# TOTAL PHENOLIC, FLAVONOID CONTENTS, REDUCING POWER AND FREE RADICAL SCAVENGING POTENTIAL OF *PLEUROSPERMUM BRUNONIS*

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**Abstract—** In the present investigation it was observed that the chloroform extract of *Pleurospermum brunonis* (PCH) possess high total phenolic content (**24.03±0.71**) whereas maximum total flavonoid content (**3.39±0.15**) was observed in butanol extract (PBU). The reducing potential of butanol extracts was better than ascorbic acid. The DPPH scavenging activity at 400 (**93.73±1.11**) and 500 µg/ml (**96.26±0.93)** is almost similar to standard in butanol extract. Antioxidants are substances that help prevent certain types of cell damage, especially those caused by oxidation. When certain types of oxygen molecules are allowed to travel freely in the body they cause what is known as oxidative damage which leads to the formation of free radicals. Free radicals are very dangerous to the body's tissues and have been connected to cancer and premature aging.

**Keywords—** *Pleurospermum brunonis*, free radicals, antioxidant assays, Phenolic compounds, Flavonoid.

## INTRODUCTION

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane [1]. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of *antioxidants.* Free radicals are known to cause body cell decomposition and therefore are the main culprit in our aging process Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases. Cancer initiation and promotion is associated with chromosomal defects and oncogene activation. It is possible that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumor formation. The highly significant correlation between consumption of fats and oils and death rates from leukemia and malignant neoplasia of the breast, ovaries and rectum among persons over 55 years may be a reflection of greater lipid per oxidation [2]. Various human diseases such as Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis and atherosclerosis [3, 4] are also initiated by free radicals. Antioxidants act as radical scavengers, and are able to protect the human body from these diseases [5, 6]. Flavonoids can be used for the antimicrobial, antioxidant and cytotoxic activities of some Yemeni medicinal plants [7]. A great number of natural medicinal plants have been tested for their antioxidant activities and results have shown that the crude extracts or isolated pure compounds from them were more effective antioxidants *in vitro* than BHT or vitamin E [8]. So, medicinal plants can be a potential source of natural antioxidants. *Pleurospermum brunonis* (Vernacular Hiyarn, Nesar, Losar) [9] is distributed in the temperate regions of Kashmir, Himachal Pradesh and in the south east. In Kashmir the plant grows at an altitude of 4500-5000m and prefers a sunny site [10, 11]. *P. brunonis* is used as an ingredient of incense prepared by the local people [12]. Gugul Dhoop locally known in Kumaun Himalaya is made from dried leaves and flowers [17]. The powder of the flowering shoot is mixed with cow's fresh butter and messaged over the entire body to allay fevers. The dried herb or the garland prepared from the plant is used to protect woolen cloths from the attack of moths and silver fish [9, 16]. Due to strong aromatic character it is used for extraction of essential oil, which is used in cosmetic, perfumes, for the preparation of wormicidal and insect repellent products [13]. The paste of leaves and terminal shoot mixed with black pepper is applied to boils, cuts, wounds, ulcer and weeping eczema. The fine paste of leaves is reported highly efficacious in the abscess of the breast and in fire burns [14]. It is used to scent butter, for incense and moth repellant [15].

## MATERIALS AND METHODS

### Plant material

The plant material of *P. brunonis* was collected from Daggan Dhar (4200-4300 m asl) Bhalessa, District Doda, Jammu & Kashmir, in July, 2010, The plant was identified by Dr. Sumer Chand, Systematic Botany Division, FRI, Dehradun. The voucher specimen (Hr. no. 61) was deposited in the herbarium of Department of Botany, Govt. P. G. College Uttarkashi, Uttarakhand.

### Preparation of extracts:

The air-dried and powdered aerial parts (3 kg) of *Pleurospermum brunonis* were exhaustively defatted with light petroleum ether ($60\text{-}80^0$). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and

a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:$H_2O$:MeOH (6:4:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (PCH) (13.5 g). The aqueous layer was partitioned with ethyl acetate and n-butanol in a separatory funnel. Each layer was separated and concentrated under reduced pressure to give EtOAc extract (PEA) (15.3g) and BuOH extract (PBU) (18.5g). Few grams of each extract were purified and subjected to carry this biological activity.

