Analysis of carbohydrate in honey is a quality criterion which is influenced by honey storage and heating thus is an indicator of honey freshness. It was observed that there is no any significant difference between the total carbohydrate contents of samples of all four locations. These findings are similar with the findings of other previous studies. The percentage of total carbohydrates was observed to be 74-80%. Results obtained are comparable to the reports of Kalimi and Sohomie (1964) who reported that the total carbohydrate contents of honey ranged between 74.7 to 80 per cent. Ivanov and Mitev (1972) analyzed 175 Bulgarian honeys and showed that amount of total carbohydrate content ranged from 68.98 to 79.80 per cent. White (1975) recorded 79.59 percent carbohydrates in honey samples. Vaoca et al. (1982) investigated 40 European honeys and obtained the percentage of total carbohydrate as 74.34 per cent. Egbagwa et al., 2011; Khalil, 2012; Estevinho et al., 2012 reported total carbohydrates of honey samples in the range of 62-70%.

**Total Reducing Sugar**

Honey is mainly a solution of glucose and fructose with other minor constituents. The major sugars present are glucose and fructose followed by lower concentrations of sucrose and maltose (Siddiqui and Furgula, 1976). It also contains vitamins such as Vitamin B1, B2, C and nicotinic acid. Results obtained for total reducing sugar revealed
that percentage of total reducing sugars in the samples collected from Gwalior, Guna, Morena & Shivpuri was in the range of 65 to 69 percent. Results obtained are comparable to that of Phadke (1967) who found reducing sugar in honey samples in the range of 66.45 to 77.79 percent. White (1975) recorded 69.47 percent reducing sugars in honey samples. Poncini et al. (1984) examined twelve honeys produced in different parts of Fogi and compared with them with the honey samples of other countries, and with Codex Alimentarius Commission, 2001. Values for total reducing sugars ranged from 61.6 to 73.8 percent. Dozo (1984) analyzed 66 samples of honey from different localities of Buenos Aires. The reducing sugars were in excess of 65.5 percent, the lowest value being 71.1 percent.

Results were also similar to the studies conducted by Kaushik (1988) who analyzed fresh Himachal honey and reported 68.33 percent total reducing sugars. Ghoshdastidar and Chakraborti (1992) reported 65.5-75.1 percent total reducing sugars in the samples received from the Central Bee Research Institute, Pune. Gomez et al. (1993) made physico-chemical analysis of Spanish Commercial Eucalyptus honeys. He found a mean of 68.62 percent reducing sugars. Cirilli et al. (1973) reported 72.3 percent total reducing sugar in honey samples. The reducing sugar contents of the samples used in this study had average value of 72.40 ± 6.65 g/100 g. Almost similar results were reported by Khalil et al., 2001, Kamal et al., 2002, Anupama et al., 2003, Cantarelli et al., 2008 and Vit, 2009 and Iftikhar et al., 2011. Fahim et al. (2014), Oshomah et al. (2015) and Hasan et al. (2015) reported total carbohydrates in Libyan honey in the range of 78 to 80%.

**Fructose**

The results of the sugar analysis of honey samples collected from all the locations showed that the fructose contents varied between 33.99 and 36.82 percent. Results obtained are similar to the observations of White and Maher (1954) who found the average fructose composition to be 39.28%. Latiff et al. (1956) reported that sample of Pakistan honey had fructose content of 39.01-53.8%. Fourteen types of Yugoslavian honeys were reported to have 35.43 to 41.37% fructose content (Murko et al., 1976). Olek et al. (1987) made a study of the chemical composition of two honey samples collected in Nepal. Honey from *A. cerana* contained 40.25 percent fructose whereas, that
of *A. laboriosa* had 36.05 percent fructose. The fructose contents of the honey samples analyzed in the study conducted by White, 1980 varied between 37.68 to 40.31 g/100 g with an average of 38.94 ± 0.40 g/100 g. The average fructose contents for the samples from the different States within the sub-region were not significantly different from each other and fall in the range of values reported by other researchers (White, 1980; Makhloufi, 2007; Finola *et al.*, 2007; Zafar *et al.*, 2008 and Amir, 2010.

