ANTIOXIDANT ACTIVITY AND CHARACTERIZATION OF ETHANOLIC BARK EXTRACT OF *BOSWELLIA SERRATA*

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Abstract

Boswellia serrata (BS) is an important traditional medicinal plant also known as Indian frankincense, *is* currently represents an interesting topic for pharmaceutical research since it possesses several pharmacological properties (e.g., anti-inflammatory, antimicrobial, and antitumour). *B. serrata* (Salai/Salai guggul), is a moderate to large sized branching tree of family Burseraceae (Genus Boswellia), grows in dry mountainous regions of India, Northern Africa and Middle East. In the present research, *B. serrata* bark was collected from Pohra forest region, Dist. Amravati Maharashtra. Aqueous and Ethanolic extract of *B. serrata* bark was prepared to study *in vitro* antioxidant activity using DPPH assay. Our results showed that Ethanolic *B. serrata* bark extract shows more antioxidant activity i.e. 80% percent inhibition as compare to aqueous extract 60%. The TLC based separation of metabolites was peformed using Choloroform : Methanol (15 : 1) solvent system which suggest the presence of metabolites of several class. The GC-MS anlaysis of the Ethanolic *B. serrata* bark extract confirms the presence of various bioactive metabolites including sydone-3-benzyl, 4-karene, trans-1,2,4,5-diepoxy –methane, 5-eicosene etc in maximum amount.

Keywords: Antioxidant activity, *Boswellia serrata*, TLC, DPPH, GC-MS.

Introduction:

Herbal medications have been used for the relief of disease symptoms since ancient times (Maqsood et al2010). As a result of many years of struggle against various illnesses, humans learned to pursue drugs in barks, seeds, leaves, fruits and other plant parts (Petrovska 2012). It has been estimated that 25% of modern medicines are made from traditionally used plants and about 80% of the world population relies on herbal medicines in dealing with some aspects of their primary health care.

The plant *Boswellia serrata* is locally known as "Salai" in Hindi and belongs to family *Burseraceae*. It is distributed in the hilly region of the country and also occurs in quite abundance in the plains of central India. The plant is of medium size and on tapping provides a very important gum known as "OlegumResine" which is commonly known as "Indian Olibanum" or *"frankincense"*.

Their use in traditional medicine and cosmetics were very popular during early days. Particularly importance is attached to gums in "Unani" and ''Ayurvedic"systems of medicine for curing many diseases such as fevers, sexual debilities, cough and cold, dysentery and diarrhoea etc.

Antioxidants are micro constituents that inhibit lipid oxidation through prevention of the initiation or propagation of oxidizing chain reactions (Irshad et al 2012), suppress the formation of reactive oxygen species, and inhibit enzymes or chelate elements involved in free radical production and scavenge reactive species (Montoro et al 2005). These mechanisms of action are necessary in cell protection, thus contributing to the prevention of cardiovascular diseases, cancer and other chronic diseases (Pham Huy et al 2008; Willcox et al 2004). The search for natural antioxidant compounds has been intensified in recent years due to the reported data that synthetic antioxidants are dangerous to human health (Lobo et al 2010). In addition, natural-based compounds are inherently better tolerated by the body than synthetic compounds (Zaid et al 2010). Natural antioxidants are believed to be safer than synthetic ones. These antioxidants of plants are non-carcinogenic and shows anticanceractivity (Afroz et al 2014).

B. serrata (Family- Burseraceae), an oleo-gum-resin, is a medium size tree lavishly full-fledged in dry undulating parts of India. *B. serrata* has been used for a variety of therapeutic purposes such as cancer, inflammation, arthritis, asthma, psoriasis, colitis and as an anti-hyperlipidemic (Marinetz et al 1988; Shao et al 1998; Gupta et al 1998). leukotrienes synthesis by inhibiting 5- ipoxygenase enzyme in an enzyme directed, nonredox, non-competitive mechanism. The induction of many chronic and degenerative diseases is a direct result of oxidative stress caused by free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS), which cause oxidative damage of amino acids, lipids, proteins, and DNA (Ioana et al 2010). The degenerative diseases include ischemic heart disease, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases, and aging. The phytocompounds of natural plant foods, antioxidants have attracted researchers as they protect the human body against oxidative stress andprevent chronic non-communicable ailments (Yahia 2010).

