DISCUSSION

Traditional medicine refers to health practice, approaches, knowledge and beliefs of incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises; applied all these individually or in combination to diagnose and treat or prevent illness. Medicinal plants can save lives, livelihood and cultures. Herbal medicines are an important part of the culture and traditions of India. It is therefore no surprise that medicinal plants have raised their importance all over the world. Recently, the renewed interest in medicinal plants as a re-emergent health aid has been fuelled by the extensive antimicrobial resistance along with rising costs of synthetic drugs in maintenance of health and the bioprospecting of new plant-derived drugs are important as described by Hoareau and Da-Silva, (1999).

At present, phytochemicals are fervently focusing on health promotion, disease prevention and the development of therapeutic interventions. The introduction of terms such as “Functional food” and “Nutraceuticals” illustrates the high expectations associated with current phytochemical research.

Keeping this in view, in the present investigation the phytochemical and antimicrobial investigation was carried out on the medicinally important members of Euphorbiaceae namely Baliospermum montanum, Drypetes roxburghii and Codiaeum variegatum.

From over the centuries, people living in intimate association with their environment have experimented and accumulated the knowledge of plant
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bioactivities. Therefore, ethno-directed research is very useful in drug discovery and development (Cox and Balick, 1994; Heinrich and Gibbons, 2001). Modern research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological and molecular techniques. In order to understand the biological activity of a plant such as medicinal, poisonous or nutritive, it is necessary to investigate its chemical constituents of primary and secondary metabolites of plants such as alkaloids, terpenoids, phenolics, gums, mucilages, carbohydrates, amino acids, proteins, fatty acids and glycolipids, as opined by Croteau et al., (2000).

In the present study, different parts such as leaf, stem, root and flower of *B. montanum*, *D. roxburghii* and *C. variegatum* extracts were prepared in seven different solvents such as water, ethanol, acetone, petroleum ether, chloroform, methanol and hexane to investigate phytochemical and antimicrobial activities.

It was observed that *B. montanum; D. roxburghii* and *C. variegatum* thrive well at 25 ± 2° C in natural conditions, though they have collected from different agro-climatic conditions.

In the present study, alkaloids are found to be present in ethanol and petroleum ether extract of leaf, stem, root and flowers of *B. montanum, D. roxburghii* and *C. variegatum* (Table 5, 6 & 7). The present finding does not coincides with the findings of Iniaghe et al., (2009) who have reported that, alkaloids were extracted from water and methanol extracts of leaves of three *Acalypha* species.

In the present study, it was found that anthraquinone was present in chloroform and hexane solvent with leaf, stem, flower and root extract of *D. roxburghii* and *C. variegatum*. Alkaloids were also found to be present in petroleum ether and ethanol extract of leaf, stem, flower and root of *C. variegatum*. However, it is totally absent in *B. montanum* (Table 8). The present finding coincides with the findings of Kamba and Hassan (2010) as they have reported the presence of anthraquinone in ethanol and petroleum ether solvent with of leaf, stem and roots of *Euphorbia balsamifera*. 


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Water, acetone, ethanol and methanol extract showed the presence of carbohydrates in leaf, stem, flower and root of *B. montanum, D. roxburghii* and *C. variegatum* (Table 9, 10 & 11). The present finding agrees with Madziha *et al.*, (2010) who have published the presence of carbohydrate in *Acalypha wilkesiana* polar solvent.

The cardiac glycosides were found to be present in the water, acetone, ethanol and methanol extract of leaf, stem, flower and roots of *B. montanum, D. roxburghii* and *C. variegatum* (Table 12). This does not coincide with the findings of Kamba and Hassan (2010) who have reported the presence of cardiac glycosides in petroleum ether extract of roots of *Euphorbia balsamifera*.

In the current study, traces of coumarins were found in chloroform root extract of *B. montanum* and chloroform stem *D. roxburghii*, hexane leaf extract of *C. variegatum* and petroleum ether stem extract of *Codiaeum variegatum* and *D. roxburghii* (Table 13). This agree with the findings of Thenmozhi and Rajan (2012) they have reported that coumarins are present in water and ethanol extract of *Acalypha indica*.