## QUALITATIVE ANALYSIS OF CRUDE EXTRACTS

Qualitative analysis of different extract like solubility test, foam test, alkaloid Meyers test, $FeCl_3$ test, carbohydrate test and ninhydrin test are shown in table 1.

**Table 1: Qualitative analysis of crude extracts of various extracts from Aerial parts of *P. brunonis.***

| Extract | Solubility Test | Foam Test | $Fecl_3$ Test | Alkaloid Meyors | Carbohydrate Test | Ninhydrin |
|---|---|---|---|---|---|---|
| **PCH** | -- | +++ | - | + | ++ | - |
| **PEA** | ++ | + | - | + | - | - |
| **PBU** | ++ | - | + | - | ++ | + |

## DETERMINATION OF TOTAL PHENOLICS AND TOTAL FLAVONOID CONTENTS:

The total phenolic content (TPC) and total flavonoid content (TFC) of the different extracts were determined by method of Folin–Ciocalteu reaction [18], using Gallic acid as standard. To the extract, Folin–Ciocalteu reagent and $Na_2CO_3$ was added. After 20 min incubation at room temperature, the absorbance was measured at 730 nm.

The total flavonoid content (TFC) of the different extracts was determined by slightly modified method (Nieva Moreno et al., 2000) [19]. To the extract, potassium acetate and aluminium nitrate was added. After 40 min incubation at room temperature, the absorbance was measured at 415 nm using quercetin as standard Table 2.

**Table 2: Total phenolic and total flavonoid contents of various extracts from Aerial parts of *P. brunonis.***

| S. NO. | *EXTRACT* | Total flavonoids mg Quercetin/100mg | Total phenolics mg GAE/100mg |
|---|---|---|---|
| 1 | **PCH** | 2.32±0.05 | **24.03±0.71** |
| 2 | **PEA** | 2.23±0.06 | 13.24±0.49 |
| 3 | **PBU** | **3.39±0.15** | 15.94±0.82 |

## ANTIOXIDANT TESTING ASSAYS:

### Determination of reducing power (RP):

The iron (III) reductive capacity was assessed as described by Oyaizu [20]. Briefly, 1 ml of extract in an appropriate solvent was mixed with phosphate buffer and $K_3[Fe(CN)_6]$solution. After 30 min at 50 ˚C, 10% TCA was added and the mixture was centrifuged for 10 min at 2000 rpm. Finally, a 2.5 ml aliquot was mixed with 2.5 ml ultra-pure water and 0.5 ml of 0.1% $FeCl_3$, the absorbance was recorded at 700 nm, The results are reported as mean ±SEM (n=3) in table 3 and fig 1. The experiments were performed in triplicate.

**Table 3: Reducing potential of different extracts of aerial parts of *P. brunonis.***

| Conc./Extract | 100 µg | 200 µg | 300 µg | 400 µg | 500 µg |
|---|---|---|---|---|---|
| STD | 0.23 | 0.43 | 0.53 | 0.63 | 0.660 |
| PCH | 0.047±0.001 | 0.08±0.002 | 0.13±0.001 | 0.206±0.001 | 0.256±0.002 |
| PEA | 0.189±0.002 | 0.28±0.003 | 0.436±0.004 | 0.469±0.01 | 0.558±0.007 |
| PBU | **0.214±0.004** | **0.313±0.004** | **0.47±0.005** | **0.617±0.006** | **0.684±0.006** |