The present study deals with antioxidant activities of the B. serrate bark extracts, Thin layer chromatographic (TLC) profiles of the ethanol extracts and also the objective of the present study is to identify the most probable antioxidant compounds present in plant part using GCMS.

MATERIALS AND METHODS

1. Collection, Extraction and Preparation of Plant Extract

In the present study, the four plants were selected assuming ethno pharmacological as well as folklore based therapeutic importance was collected from Pohra forest region with the help of local people Amravati (M.S.) India. The herbarium specimen was prepared, authenticated by Dr.–S. M. Bhuskute–from Department of Botany Bhawbhuti Mahavidyalaya, Amgaon, Gondia District, Maharashtra; India.

The Dry bark of *B. serrata* was cleaned, dried and grinded as a powder and stored for experimentation.

Cold extraction process was used for the preparation of drug. About 20 g powdered material was mixed in 100 ml distilled water and ethanol in conical flasks separately; both flask were kept for 24 hours for maximum drug extraction from bark. After 24 hours both extracts were filtered through muslin cloth and centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatant was allowed to evaporate under the vacuum conditions at 50 ± 2°C. During the experimentation stock solution (100 mg/ml) of tri-sodium citrate (positive control) and each extract was diluted to working range 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml.

2. Characterization of Plant Drug Extract:

a) DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The anti-oxidant activity of each of the plant extracts was determined using the colorimetric DPPH assay, as described by Shimada et al. (1992) to determine the radical scavenging activity of the plant extracts.

0.1 mM solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol and Ascorbic acid (1 mg/ml) standard was prepared.

The hydrogen donating capacity of test samples is quantified in terms of their ability to scavenge the relatively stable, organic free radical DPPH and by consequent reduction. The absorption of the deep violet DPPH solution is measured at 517 nm, after which absorption decreases due to decolorization to a yellow-white colour, in the event of reduction. This decrease in absorption is stoichiometric according to the degree of reduction (Arulpriya et al 2010).

The free-radical scavenging activity was estimated by DPPH assay. The reaction mixture contained 100 µl of test extracts and 2.9 ml of methanolic solution of 0.1 mM DPPH radical. The mixture was then shaken vigorously and incubated at 37° C for 30 min. The absorbance was measured at 517 nm using ascorbic acid as positive control lower absorbance of the reaction mixture indicated higher free radical scavenging activity, which was calculated using the equation given below. The steps were used to study (%) DPPH scavenging activity of both extracts along with standards different concentrations 0.2 to 1.0 mg/mL.

(%) DPPH scavenging effect = (Ac - As)/ (Ac) x100

Where,

Absorbance of control = Ac Absorbance of sample = As

b) Thin layer chromatography (TLC) profile of potent extract:

TLC isantechnique in which substances migrates when a solvent flows along thin layer of fine powder spread on glass or plastic plate. The well known coating materials are silica gel, alumina and cellulose to give stable layers whichoften contain binders like gypsumor starch.

Preparation of Plate: The pre-coated plates of adsorbent on glass, aluminum or plastic are accessible. The size of glass plate are characteristically 20 x 5 cm and the thickness of moist layer should be about 0.25 mm. Plates are dried in air for 30 min and then dried at 100-120°C for 1 h to activate the adsorbent.

TLC Separation: For further characterization only ethonolic extract was used as it was found more antioxidant as compare to aqueous extract.

The presence of active metabolites in *B. serrata* ethanolic extract was evaluated by TLC fingerprinting. The preparative TLC was performed on 15x10 cm glass plates coated with 1 mm layer of silica gel, the plates were dried in room temperature for 30 minutes and then activated in the oven at 110˚ C for next 30 minutes.

Different solvent systems were used and about 20 µl of extract was spotted on the silica plates. The experimental plates were developed in a chromatographic chamber contain saturated the solvent system. Plate development requires 15 to 20 minutes; the plates were visualized at 365 nm under UV transilluminator (Cleaver). Rf values of bands were recorded by using formula and noted down.

$$Rf = \frac{\text{Distance traveled by sample}}{\text{Distance traveled by Solvent}}$$

c) GCMS analysis of bioactive fraction:

The Ethanolic extract was diluted in ethanol, centrifuged at 4,000 r.p.m. and the supernatant was used for the HR-LCMS analysis to get the idea of presence of several biochemical present in the extract. The sample was sent to BioRajLab Nagpur for GCMS analysis.