The present investigation revealed that fatty acids are absent in water, acetone, ethanol, methanol, chloroform, hexane and petroleum ether solvent with leaf, stem, flower and root of *B. montanum, D. roxburghii* and *Codiaeum variegatum* (Table 14).

It was found that flavonoids were present in water, acetone, ethanol, methanol and chloroform of leaf, stem, flower and root of *B. montanum, D. roxburghii* and *Codiaeum variegatum* (Table 15, 16 and 17). This finding agrees with Iniaghe *et al.*, (2009) as they have reported the presence of flavonoids in methanol solvent with leaf of *Acalypha species*.

It was noticed in the present study, that gum and mucilage are present only in water extract of *B. montanum* flower and acetone leaf extract of *D. roxburghii* (Table 18). This coincides with the findings of Surabhi and Leelavathi (2010), they have reported that gum and mucilage are absent in leaf of *Catunaregum spinosa*. 
In the present investigation, it was found that proteins and amino acids are major constituents present in water extract of leaf, stem, flower and root of *B. montanum, D. roxburghii* and *C. variegatum* (Table 19). This disagrees with the finding of Rajanisrosha and Ananthi (2013), as they have published that proteins are present in water and ethanol extract of *Jatropha curcas*.

It was found that phenols are present in ethanol, methanol and acetone extract of leaf, stem, flower and root of *B. montanum, D. roxburghii* and *Codiaeum variegatum* (Table 20). This does not coincide with the findings of Mamatha et al., (2014), as they have published the presence of phenols in chloroform and ethanol leaf extract of *Euphorbia thymifolia*.

It was found that saponins are present in water leaf, stem, flower and root extract of *B. montanum, D. roxburghii* and *Codiaeum variegatum*, methanol extract of leaf, stem, flower and root of *D. roxburghii* and *Codiaeum variegatum*. The presence of saponins was also noticed in ethanol extract of leaf, stem, flower and root of *Codiaeum variegatum* (Table 21). This coincides with the findings of Madane et al., (2013). They have reported the presence of saponins in aqueous leaf extract of *Euphorbia tirucalli* and *Chrozophora rottleri*.

The current investigation revealed the presence of steroids in chloroform and methanol extract of roots, stem of *B. montanum* and *Codiaeum variegatum* whereas chloroform, hexane and petroleum ether extract of *D. roxburghii* roots showed the presence of steroids (Table 22), this does not coincide with the findings of Mamatha et al., (2014) who have reported the presence of steroids in alcohol and water extract of *Euphorbia thymifolia*.

It was noticed that tannin are present in ethanol and methanol extract of leaf, stem, flower and root of *B. montanum, D. roxburghii* and *Codiaeum variegatum* (Table 23) the present findings agrees with finding of Rajanisrosa and Ananthi (2013), as they have showed that tannin are present in alcoholic leaf extract of *Jatropha curcas*. 

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The present study reveals that terpenoids are present in water, ethanol, methanol, chloroform, hexane and petroleum ether extracts of leaf, stem, flower and root of \textit{B. montanum} and \textit{D. roxburghii} (Table 24). The present findings coincide with the findings of Egwaikhide and Gimba (2007) and they have reported the presence of terpenoids in hexane and ethanol leaf extracts of \textit{Plectranthus glandulosus}.

In the present investigation, it was found that the alkaloids, anthraquinone, carbohydrates, cardiac glycosides, coumarins, flavonoids, gum and mucilage, phenols, protein and amino acids, saponins, steroids, tannin and terpenoids are present in the leaf, stem, flower and roots of \textit{B. montanum}, \textit{D. roxburghii} and \textit{Codiaeum variegatum}. This indicates that these medicinal plants are rich in phytochemicals compounds. Hence, presence of these compounds in three plants enhances their use in commercial exploitation for medicinal purposes. The present finding coincides with Shruthi \textit{et al.}, (2012), as they had estimated the presence of phytochemicals in ethanol, methanol, chloroform, acetone and hexane extract of \textit{Kirganelia reticulata} (Euphorbiaceae), they also reported that alkaloids, phytosterols, deoxysugars, saponins, phenol, tannins, flavonoids, proteins, amino acids, gum, glycosides, anthraquinone glycosides are present in \textit{Kirganelia reticulate}. Whereas, the present findings does not coincides with Kothale \textit{et al.}, (2011) as they have reported absence of saponins, tannin, cardenolides, steroids in some Euphorbiacea members.