**Glucose**

In honey, concentration of glucose is lower than fructose (Siddiqui and Furgula, 1976). In the present study, maximum percentage of glucose was analyzed in the samples collected from Morena region and minimum value of glucose was obtained in samples procured from Shivpuri.

The results are concurrent with many researchers who reported glucose content of various honey samples obtained from the various locations in the different States of the sub-region as non-significant. In a similar manner, the glucose contents of the honey samples obtained from the various locations in the different States of the sub-region were not significantly different from each other. The glucose contents of the samples which varied from 27.25 to 39.56 g/100 g with an average of 31.65 ± 2.79 g/100 g were significantly (P<0.05) lower than the fructose contents (Buba *et al.*, 2013). Similar results were reported by Latiff *et al.*, 1956; Cirilli *et al.*, 1973; Lin *et al.*, 1977; Salashinskii *et al.*, 1980; Chang *et al.*, 1988; Ghoshdastidar and Chakrabarti, 1992; Gomez *et al.*, 1993; Finola *et al.*, 2008; Agbawa, 2011; Cherian *et al.*, 2011; Anupama, 2011 and Buba *et al.*, 2013.

**Sucrose**

The percentage of sucrose in honey is generally very less. It contributes only 2 – 3% of total honey composition. In the present study also, the average percentage of sucrose is calculated in the range of 2 – 3% for all samples except for Gwalior Market where the value of sucrose was found higher and found to be (4.5%). The reason behind high percentage of sucrose in Gwalior samples might be prolonged storage & method of processing of honey in industry. Similar results were obtained by White and Maher (1954) who found the average sucrose percentage in honey samples as 1.62%. Latiff *et al.*
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*al. (1956) reported 1.9-2.7 percent sucrose in samples of Pakistan honey. Baculinschi (1965) reported sucrose 2.34 percent sucrose in Sunflower honey, 3.38 percent in Swamp flower honey and 2.58 percent in Raspberry honey. Salashinskii *et al.* (1980) made a three year study of U.S.S.R. honeys. Mean results for sucrose content were 4.15±0.61 percent. Anupama (2003) reported 1.2 to 4.5% sucrose in honey samples obtained from Mysore, India. Similar results were reported by Agbagwa *et al.* (2011), Iftikhar *et al.* (2011) & Khalil *et al.* (2012). Habib *et al.* (2014) and Ahmed *et al.* (2015) who analyzed minimum of 1.65% of sucrose in Nigerian honey.

**Total non-reducing sugar**

Total non-reducing sugars of honey samples collected from all four locations was in the range of 9-11%. It was observed that Gwalior samples have maximum percentage of TnRS. Reason behind this might be the anomaly in the processing of honey samples in industry which may lead to variation in the concentration of glucose, fructose & sucrose.

Results obtained are concurrent to that of Buba *et al.*, 2013 who reported 0.5-3.2% TnRS in honey samples collected from North East Nigeria.

**Fructose-Glucose ratio**

In addition to the percentages of reducing and non-reducing sugars, other important factor that relate to honey quality include the fructose/glucose ratio. The ratio of F/G affects the crystallization of honey. Honey crystallization is slower when the fructose/glucose ratio is more than 1.3 and it is faster when the ratio is below 1.0 (Amir *et al.*, 2010).

In the present study, the fructose/glucose ratio was in the range of 1.00 to 1.20. Results obtained are similar to study conducted by Buba *et al.*, 2013 who reported the fructose/glucose ratio in the range of 1.00 to 1.45.