Gas chromatograph with a mass spectrometer (GC-MS/MS) instrument (Make-Bruker Scion, Model- TQ MS System) was used for the analysis of plant extract sample by attempting following conditions: Column DB-5MS Agilent (30m x 0.25mm1D), composed of 100% dimethyl polysiloxane). The sample was filtered with 0.45 micron and it was injected into GC-MS instrument. For GC-MS detection, electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1.0 ml/min with a split ratio of 10:1. The oven temperature was operated according to the following oven temperature: 40°C held for 1 min, raising at the rate of20°**C** min^{-1} up to 150°**C** then, raising at the rate of 3°**C** min^{-1}, hold for 0 min and raising at the rate of 20°**C** min^{-1} up to 300°C with 10 min held, injector temperature and volume 250°C and 2µL, respectively. The total GC running time was about 50 min. The MS operating conditions were ionization voltage 70 eV, source temperature of 250°C,inlet line temperature 280°C, mass scan (m/z)-30-500, solvent delay: 3.0 min, total MS running time 47 min. The mass spectra of compounds were identified by comparing the mass spectra obtained from their related chromatographic peaks with NIST mass spectral libraries.

RESULTS AND DISCUSSION

1. Collection, Extraction and Preparation of Plant Extract:

Bark sample of *B. serrata* was collected successfully using ethnobotanical knowledge and earlier reports from Pohra forest, Amravati. Both extract prepared using the above explained method were gelly like, brownish dark in color. Extracts and different dilutions were prepared successfully and stored for future use.

Figure 1: Bark of *B. serrata* plant

2. Characterization of Plant Drug Extract:

a) Antioxidant activity using DPPH free radical scavenging assay

The antioxidant activity of aqueous and ethanolic extracts of *B. serrata* and ascorbic acidwas evaluated in *in vitro* models using different concentrations. The antioxidant reacts with DPPH radical (purple colour) and converts it into a colourless DPPH. The amount of DPPH reduced could be quantified by measuring decrease in absorbance at 517 nm. The aqueous extract significantly reduces DPPH radical in dose dependent manner. At the concentration of 1.0 mg/mL Ethanolic extract of *B. serrata* shows maximum % inhibition i.e. 84.51±6.97 as compare to aqueous extract (Table 1).

DPPH is a commonly used for evaluation of antioxidant activity because of its stability and simplicity during the assay. This assay gives reliable information related to the antioxidant ability of the compounds.

The medicinal ability of *B. serrata*hasstudied for arthritis, asthma, diabetes mellitus, colitis, and cancer (Azemi et al 2012)in relation of their antioxidant and anti-inflammatory properties (Florean and Diederich 2012).The antioxidant andfree radical scavenging property extracts of *B. serrata* are directly relates with total phenolic and flavonoid presence (Singh et al 2012). Extraction procedures suggest that the antioxidant activity depends on the polarity of the solvent. Earlier reports suggest that the essential oil of *B.dalzielii* was characterized by low antioxidant activity (Paul et al 2012) and that this was due to the extraction method adopted (e.g., low polarity of the solvent) that determined the absence of phenolics, especially flavonoids.

Table 1 :DPPH free radical scavenging percent inhibition of *B. serrata* extracts

Concentration (in mg/mL)	**% Inhibition**		
	Ascorbic acid	**Ethanolic Bark Extract**	**Aqueous Bark Extract**
0.2	84.07±4.07	35.84±3.36	63.27±5.55
0.4	84.51±3.58	76.54±5.56	67.24±4.21
0.6	85.39±7.76	77.43±4.13	68.14±4.30
0.8	85.84±7.88	80.53±5.88	75.66±5.60
1.0	86.30±5.46	84.51±6.97	78.76±6.12

* All the data statistically analyzed with mean±SEM (n=3)

b) Thin layer chromatography (TLC) profile of potent extract:

The DPPH assay results suggests that the ethanolic bark extract shows more antioxidant activity as compare to aqueous bark extract. Thus for further study ethanolic extract is used. The ethanolic extract of bark was characterizes for the prsence of various metabolites fingerprint. For these purpose 2 different solvent systems were optimized which shows proper separation and pattern of metabolites on TLC (Table 2 and figure 2).