Plants are the rich source of secondary metabolites such as alkaloids, flavonoids, terpenoids and saponins. The nutraceuticals industries have increased their attention over secondary metabolites due to its antioxidant activity (Wan and Diaz-Sanchez, 2007). There are many reports available on medicinal properties of alkaloids, saponins, tannin, terpenoids, phenols and flavonoids in the review by Mwine and Demme, (2011).

\textbf{Alkaloids} are one of the largest groups of chemical produced by plant and their amazing effects on humans have led to the development of powerful painkiller medications (Raffauf 1996), They are known to have anticholinergic and spasmolytic properties (Tyler \textit{et al.}, 1988), antimalarial (Banzouzi \textit{et al.}, 2004 and Karou \textit{et al.}, 2003).
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**Saponins** are plant glycosides with a triterpene or steroids aglycone. Saponins are known for its anti-inflammatory, anti-parasite and antivirus activity as reported by Just *et al.*, (1998) and Traore *et al.* (2000). Recently, there have been tremendous commercially driven promotions of saponins as dietary supplement and nutraceuticals (Repetto and Llesuy, 2002).

The name ‘**tannins**’ is derived from the French word ‘tannins’ (tannins substance) and is used for a range of natural polyphenols. The astringency from the tannin is that which causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripened fruit (McGee, 2004). The antiinflammatory effects of tannin help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders (Cheng, 2002).

**Terpenoids** are defined as secondary metabolites consist of two isoprene units. The classification of terpenoids is based on the number of isoprene units (Ashour, 2010). The terpenoids group show significant pharmacological activities, such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer (Mahato and Sen 1997).

Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants (Kim *et al.*, 2003). These polyphenolics compounds display a remarkable spectrum of biological activities including those that might be able to influence processes that are dysregulated during cancer development. They are antiallergic, anti-inflammatory, antioxidant, antimutagenic, anticarcinogenic, and also modulate enzymatic activities. They may therefore have beneficial health effects and can be considered possible chemopreventive or therapeutic agents against cancer (Wenying Ren *et al.*, 2003).

In the current investigation, leaf, stem, root, flower methanolic extract and latex of *B. montanum* and leaves, stem and root of *D. roxburghii* and *C. variegatum* was utilized for quantitative estimation of alkaloids, flavonoids, Saponins, phenolics, tannin and terpenoids. The results of quantitative estimations are discussed below with the relevant literature.
The analysis of the quantitative results revealed the presence of alkaloids, flavonoids, saponins, phenolics, tannin and terpenoids in methanol leaf, stem, root, flower extracts of *B. montanum*, *D. roxburghii* and *C. variegatum* and in latex of *B. montanum* with varying range of percentage such as alkaloids (6.2 to 9.5%); flavonoids (35.63 to 43.33%); Saponins (9.9 to 13.2%); phenolics (38.43 to 43.44%); tannin (13.26 to 18.3%); terpenoids (25.36 to 33.4%) in *B. montanum*, alkaloids (9.5 to 9.6%), flavonoids (18.63 to 21.23%), saponins (11.36 to 5.13%), phenolics (28.36 to 31.1%), tannin (13.16 to 15.38%) and terpenoids (18.8 to 21.56%) in *D. roxburghii*. In *Codiaeum variegatum* alkaloids (4.66 -10.2%), flavonoids (33.1-37.63%), saponins (11.36-13.76%), phenolics (35.43-39.76%), tannin (10.5-18.5%), terpenoids (27.56-30.3%) are present. It was observed that phenols, flavonoids and terpenoids are predominantly present in leaf, stem, flower and root of *B. montanum*, *D. roxburghii* and *Codiaeum variegatum* and also in latex of *B. montanum*. The present finding does not coincide with the findings of Sutharsingh *et al.*, (2011) they have reported that *Naravelia zeylanica* contain low content of phytochemicals such as alkaloids (0.86 ± 0.023), total phenols (0.72 ± 0.012), tannins (8.72 ± 0.044), flavonoids (0.56 ± 0.037), saponins (2.86 ± 0.023).