**Ash**

Ash content is an important quality criterion for estimating botanical origin of honey. The floral origin has been reported to be responsible for the difference in ash content (Fredes and Montenegro, 2006) and maximum limit of ash contents is 0.6% (Codex Alimentarius, 2001).
The ash content of samples investigated in this study was found within acceptable limits i.e. less than 0.6%. Results are comparable with the findings of Taormina et al., 2001; Fredes and Montenegro, 2006, Finola et al., 2007, Agbagwa et al., 2011. Buba et al., 2013, Kayode et al. 2014, Boussaid et al. 2014, Prica et al., 2015 and Umarani et al. (2015).

**Free acidity**

Acidity is one of the most essential chemical characteristic which influences the honey quality. The maximum limit of 0.2 percent acidity has been prescribed in the ‘Agmark’ specifications (Phadke, 1962) and also in I.S.I., 1968 for “A” grade and “Standard” grade honeys.

It was observed that acidity was maximum in samples procured from Gwalior market i.e. 32.02±1.22 meq/kg. The value found to be significantly maximum as compared to the values obtained for Guna, Morena & Shivpuri region where acidity was recorded in the range of 16-24 meq/kg. Latif et al. (1956) carried out researches on the composition of Pakistan honey and found acidity in the range of 0.07-0.16 percent. Kaushik (1988) found average acidity (as formic acid) 0.11 per cent in the multifloral Indian honeys.

Similar results were obtained by Cherian et al., 2011 who reported acidity in the range of 15-47 meq/kg. Finola et al., 2007 analyzed 11-30 meq/kg acidity in Indian honey samples and Babarinde et al., 2011 reported 22-37 meq/kg acidity in Nigerian honey. Results were also concurrent with that of Kayode et al. (2014), Habib et al. (2014), Oshomah et al. (2015) and Ahmed et al., 2015 who reported the free acidity in honey in the range from 10.2 to 26.1 meq/kg.

**Total Protein**

Honey is known to be rich in both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins (Aljadi & Kamaruddin, 2004; Al-Mamary et al., 2002; Gheldof and Engeseth, 2002; Schramm et al., 2003).
In the present study, total protein content in honey samples was very less and below one percent at all the four locations. It has already been reported by White, 1975 that filtered honey is devoid of desirable enzymes and proteins. Azeredo et al. (2003) also determined protein contents in honey to be less than 1%. Results were concurrent to the reports of Habib et al. (2014), Boussaid et al. (2014), Linkon et al. (2015) and Hasan et al. (2015) who reported total protein in honey sample in the range of 0.49 to 0.53%.

**Hydroxymethyl furfural (HMF)**

Hydroxymethyl furfural aldehyde is the compounds formed during commercial conversion of sucrose to form invert sugar. Higher percentage of HMF absence has been used as a test for determining purity of honey.

Data obtained for percent hydroxymethyl furfural content is presented in table 23. HMF of samples collected from Gwalior market was calculated in the range of 12 to 34 percent with an average of 23.13%. Reason behind this might be storage of honey samples since long as well as heating of honey during processing. It has already been reported that HMF content tends to increase with the passage of time. Samples collected from apiaries of other three locations have lower HMF content (<10%) as these were fresh samples. As per USDA, HMF content must not be more than or equal to 60 µg/kg after processing and blending (Codex) or 40 µg/kg for all retail honey (Bogdanov et al., 2001). In fresh honeys 10µg/kg HMF levels are found, but increases with storing time & temperature (Tosi et al., 2004).

Results were comparable with that of Frias-Tejera et al., 1991, Finola et al., 2007, Feas et al., 2010, Iftikhar et al., 2011 and Cherian et al., 2011. Frias-Tejera et al., 1991 reported results for 38 honey samples; mean HMF content was 16.9 gm/kg. Iftikhar et al., 2011 analyzed honey samples from Pakistan & reported the value as 23-29%. Habib et al. (2014) reported HMF in Arid regions honey sample range from 0.17 to 79.26mg/kg. Similar results were obtained by Boussaid et al. (2014), Akhtar et al. (2014), Umarani et al. (2015) and Ahmed et al. (2015) who reported HMF in honey in the range of 3.8 to 21.4 mg/kg.
5.2. Qualitative analysis of pesticides residues in honey samples:-

Honey, being a natural produce is considered to be free from any extraneous material but residues of pesticides were recorded by several workers.