Fighure 2: TLC separation of metabolites of *B. serrata* of ethanolic extract

(a) Toluen : Ethyl acetate : Hexane : Formic acid (8:2:1:0.3) (b) Chloroform : Methanol (15:1)

Table 2: Observation of separated metabolites by TLC under UV transilluminator

Sr. No.	Mobile phases	No.of bands observed in Ethanolic extract	Colour of bands	Rf
1.	Toluen : Ethyl acetate : Hexane : Formic acid (8:2:1:0.3)	4	Light blue	0.52
			Light green	0.57
			Dark green	0.77
			Red	0.84
2.	Chloroform : Methanol (15:1)	9	Light green	0.03
			Light green	0.05
			Light green	0.11
			Light yellow	0.25
			Light blue	0.30
			Yellow	0.42
			Blue	0.54
			Dark blue	0.69
			Sky blue	0.92

D) GCMS Analysis of *B. serrata* Ethanolic Extract

The Ethanolic extract of *B. serrata* was sent for GCMS analysis. The large range of compounds in the extract was determined by the peak and retention times. The high number of peaks showed the complexity of compounds in the plant extract. Some compounds were identified in high concentration in the extract at specific retention time.

This results support the presence of long range of compounds present in the Ethanolic extract of *B. serrata*. The chromatogram of the GCMS analysis of Ethanolic extracts of *B. serrata* is shown in figure 3 and the compounds in the extracts were noted down in Table 3. The present study suggests the most probable components of bark of *B. serrata.* In earlier reports most of the workers has studiedthe phytochemical content of *B. serrata* gum resin which also depends on origin and the geography(Gupta et al 2016). The chemicals include 30–60% triterpenes (such as α- and β-boswellic acids, lupeolic acid), 5–10% essential oils, and 20–35% polysaccharides (Catanzaro et al 2015). In aqueous and ethanolic extracts of *B. serrata*, alkaloids, carbohydrates, phytosterols, terpenoids, phenolic compounds, flavonoids, tannins, glycosides, proteins, and saponins has also reported(Singh et al 2012; Barik et al 2016).

