*Asian Journal of Chemistry* Vol. 21, No. 5 (2009), 3529-3534

# Stability Study on *Boswellia serrata* (Hydro-alcoholic) Extract

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*Boswellia serrata* Roxb. (family: Burseraceae) are reported to have antioxidant, antiarthritic, immunomodulatory and anticancer activity. The present study deals with elucidate the physical, chemical, pharmacological and microbiological attributes of the *Boswellia serrata* extract filled in double polythene bag and in capsule with respect to real and accelerated storage conditions on 6 months storage. In this study, extract as a whole was considered as active substance and physical, chemical, pharmaceutical and microbiological characteristic were studied at predetermined intervals.

**Key Words: *Boswellia serrata* (hydro-alcoholic) extract, Stability study.**

## INTRODUCTION

The role of herbals as drugs, neutraceuticals and dietary supplements is gaining popularity. There have been several examples of poor quality of this product. The reasons for quality are many but most important one is formidable challenges faced in formulating herbal products. These challenges are due to poor physical properties namely hygroscopicity, low bulk density, variable particle size distributions and chemically complex nature of herbals. Hygroscopicity is a crucial for product physical and chemical stability while, flow ability and compression properties are important for processing. Herbals contain diverse chemical classes and there are several reports of instability of chemical groups to heat[1], light[2], pH[3,4] and oxygen[5]. Due to this reasons, few studies have been reported on stability testing of herbals. Stability testing of herbals is important, as instability modifies 3 important attributes of the product *i.e.* quality, safety and efficacy. The present study attempts to study physical, chemical, pharmaceutical and microbiological stability of *Boswellia serrata* extract at real time and accelerated condition.

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## EXPERIMENTAL

**Procurement of extract:** *Boswellia serrata* extract (BSE) was purchased from Natural Remedies Pvt. Ltd. Bangalore Batch no BSE/2005. Extract was stored in double polythene bags and in '0' shape hard gelatin capsules on real (25 ºC/60 % RH) and accelerated (40 ºC/75 % RH) stability for six months immediately.

**Mobile phase optimization:** Initially chloroform and methanol were tried in different ratios. The developed spots lack compactness and were diffused. After that hexane, chloroform and methanol were used at different ratios, but it was found that developed spots were not so resolved because of methanol and chloroform in mobile phase help in migration of drug where as hexane retards the migration of drug. It was observed that drying of TLC plate under I.R. lamp after spotting and presaturation of TLC chamber with mobile phase for 45 min ensured the reproducibility of $R_f$ value but the typical peak nature was missing with dragging of spots. Then hexane and ethyl acetate were tried at different ratios (6:4, 8:2 and 8.5:1.5). In case of mobile phase consisting of ratios (6:4 and 8:2), the $R_f$ values were not too high. Finally by changing the solvent system to non-polar (7:3), the mobile phase was optimized producing good resolution with $R_f$ value of 0.62 for β-boswellic acid (BA) and 0.41 for acetyl-β-boswellic acid (ABA). When densitometry scanning was performed at 260 nm, the spot appeared more compact and peak shape more symmetrical. Then the TLC plates were pretreated with methanol and activated at 110 ºC for 10 min. The chamber saturation time was optimized at 20 min at room temperature. It was required to eliminate the edge effect.

**Sample preparation:** For preparation of samples, 20 mg of *Boswellia serrata* extract weighed accurately were taken from both polythene bags and capsule stored at real time and accelerated conditions from 0 month to up to 6th month storage and then transferred to a 10 mL volumetric flask containing 5 mL of methanol producing the resulting solution 4 mg/mL *i.e.*, 4 µg/µL and then sonicated for 15 min. The resulting solution was centrifuged at 3000 rpm for 5 min and filtered using 0.45 micron filter (Millipore, Milford, MA). 10 µL of each sample solution was applied 6 times to the HPTLC plate to give the concentration of 40 µg/spot or 40,000 ng/spot and spots was measured at 260 nm.