In the present study, some organochlorine & organophosphates residues were analyzed in honey samples. Traces of residues were maximum in Gwalior market samples. The results obtained for freshly collected honey samples indicated a low level of contamination by pesticide residues.

A few similar kind of works on the monitoring of pesticide residue levels in honey have been previously published (Estep et al., 1977; Gayger and Dutmann, 1985; Jan and Cerne, 1993; Al-Rifai and Akkel, 1997; Blasco et al., 2003, 2004 and Khan et al., 2004. MRL’s (Maximum Residue Limit) have been framed by some countries but for insecticides these are neither included nor in the Codex Alimentarius nor in the EU (European Union) (Al-Rifai and Akkel, 1997; Blasco et al., 2003; Khan et al., 2004).

Pesticides consumption in the state of Himachal Pradesh is very low as compared to other states of India. So it is speculated that honey collected from the state is free from the pesticide residues. In contrary to this Sharma and Kashyap (2002) recorded pesticide residues in honey. Muino and Lozano (1991) determined organochlorine pesticides (lindane, heptachlor, aldrin, heptachlor epoxide, dieldrine, endrin, p,p’ DDT, and methoxychlor) in honey samples from Spain. Studies of Spanish honeys showed the presence of few acaricides, amitraz, coumaphos, and fluvalinate (Garcia et al., 1996). Some organophosphorus pesticides: azinphos-methyl, diazinon, ethion, methamidophos, and phosalone were found in Spanish honeys at low microgram per kilogram levels (Garcia et al., 1995). In a study on Portuguese and Spanish honeys (Blasco et al., 2003), organochlorine pesticides were the compounds most frequently detected at concentrations from 0.01 to 4.3 µg/g, mainly in Portuguese honeys, with lindane presenting the highest levels. Blasco et al. (2004) determined nine organochlorine pesticide residues (α–β– and γ–HCH, hexachlorobenzene, aldrin, p,p’ DDE, p,p’ DDD, o,p’ DDT, and p,p’ DDT) in 49 samples of honey collected from markets of Portugal and Spain. Erdogruk, 2007 has determined levels of pesticides in nine honey samples. Guler et al., 2010 have also reported the presence of pesticide residues in honey samples obtained from Turkey.
Choudhary and Sharma, 2008 have also reported the presence of pesticide residues in honey samples from Himachal Pradesh (India).

5.3. **Antibiotics analysis**

Antibiotic residues in honey have recently become a major consumer concern. It has become evident that residues of antibiotics in honey originate mostly not from the environment but from improper beekeeping practices. There are several international reports of antibiotic residues in honey samples, however there are very few reports of antibiotics in honey sold in domestic market in India.

Oxytetracycline, Chloramphenicol, Ampicillin, Erythromycin and Ciprofloxacin antibiotics were found in all almost all samples analyzed. Presence of traces of Oxytetracycline might be due to fact that it is most commonly used against bacterial foul brood diseases by beekeepers. So from there minute traces may enter the honey stored in combs. Presence of ampicillin can be due to its wide use in veterinary medicine for the treatment and prevention of bacterial diseases. Some beekeepers use this antibiotic to prevent the colonies from various diseases. Erythromycin & ciprofloxacin are two important antibiotic widely used to protect honey bees from bacterial diseases.

So it was concluded at the end of study that while spraying the antibiotics in and around the apiary, traces of antibiotics might get entered in the comb accidently which got analyzed in honey stores later. Also on commercial scales, honey is collected from different sources and then pooled before packing. Widespread contamination of different components of environment by antibiotics has been reported including milk, eggs, meat and honey etc. (Gunes, 2008).