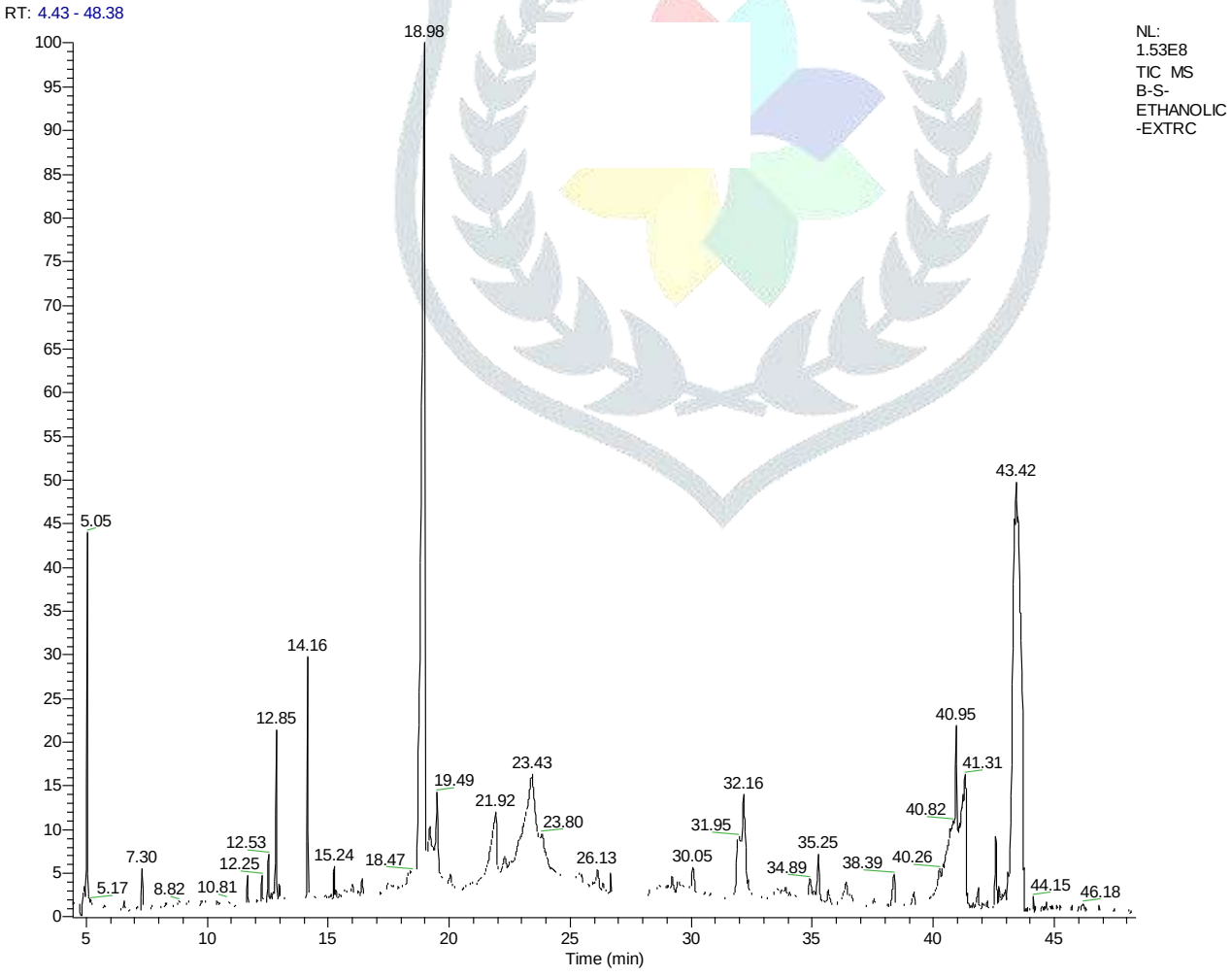

Figure 3: GC-MS Spectra of ethanolic extract of sample

Table 3: List of identified compounds from ethanolic extract of sample

Sr. No.	Name of compound	RT (min)	Peak area	%
1	Methylglyoxal	5.05	2849941	0.04049384
2	Diacetyl sulfide	7.3	26480154	0.376247484
3	1-Methylbutyl Methacrylate	11.66	17329731	0.246232242
4	Azidamantane	12.25	21525616	0.305850142
5	a-cis-Ocimene	12.53	26207412	0.372372185
6	**Sydone 3-benzyl**	**12.85**	**589247811**	**8.372421326**
7	**4-Carene**	**14.16**	**793471028**	**11.27415942**
8	**trans-1,2,4,5-Diepoxy p-Menthane**	**18.98**	**3325892331**	**47.25647064**
9	4-Methoxyphenyl heptanoate	19.49	128334364	1.823459241
10	Lilac aldehyde A	21.92	341264833	4.848915706
11	5-Eicosene	23.43	793471028	11.27415942
12	Decylhydroxylamine	32.16	322499912	4.582291339
13	Di iso octyl phalate	36.25	133432864	1.89590209
14	Ethyl iso-allocholate	38.39	125142580	1.77810827
15	All trans squalene	40.95	390812151	5.552916662

Conclusion

B.sarrata plant is found in plenty in the Pohra region and it is found to be one of the most dominant plant of Pohra region; it might be due to suitable environment availability for this plant.Out of the many extraction processes, cold extraction is one of the most patent method of extraction as it does not harm to the compounds present in the sample, and of course it is simple, cheapest and relative effective method. Ethonol was found suitable solvent for extraction as it shows better separation of metabolites and more % inhibition. TLC was optimized and Choloroform : Methanol (15 : 1) solvent system was found most suitable for separation of metabolites.GCMS analysis suggests a long range of most probable metabolites present in plant extract with different concentrations. These data can be used to verify the further future and past research related to the metabolites of *B. serrata.*

Refferences:

1. Afroz R, Tanvir ME, Islam MDA, Alam F, Gan SH, Khalil MDI. 2014. Potential antioxidant and antibacterial properties of a popular JUJUBIZ fruit: apple Kul (*Zizyphusmauritiana*). J Food Biochem. 38:592–601.

2. Arulpriya P, Lalitha P, Hemalatha S. 2010. Antioxidant activities of the extracts of the aerial roots of *Pothosaurea*. Der Chem. Sin. 1:73–79.