In the present investigation, for the first time phytochemical fingerprint was developed by computing all the phytochemical qualitative data. The fingerprint pertaining to fourteen different phytochemical compound such as alkaloids, anthraquinone, carbohydrates, cardiac glycosides, coumarins, fatty acids, flavonoids, gum and mucilage, phenols, protein and amino acids, saponins, steroids, tannin and terpenoids with their presence or absence in leaf, stem, roots and flower of water, acetone, ethanol, methanol, chloroform, hexane and petroleum ether extract of *B. montanum*, *D. roxburghii* and *C. variegatum*. This results have been published in Bijekar and Gayatri (2014); Bijekar *et al.*, (2014) and Bijekar *et al.*, (2015).

Although steroidal antiinflammatory drugs and Non Steroidal Anti-inflammatory drugs (NSAIDs) are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders while such compounds are accompanied by side effects. Therefore, there is an urgent need to find safer antiinflammatory compounds as opined by Yoon *et al.*, (2005). The use of natural products with therapeutic properties is as ancient as human civilization.
and, for a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale 1984).

In traditional medicine extracts of different plants are widely used for the treatment of a wide variety of disorders including acute and chronic inflammation. Among the active constituents of these extracts, flavonoids are a family of substances whose members are having many interesting biological properties including anticancer, antimicrobial, antiviral, anti-inflammatory, immunomodulatory and antithrombotic activities (Robak 1996 and Havsteen, 1983).

In the present investigation, it was found that the methanolic leaf extract of *B. montanum*, *Drypetes roxburghii* and *Codiaeum variegatum* contains three flavonoids Fractions (I, II and III). The present findings disagree with the findings of Yahaya, (2015), and they have reported the presence of only one Fraction of flavonoids in methanol leaf extract of *Mitracarpushirtitus*. These flavonoids Fractions were tested against RAW 264.7 cell lines (Mouse Leukaemic Monocyte Macrophage) for cytotoxic and anti-inflammatory study, antimicrobial and antioxidant activity.

The *in vitro* cytotoxic activity of flavonoids Fraction I from *B. montanum* against RAW 264.7 cell lines (Mouse Leukaemic Monocyte Macrophage) was found to be least when compared to other two Fractions (II and III), it means that Fraction I showed maximum cell viability in RAW 264.7 (Mouse Leukaemic Monocyte Macrophage) cell lines as compared to Fraction II and III with more effective anti-inflammatory property. In *D. roxburghii*, Fraction III showed least cytotoxic activity against RAW 264.7 cell lines as compared to others Fractions and Fraction I showed highest anti-inflammatory activity against RAW 264.7 cell lines. In *C. variegatum*, Fraction I revealed highest cell viability in RAW 264.7 cell lines and Fraction III showed least. Fraction II was found to have most effective anti-inflammatory activity against RAW 264.7 cell lines whereas Fraction III was least effective. In the present study, it was found that the Fraction I and III exhibit highest cytotoxic activity against RAW 264.7 cell lines because of least Nitric Oxide (NO) production and Faction I, II and III revealed highest anti-inflammatory activity in all the members tried. The present result coincides with result of Vichitra *et al.*, (2009), and they had tested the anti-inflammatory property of various leaf extracts (petroleum ether, chloroform,
acetone, methanol and aqueous) of *C. variegatum* in some acute models viz. carrageenan induced and dextran induced paw oedema and found acetone extract of *C. variegatum* leaves possesses significant anti-inflammatory activity where as it does not coincides with the findings of Lalitha and Gayathiri (2013); Kumar et al., (2011). They have reported that *B. montanum* is effective against acute inflammation (carrageenan paw edema, and Ibuprofen-induced paw edema) in a dose related manner. Reanmongkol et al. (2009) has reported antinociceptive, antipyretic, and anti-inflammatory activities of ether leaf extracts of *Putranjiva roxburghii* Wall. (*Drypetes roxburghii*) in experimental animals and found that, extract exhibits moderate inhibitory activity of inflammation in carrageenan-induced paw edema in rats.