Weigel *et al.*, 2004 reported the presence of chloramphenicol, streptomycin, sulphonamides and tetracyclines in honey samples. Vidal *et al.* (2009) reported presence of up to 8.6 g/kg erythromycin in three out of 16 samples of honey in Granada & Almeria. There are reports of tests conducted by Agricultural Processed Food Product Export Development Agency (APEDA) and EIC from 2005 onwards show high levels of antibiotics and heavy metals in Indian honey. Draiaia *et al.* (2015) reported of antibiotics in Algerian honey was tetracycline and streptomycin are absent and oxytetracycline present 0.03ppb.
In a study from Tamil Nadu, India Ampicillin examined in honey collected during peak flowering seasons of rubber (March and April) and banana (December and January) was detected in the range of 3-44 µg/kg (Solomon et al. 2006). In a report by Jadon & Johnson, multiple antibiotics were detected in all domestic and imported brands of honey tested except Hitkari Honey of Hitkari Pharmacy, Delhi was found to be free of antibiotics. Highest number of antibiotics - 5 out of 6 were detected in imported Nectaflor Natural Blossom Honey, followed by Patanjali’s Pure honey which had 4 antibiotics. The number of antibiotics in other brands was 3 each in Dabur, Himalaya Forest and Khadi Honey, 2 each in Mehsons Pure Honey, Himflora Gold, Umang Honey and Baidyanath Wild flower Honey. No antibiotic was detected in Hitkari Honey. Three antibiotics were detected in imported brand from Australia (Capilano Pure and Natural Honey).

The concentrations detected in the present study honey samples are low and not likely to cause any acute effect but chronic health effects cannot be ruled out. There is a need to regulate and monitor the level of antibiotics in honey as continuous long term exposure to low levels of antibiotics could in due course of time lead to antibiotic resistance in pathogenic bacteria making their treatment difficult.

5.4. Minerals

Many trace elements in different types of bee honeys, have been analyzed (Rodriguez-Otero et al., 1992; Chung & Tsai, 1992; Stein & Umland, 1986; Vinas et al., 1997; Caroli et al., 1999; Taddia et al., 2004; Gonzalez-Miret et al., 2005; Ioannidou et al., 2005).

In the present studies, thirteen trace elements were analyzed in forty eight honey samples collected from four different locations in M.P. Results indicate the trace elements found in Gwalior market samples. It was observed that all 13 trace elements are present in these samples in minor concentrations. Calcium was found in all the samples in highest amount.

The concentrations metals in the present study are low and not likely to cause any acute effects. There is a need to regulate and monitor the level of heavy metals in commercial honey samples so as to maintain the standards of export quality honey.
5.5. *In-vitro* antioxidant potential

**DPPH assay**

The radical scavenging activity in 48 honey samples varied from 46% to 70% in the DPPH reaction system. The results showed that samples from Gwalior market have maximum average free radical scavenging activity i.e. 70.00±0.52 followed by 52.00±0.64 in Guna samples, 49.00±0.66 in Morena samples and 46.00±0.65 percent in Shivpuri samples. However, it was observed that except for Gwalior region samples, percent radical scavenging activity was statistically similar among the samples for all the three locations. The difference in radical scavenging activity of and among Gwalior samples can be due to dark color (long preserved samples) as it has already been reported that dark colored honey tended to be highly active in the reaction with DPPH (Bertoncelj *et al.*, 2007; Blaska *et al.*, 2006; Meda *et al.*, 2005). Antioxidant capacity was reported higher for darker honey samples (Chen *et al.*, 2000; Frankel *et al.*, 1998; Gheldof and Engeseth, 2002 and Nagai *et al.*, 2001) as well as in honey with higher content of water (Frankel *et al.*, 1998 and Aljadi and Kamaruddin, 2004).