3. Azemi ME, Namjoyan F, Khodayar MJ, Ahmadpour F, Padok AD, Panahi M. 2012. The antioxidant capacity and anti-diabetic effect of *Boswellia serrata* triana and planch aqueous extract in fertile female diabetic rats and

the possible effects on reproduction and histological changes in the liver and kidneys. Jundishapur Journal of Natural Pharmaceutical Products. 7(4)168–175.

4. Barik CS, Kanungo SK, Tripathy NK, Panda J, Sahoo B. 2016. Evaluation of free radical scavenging activity of polyherbal formulations containing four different plant extracts. Der Pharmacia Lettre. 8(2)496–501.

5. Catanzaro D, Rancan S, Orso G et al. 2015. *Boswellia serrata* preserves intestinal epithelial barrier from oxidative and inflammatory damage, PLoS ONE. 10(5).

6. Florean C and Diederich M. 2012. Redox regulation—natural compound as regulators of inflammation signaling," Biochemical Pharmacology. 84(10)1223–1224.

7. Gupta I, Gupta V, Gupta S, Parihar A, Ludtke R, Safayhi H and Ammon HP. 1998. Effects of *Boswellia serrata* gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study. European Journal of Medical Research. 3:511.

8. Gupta M, Rout PK, Misra LN et al. 2016. Chemical composition and bioactivity of *Boswellia serrata* Roxb. essential oil in relation to geographical variation," Plant Biosystems. 1–7.

9. Ioana I, Volf I, Popa VI. 2010. A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chem. 126:821–35.

10. Irshad M, Zafaryab M, Singh M, Rizvi MMA. 2012. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. International Journal of Medicinal Chemistry. 1-6.

11. Lobo V, Patil A, Phatak A, Chandra N. 2010. Free radicals, antioxidants and functional foods: impact on human health. Pharmacognosy Reviews. 4:118-26.

12. Maqsood S, Singh P, Samoon MH, Balange AK. 2010. Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio*challenged with *Aeromonas hydrophila.* International Aquatic Research. 2:77-85.

13. Marinetz D, Lohs K., and Janzen J. 1988. Weihrauch and myrrhe: Kulturgeschichtliche and wirtschaftl. BedeutungBotanikChemirWissVertgesStuttgast. 153.

14. Montoro P, Braca A, Pizza C, De Tommasi N. 2005. Structure antioxidant activity relationships of flavonoids isolated from different plant species. Food Chemistry. 92:349-55.

15. Paul M, Brüning G, Bergmann J, Jauch J. 2012. A thin-layer chromatography method for the identification of three different olibanum resins (*Boswellia serrata, Boswellia papyrifera* and *Boswellia carterii,* respectively, *Boswellia sacra*)," Phytochemical Analysis. 23(2)184–189.

16. Petrovska BB. 2012. Historical review of medicinal plants' usage. Pharmacognosy Reviews. 6:1-5.

17. Pham-Huy LA, He H, Pham-Huy C. 2008. Free radicals, antioxidants in disease and health. International Journal of Biomedical Science. 4:89-96.

18. Shao Y, Ho CT, Chin CK, Badmaev V, Ma W and Huang MT. 1998. Inhibitory activity of boswellic acids from *Boswellia serrata* against human leukemia HL-60 cells in culture. Planta Medica.**64:** 328.

19. Singh HP, Yadav IK, Chandra D, Jain DA. 2012. *In vitro* antioxidant and free radical scavenging activity of different extracts of *Boerhaviadiffusa* and *Boswellia serrata*. International Journal of Pharma Sciences and Research. 3(11)503–511.

20. Willcox JK, Ash SL, Catignani GL. 2004. Antioxidants and prevention of chronic disease. Critical Reviews in Food Science and Nutrition. 44:275-95.

21. Yahia EM. 2010. The contribution of fruit and vegetable consumption to human health. In: Rosa LA, Alvarez-Parrilla E, Gonzalez-Aguirala GA, editors. Fruit and vegetable phytochemicals chemistry, nutritional value and stability. Hoboken: Wiley-Blackwell.

22. Zaid H, Raiyn J, Nasser A, Saad B, Rayan A. 2010. Physicochemical properties of natural based products versus synthetic chemicals. Open Nutraceuticals Journal. 3:194-202.