Among all three selected medicinal plants *B. montanum* was found to have least cytotoxic effect with maximum cell viability and highest anti-inflammatory activity as compared to other. The present finding agrees with the findings of Sharma et al. (2014) and they have reported cytotoxic and anti-inflammatory activity of *Euphorbia hirta* (Euphorbiaceae). Further, the present findings are in agreement with Raju et al., (2005) and they have revealed that flavonoids and alkaloids are responsible for anti-inflammatory reactions. Similarly, flavonoids with anti-inflammatory potential were reported from *Morindatinctoria roxb,* and *Vernonia amygdalina* by Sivaraman and Muralidharan (2010) and Udeme et al., (2009).

Free radicals play an important role in many physiological and pathological activities of living organisms. Any imbalance in the generation and scavenging of free radicals causes diseases. Free radical reactions make significant impact on membrane proteins, enzymes and DNA as reported by Vandemiale et al., (1999); Valko et al., (2006) and Chen et al., (2011). Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress as reported by Zengin et al., (2011). Study of antioxidant efficiency in oil using (2-2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2- diphenyl-1 picrylhydrazyl (DPPH) radical scavenging assay is an important *in-vitro* analysis with which the total
antioxidant stability in oils can be studied for their high quality reproducibility and simple eminence control was opined by Bakkali et al., (2008); Rubalya and Neelamegam, (2012b). Both the methods apply decolorization assays to identify the existence of antioxidant which annul the development of the ABTS radical cation and DPPH radical (Tomaino et al., 2005). ABTS (2-2’-azinobis (3-ethylbenzothiazoline-6 and DPPH (2,2- diphenyl-1-picrylhydrazyl were used to predict antioxidant capacity of fresh fruits, beverages and food (Thaipong, 2006).

Both ABTS and DPPH are stable free radicals which dissolve in methanol or ethanol, and their colours show characteristic absorption at wavelength 734 nm or 516 nm, respectively. Colours of ABTS and DPPH would be changed when the free radicals were scavenged by antioxidant (Li, 2011 and Apak, 2007). The DPPH method is a simple, rapid and convenient method for screening of polarity and radical scavenging activity (Koleva et al. 2001). Generally, the DPPH absorbance is measured at a wavelength of 515 - 520 nm (Bandoniene et al. 2002, Pavlov et al. 2002, Gazi et al. 2004). DPPH is a stable free radical in a methanolic solution. In its oxidized form, the DPPH radical has an absorbance maximum centered at about 520 nm (Molyneux, 2004). The DPPH assay method is based on the reduction of DPPH, a stable free radical (Warrier, 1994).

In the present study the free radical scavenging activity of flavonoids Fractions isolated from methanol leaf extract of B. montanum, D. roxburghii and C. variegatum and compared with standard Quercetin. It was found that the IC50 value of B. montanum flavonoids Fraction I was found to be 38.78 when compared to standard quercetin 9.116. As the flavonoids Fractions Fraction I, II and III of Drypetes roxburghii and Codiaeum variegatum and Fraction II and III of B. montanum showed less than 50% inhibition, hence their IC50 value was not calculated. Similar antioxidant activity was reported by Prasad and Rajkumar, (2014.) They have studied and calculated the IC50 value for the DPPH scavenging assay for three solvent extracts of the Citrus species. The highest IC50 values of 0.647 mg/ml (Murraya koenigii), 0.58 mg/ml (Citrus aurantifolia) and 0.73 mg/ml (Cucurbita maxima) were observed. The present investigation coincides with the findings of Basma et al., (2011) and Shahwar et al., (2010).
The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are increasing at present. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988; Ng, 1994; Dean and Burchard, 1996; Gonzalez et al, 1996). Since, the secondary metabolites are synthesized by plants in response to microbial infection, it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms as reported by Harborne (1993); Cowan (1999); Middleton and Kandaswami (1993).