**Selection of analytical wavelength:** In order to determined the absorbance maxima, same concentrations of the prepared samples were spotted on the pre coated HPTLC plate and developed. After developing, the plate was dried and then scanned for spectrum in the range of 200-360 nm.

**Physical characters:** Physical constants such as organoleptic characters (colour, odour, taste and physical taste), total ash and acid in-soluble ash of Boswellia hydro-alcoholic extract on real time and accelerated time studies were determined. The total ash method was designed to measure the total amount of material remaining after ignition. About 4 g of the Boswellia extract on zero time at real time and accelerated time were accurately weighed. Then in a previously ignited and tarred silica crucible spread the material in an even layer and ignited it by gradually

increasing the heat to 500-600 ºC until it is white, indicating the absence of carbon. Cool in desiccators and then weighed without delay. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth[6]. The moisture content of Boswellia (hydro-alcoholic) extract was determined by Karl Fischer titration method[7].

**Chemical and microbiological stability:** Chemical stability of Boswellia extract was studied by developed HPTLC method[8,9]. Methanolic solution of 4 mg/mL of extract on room temperature and accelerated condition was prepared and 4 µg/µL spot was applied to TLC plate at every time interval in study. Total peak area, acetyl-β-boswellic acid peak area at $R_f$ 0.41 and β-boswellic acid peak area at $R_f$ of 0.62 were evaluated by densitometry analysis. Microbiological stability of Boswellia extract was determined by plate count method[10]. In pharmaceutical properties, angle of repose was determined by fixed funnel method and density of powder were determined by Density tester USP[11].

## RESULTS AND DISCUSSION

**HPTLC method optimization:** The mobile phase was optimized with suitable method and same concentration of samples prepared for extract at (zero time), extract at real time and accelerated storage condition filled both in double polythene bag and capsule from 0 to 6 month were applied on HPTLC plate.

**Selection of λ maxima:** The maximum absorbance of β-boswellic acid (peak 2) and acetyl-β-boswellic acid (peak 1) in extract was found to be 260 and 257 nm. respectively in densitometry scanning.

**Stability study:** In stability studies, the study duration was of 6 months and was carried out at accelerated and real time by testing the extract after each month.

**Physical characteristic and pharmaceutical characteristic:** In physical studies, (Table-2) suggest no significant change in moisture content at real time (4.67-5.67 %) and accelerated conditions (4.68-5.97 %) in case of *Boswellia serrata* extract in polythene bag and it was found 4.65-5.97 % (in real capsules) and 4.93-5.99 % (in accelerated capsules). Significant change in form was also observed at real time (little clumps) and accelerated (clumps) at end of 6 months in case of *Boswellia serrata* extract in polythene bag. Pharmaceutical stability testing of extracts was found failing at real time (after 4th month) and accelerated storage (after 3rd month) due to clump formation (Table-1).

**Chemical characteristic:** The result suggests that at real time, the relative % change in peak 1 area for the (extract in polythene bag) decreased up to 87.89 % but 86.89 % in case of capsule, while in accelerated condition decreased up to 87.34 % (extract filled in bag) but 84.68 % (capsule) from zero (100 %) to 6 month (Fig. 1). The peak 2 area also decreased up to 56.24 % (real time extract) and 59.84 % (real time capsule), while in accelerated condition decreased (extract in bag) up to 82.63 and 67.54 % (extract in capsule) (Fig. 2) and the result so obtained are recorded in (Tables 3 and 4).