It has also been observed that the composition and antioxidant capacity of honey depend on the floral source used to collect nectar; seasonal and environmental factors, as well as processing may also have an effect on honey composition and antioxidant activity (Al-Mamary *et al.*, 2002; Chen *et al.*, 2000; Frankel *et al.*, 1998; Gheldof and Engeseth, 2002; Gheldof *et al.*, 2002 and Yao *et al.*, 2003). DPPH directly proportional to HMF, inversely proportional to pH, directly proportional to OD.

**ABTS assay**

Honey serves as a source of natural antioxidants (Al-Mamary *et al.*, 2002; Aljadi & Kamaruddin, 2004; Antony *et al.*, 2000; Beretta *et al.*, 2005; Gheldof *et al.*, 2002; Kucuk *et al.*, 2007; Nagai *et al.*, 2001 and Vit *et al.*, 1997), which play an important role in food preservation and human health. The percent antioxidant activity in honey samples varied between 40 to 54%. The data shows that Gwalior market samples have maximum average antioxidant activity whereas; freshly collected honey samples have lower antioxidant activity.
The difference in antioxidant activity can be due to the dark color of samples. Beretta et al., 2005 and Bertoncelj et al., 2007 declared that dark colored honey obtained from forest, honeydew and buckwheat had highest antioxidant activity and pale or light color honey of Acacia had low antioxidant activity. In the literature, several studies for the identification and quantification of antioxidant components of honeybee products have been reported (Buratti et al., 2007; Ferreres et al., 1994 and Gheldof et al., 2002). ABTS directly proportional to HMF, inversely proportional to pH, directly proportional to OD.

**5.6. Total phenolic content estimation:**

Honey samples obtained from Gwalior Market were found to have maximum phenolic content, reason being they were darker in appearance (long term storage) as compared to other samples. It has already been reported that dark honey samples tends to have high phenolic content.

Total phenolic contents of the honey samples used in this study are similar to those reported by Vit et al., 2009 for Venezuelan Apis Mellifera honeys (38.15 to 182.10 mgGAE/100 g, with an average of 93.50 ± 51.62 mgGAE/100 g). The values of phenolic contents in this study are, however, higher (P<0.05) than those reported by Adetuyi et al., 2009 for Apis Mellifera honey samples in Owo community, Ondo State in south west Nigeria (0.75 to 2.85 mgGAE/100 g). Phenols are reported to have antioxidant capacities that are much stronger than those of vitamins C and E (Oboh, 2005). According to Blasa et al., 2006, raw honey contains copious amounts of compounds such as flavonoids and other polyphenols which may function as antioxidants. Honey polyphenols are said to originate from nectar, pollen or propolis (Makawi et al., 2009). Phenolic content directly proportional to HMF, inversely proportional to pH, directly proportional to OD.

**5.7. Development and standardization of purity check method of honey on the basis of antioxidant and phenolic content**

**Fiehe’s test and aniline chloride test**

On the basis of higher concentration of HMF, these tests ensure the adulteration of given honey samples. In this study fiehe’s test was failed to differentiate between fresh pure samples and adulterated samples because it gave negative results for both type of samples and confirmed old pure honey samples as positive. In aniline chloride test same results
were detected as fiehe’s test. The dissolving, flame and absorption tests do not consider as scientific tests by scientific community however they differentiate pure and adulterated honey samples to some extent. Due to this insufficiency of these tests another tests were required to confirm adulteration in honey samples.

**Purity check at laboratory level**

In searching of a new method to find out adulteration in honey, success was achieved with the help of tests of antioxidant. Results of these tests confirmed the clear cut difference between pure and adulterated honey samples. The reduction of DPPH gave a range between 46 to 78% when pure and old honey samples were tested but an ascending increase (in a certain ratio) of sugar compounds in samples gave a descending decrease percentage of DPPH reduction. Same results were also found with the results of ABTS reduction and phenolic content estimation.