In the present study, it was found that Fraction III of *B. montanum* revealed highest antibacterial activity against *E. coli* when compared to flavonoids fractions II and III in *D. roxburghii* and *C variegatum*. This finding does not coincide with the Audipudi et al., (2010). They have reported that the antimicrobial activity of flavonoids is inactive against *E. coli*. In the present study significant antibacterial activity against *E. coli* has been shown and this agrees with the findings of Vandana Mathur, (2007).

In the present study, it was found that fraction III of *B. montanum* showed highest antibacterial activity against *S. typhimurium*. This finding coincides with the findings of Doss et.al. (2011), they reported antimicrobial work of flavonoids fractions obtained from *Mimosa pudica* and *Panicum* maximum against *S. typhimurium*.

It was found that fraction I of *B. montanum* showed highest antibacterial activity against *P. aeruginosa*. The present finding coincides with the findings of Edziri et al., (2010); Elsa Varghese et al., (2012) they have reported zone of inhibition against *Pseudomonas aeruginosa*.

It was found in the present investigation that flavonoids fractions I, II and III from *B. montanum* exhibit highest antibacterial activity against *Aspergillus niger*, *Aspergillus fumigates* and *Microsporum gypseum* when compared to other species tried.
Secondary metabolites are non nutritive phytochemicals which are produced at different developmental stages, under stress conditions and it plays important role in giving protection against pathogen attack. When these phytochemicals are ingested by humans, it enhances their resistance power. Wei Lei *et al.*, (2011) has reported that flavonoids are of immense economic value in drugs, food and nutraceuticals industries. The medicinal value of a plant was assumed by estimating the presence of total flavonoids content (Ganesh *et al.*, 2011). Therefore, different methods are being employed to accelerate the phytochemical production. Many successful attempt of accelerating phytochemical has been reported in *Cephalocereus senile* (Qin *et al.*, 1993), *Andrographis paniculata* (Moinuddin and Mendhulkar, 2013), *Morinda citrifolia* (Md. Abdullahil Baque *et al.*, 2012), *Citrus hystrix* (Suri *et al.*, 2002), *Marsilea quadrifolia* (Manjula and Mythili, 2012). However, this has not been reported in *B. montanum*, *D. roxburghii* and *C. variegatum*.

In the present investigation, it was found that the content of flavonoids varies with type, concentration and duration of elicitor treatment. In the present study, it was found that all the three elicitors revealed uniform acceleration pattern. Phenylalanine showed maximum elicitation of flavonoids at 25 μg/mL and at duration of 72 h in *B. montanum* (57.12 ± 0.09) when compared to *D. roxburghii* (35.26 ± 0.02) and *C. variegatum* (45.33± 0.14); CuSO₄ showed maximum elicitation of flavonoids at 20 μg/mL and at duration of 72 h in *B. montanum* (49.74 ± 0.13) when compared to *D. roxburghii* (31.59 ± 0.02) and *C. variegatum* (43.51± 0.13); *Pseudomonas aeruginosa* showed maximum elicitation at 3 μg/mL and at 48 h in *B. montanum* (55.93 ± 0.04), *C. variegatum* (48.37 ± 0.03) and *D. roxburghii* (35.15 ± 0.03). It was found that elicitor, Copper sulphate (CuSO₄) showed maximum elicitation at low concentration with duration of 72 h in *B. montanum* and *P. aeruginosa* showed maximum elicitation at highest concentration 3mg/L with 48hrs of duration in *B. montanum*. Overall it was analyzed that all the three elicitors accelerate the production of flavonoids but the phenylalanine was found to be the best and exhibited maximum elicitation in *B. montanum*. This present findings agrees with finding coincides with Cristina and Constantin (2011), Tumova and Polivkova (2006), Savitha *et al.*, (2006) and Maojun *et al.*, 2006.
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The methanolic leaf extract of *B. montanum* (sample name- SB3), *D. roxburghii* (sample name- SB2) and *C. variegatum* (sample name- SB1) were revealed through GC-MS, 15 major phytocomponents in *B. montanum and were identified for the first time namely 3-methyl-4-methylidenehexan-2-one, 2,4-Pentanedione, 3-diazo-, 1-(2,3-Dimethyl-6-oxabicyclo[3.1.0]hex-1-yl)ethanone, 6-Amino-1,3-dimethyl-2,4(1H,3H)-pyrimidinedione, 1-(2-Methyl-2H-tetrazol-5-yl)viny acetate, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one, 2,4,5-Trimethyl-1,3-dioxolane, 1,2,4,5-Tetramethyl-1,2,4,5-tetrazinane, (2R,3S)-2,3-Dimethyloxirane, Hydroxymethylfurfural, -Methyl-4-hepten-3-one, Sucrose, 2-(Hydroxymethyl)-2-nitropropan-1,3-diol, Propylene carbonate and Butoxyacetic acid, Whereas in methanolic leaf extract of *D. roxburghii* and *C. variegatum* no peaks were observed. This may be due to the absence of non volatile phytoconstituent. The present finding agrees with the findings of Wajahat Shah and Mahahpara Qadir (2014), Prabodh Satyal et al. (2013). Chitra et al., 2011; Elfahmi et al., (2011), they have reported 7, 17 and 15 compounds in *Euphorbia hirta* and *Jatropha curcas*.