TABLE-1
PHARMACEUTICAL PROPERTIES
(REAL TIMES AND ACCELERATED TIME EXTRACTS)

| Parameter | Real time extract for pharmaceutical properties | | | | | | |
|---|---|---|---|---|---|---|---|
| Months | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Bulk density | 0.34 | 0.34 | 0.35 | 0.35 | 0.35 | NA | NA |
| Tapped density | 0.51 | 0.53 | 0.52 | 0.5 | 0.53 | NA | NA |
| Carr's index | 33.34 | 35.84 | 32.69 | 31.78 | 33.96 | NA | NA |
| Angle of repose | 29.14º | 27.34º | 28.15º | 26.72º | 26.18º | NA | NA |
| | Accelerated time extract for pharmaceutical properties | | | | | | |
| Bulk density | 0.34 | 0.34 | 0.35 | 0.35 | NA | NA | NA |
| Tapped density | 0.51 | 0.53 | 0.52 | 0.5 | NA | NA | NA |
| Carr's index | 33.34 | 35.84 | 32.69 | 31.78 | NA | NA | NA |
| Angle of repose | 28.344º | 27.45º | 26.78º | 25.55º | NAº | NA | NA |

TABLE-2
PHYSICAL PROPERTIES OF (REAL TIME EXTRACTS, ACCELERATED TIME EXTRACTS, REAL TIME CAPSULE, ACCELERATED TIME CAPSULE)

| Parameter | Physical properties Real time extract | | Physical properties Accelerated time extract | |
|---|---|---|---|---|
| | 0 month | 6 month | 0 month | 6 month |
| Colour | Light yellow | Light yellow | Light yellow | Faint yellow |
| Apparance | - | - | - | - |
| Odour and taste | Light yellow Powder | Light yellow Powder | Light yellow Powder | Light yellow Powder |
| Physical state | Free flowing | Little clump formation | Free flowing | Clump formation |
| Ash content (% w/w) | 1.60 | 1.60 | 1.60 | 1.70 |
| Acid insoluble ash (%w/w) | 0. 40 | 0.40 | 0.40 | 0.30 |
| Moisture content (% w/w) | 4.67 | 5.67 | 4.68 | 5.97 |
| pH of 5 % w/v suspension | 5.60 | 5.70 | 5.60 | 5.70 |
| Disintegration time (min) | - | - | - | - |
| | Physical properties Real time capsule | | Physical properties Accelerated time capsule | |
| Colour | Light yellow | Light yellow | Light yellow | Light yellow |
| Apparance | Hard gelatin capsule of size 0 | Hard gelatin capsule of size 0 | Hard gelatin capsule of size 0 | Hard gelatin capsule of size 0 |
| Odour and taste | Light yellow Powder | Light yellow Powder | Light yellow Powder | Light yellow Powder |
| Physical state | Fine powder | Little clump formation | Fine powder | Clump formed |
| Ash content (% w/w) | 1.60 | 1.70 | 1.70 | 1.60 |
| Acid insoluble ash (%w/w) | 0.40 | 0.40 | 0.40 | 0.40 |
| Moisture content (% w/w) | 4.65 | 5.97 | 4.93 | 5.99 |
| pH of 5 % w/v suspension | 5.60 | 5.60 | 5.60 | 5.70 |
| Disintegration time (min) | 12 | 12 | 11 | 11 |

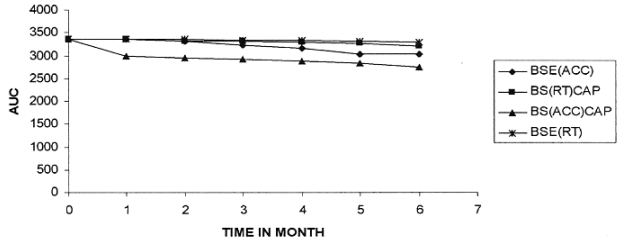


Fig. 1

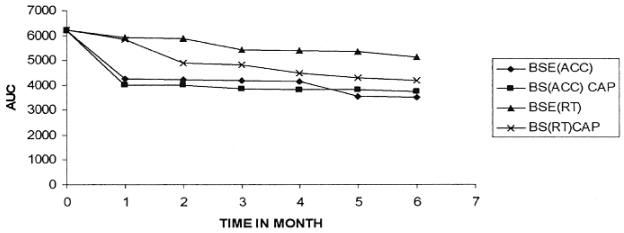


Fig. 2

TABLE-3
CHANGE AND RELATIVE % CHANGE OF PEAK 1 AREA

| Time | BSE (RT) | | BSE (ACC) | | RT CAP | | ACC CAP | |
|---|---|---|---|---|---|---|---|---|
| | Peak area | Relative % change in peak area | Peak area | Relative % change in peak area | Peak area | Relative % change in peak area | Peak area | Relative % change in peak area |
| 0 | 3357.60 | 100.00 | 3357.6 | 100.00 | 3357.6 | 100.00 | 3357.6 | 100.00 |
| 1 | 3318.70 | 98.71 | 3348.1 | 97.45 | 2980.7 | 93.56 | 3351.7 | 91.67 |
| 2 | 3313.80 | 97.45 | 3318.6 | 96.34 | 2948.6 | 92.34 | 3347.9 | 90.45 |
| 3 | 3216.40 | 95.32 | 3313.8 | 95.23 | 2931.5 | 91.45 | 3340.9 | 89.34 |
| 4 | 3431.40 | 92.46 | 3287.0 | 92.56 | 2874.4 | 91.97 | 3334.4 | 87.35 |
| 5 | 3041.00 | 88.78 | 3270.8 | 89.56 | 2846.4 | 88.34 | 3313.4 | 85.56 |
| 6 | 3033.61 | 87.89 | 3199.5 | 87.34 | 2762.8 | 86.89 | 3298.2 | 84.68 |

BSE = *Boswellia serrata* extract; RT = Real time, ACC = Accelerated, CAP = Capsule

TABLE-4
CHANGE AND RELATIVE % CHANGE OF PEAK 2 AREA

| Time | RT Extract | | ACC Extract | | RT CAP | | ACC CAP | |
|---|---|---|---|---|---|---|---|---|
| | Peak area | Relative % change in peak area | Peak area | Relative % change in peak area | Peak area | Relative % change in peak area | Peak area | Relative % change in peak area |
| 0 | 6204.9 | 100.00 | 6204.9 | 100.00010 | 6204.9 | 100.00000 | 6204.90 | 100.00000 |
| 1 | 4249.3 | 68.48 | 5919.4 | 95.39880 | 3997.2 | 64.42006 | 5842.00 | 94.15140 |
| 2 | 4232.9 | 68.21 | 5873.7 | 94.66228 | 3987.5 | 64.26373 | 4907.70 | 79.09394 |
| 3 | 4191.7 | 67.55 | 5427.2 | 87.46636 | 3828.1 | 61.69479 | 4818.40 | 77.65476 |
| 4 | 4151.5 | 66.90 | 5399.4 | 87.01832 | 3785.4 | 61.00662 | 4496.70 | 72.47014 |
| 5 | 3526.3 | 56.83 | 5329.2 | 85.88696 | 3755.1 | 60.51830 | 4494.70 | 72.43791 |
| 6 | 3489.8 | 56.24 | 5127.3 | 82.63308 | 3713.1 | 59.84142 | 4185.54 | 67.45540 |

RT = Real time, ACC = Accelerated, CAP = Capsule.

**Micro-biological analysis:** No significant change was found in total microbial load of the extract in both real time and accelerated conditions on 6 months storage. In conclusion, extract when stored at real time showed no significant change in physical (moisture content, form) and pharmaceutical (flow and compressibility) properties but having significant changes in chemical (β-boswellic acid and acetyl-β-boswellic acid peak areas at $R_f$ 0.62 and 0.41, respectively) on 6 months storage. In accelerated conditions, these changes were seen after 3rd months of storage. It was also observed that acetyl-β-boswellic acid was much protected than β-boswellic acid. The extract in capsule is also more protected than that of in double polythene bag.

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(*Received*: 19 May 2008; *Accepted*: 10 February 2009) AJC-7221