At present much work has been done on quercetin among bioflavonoids and is considered as an active ingredient which has many roles which includes including anti-inflammatory, anti-cancerous, antibacterial, antiviral, anti-gonadotropic and anti-hepatotoxic activities (Lamson and Brignale, 2000). It is also considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation (Hollman and Katan 1997; Sakanshi et al., 2008). Ong et al., (2004) has checked the anticancer activity of quercetin.

In the present investigation quercetin quantification was performed through HPLC and found 9 peaks in methanol leaf extracts of *B. montanum* and 5 peaks of quercetin each in *D. roxburghii* and *C. variegatum* and these peaks were compared with the standard peak 3.2 at 254 nm, this indicates that significant amount of quercetin is present in methanol leaf extract of *B. montanum, D. roxburghii* and *C. variegatum*. This finding coincides with Neelam Verma and Nitu Trehan (2013); Garima and Baghel (2012), and they have also quantified quercetin in medicinal plants.
Nanotechnology is now creating new excitement in biological sciences especially in biomedical devices and Biotechnology as opined by Prabhu et al., (2010). Silver nanoparticles can be synthesized from biological or non-biological substrates. Non-biological substrates could lead to the synthesis of toxic by-products. Nanoparticles synthesis from plant extracts, micro-organisms and enzymes are safe and has plentiful benefits like ecofriendliness, biocompatibility, non-toxic, and cost effective (Saifuddin et al., 2009; Verma et al., 2010; Willner et al., 2007; Mohanpuria et al., 2008). The use of plant extracts for synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of scaling up the biohazards and elaborates process of maintaining cell cultures (Njagi, et al., 2011; Zargar et al., 2011). The unique properties of silver nanoparticles make them useful in different sectors like catalysis (Jana et al., 1999), chemical sensing (Frederix, 2003), biosensing (Songping, 2005), photonics (Velicov et al., 2003), electronics (Kreibig, 1974) and pharmaceuticals (Galletto et al., 1999).

In the present study, the silver nanoparticles were successfully synthesized and its antibacterial activity was observed. It was found that the synthesized silver nanoparticles inhibits the growth of $E. \text{coli}$. This is in complete agreement with Gardea-Torresdey et al., (2003) in Emblica officinalis, Ankamwar, et al., (2005a) and Shankar et al., (2003b) in Pelarogonium graveolens, Shankar et al., (2003a) in Cinnamomum camphora and Watt and Breyer-Brandwijk, (1962) in Jatropha curcas (latex).

Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. Jeong et al., (2005) has reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects. Further Mambio-Jones and Hoek, (2010) reported that because of antimicrobial capability of SNPs they are used in numerous products such as textiles, food storage containers, home appliances and also in medical devices.