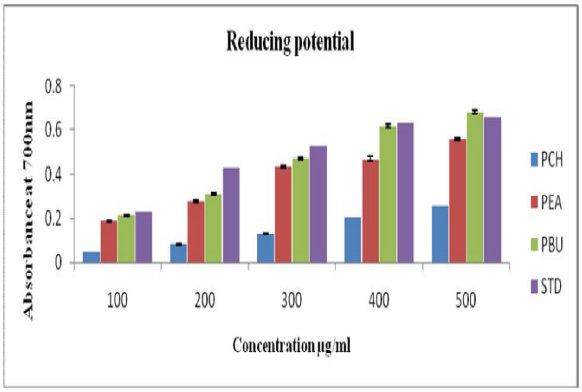


**Figure 1. Reducing potential of *P. brunonis* extract.**

### 1, 1-Diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH):

The ability of the extract to scavenge DPPH radicals was assessed as described by Ohinishi et al. (1994) [21]. To the different concentrations of extract, 3 ml of freshly prepared ethanolic DPPH (0.1 m mol/L) solution was added. After 30 min of incubation in dark, the absorbance was recorded at 517 nm. Results were expressed as percentage inhibition of DPPH.

$$\%\text{Inhibition} = [(\text{Abs.}_{\text{control}} - \text{Abs.}_{\text{sample}})/\text{Abs.}_{\text{control}}] \times 100$$

The percentage inhibition was plotted against the sample extract concentration in order to calculate the IC50 values, which is the concentration (lg/ml) of extract that causes 50% loss of DPPH activity. Results were compared with the positive control ascorbic acid table 4 fig 2.

**Table 4. DPPH assay of different extracts of aerial parts of *P. brunonis.***

| Conc. | 100 µg/ml | 200 µg/ml | 300 µg/ml | 400 µg/ml | 500 µg/ml |
|---|---|---|---|---|---|
| STD | 77.04±0.36 | 83.99±0.47 | 91.31±0.71 | 96.41±0.34 | 101.5±0.56 |
| PCH | 49.05±0.44 | **79.38±0.54** | **86.62±0.34** | 88.97±0.40 | 89.70±0.32 |
| PEA | 52.21±0.71 | 56.27±0.41 | 63.45±0.51 | 69.30±0.47 | 75.25±1.01 |
| PBU | **65.04±0.58** | 70.64±0.78 | 79.30±0.87 | **93.73±1.11** | **96.26±0.93** |

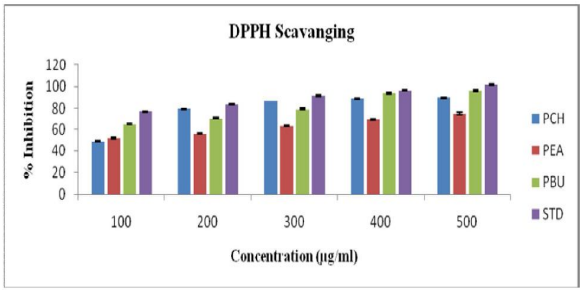


**Figure 2 DPPH Scavenging potential of *P. brunonis* extract.**

## RESULTS AND DISCUSSION:

### Total phenolic and flavonoids contents

The Total phenolic and total flavonoid contents of various extracts from aerial parts of *P. brunonis* are shown in table 2. Total phenolics are expressed as Gallic acid equivalent (GAE) and total flavonoids are expressed as quercetin equivalent (QE) per 100 mg of samples. Values are reported as mean ±SEM (n = 3). The chloroform extract (PCH) was found to have highest total phenolic content (**24.03±0.71**) whereas maximum total flavonoid content (**3.39±0.15**) was observed in butanol extract (PBU). Ethyl acetate extract (PEA) had the lowest content of total phenolic as well as total flavonoids 13.24±0.49 mg GAE/100 mg, and 2.23±0.06mg QE/100 mg respectively.

### Antioxidant Activities:

The reducing potential of different extracts of *P. brunonis* at five different concentrations (100 µg, 200 µg, 300 µg, 400 µg and 500 µg) was compared with ascorbic acids as standard (ascorbate equivalents). Butanol extract showed maximum reducing potential at all concentration **w**hile chloroform extract showed minimum reducing power at all the five conc. The reducing potential PBU at 100 µg, 200 µg, 300 µg, 400 µg and 500 µg were found to be **0.214±0.004, 0.313±0.004, 0.47±0.005, 0.617±0.006, and 0.684±0.006, respectively,** while the RP of ascorbic acid at these concentration was found to be 0.23, 0.43, 0.53, 0.63 and 0.66. These results showed that at higher concentrations butanol extracts has better reducing ability then ascorbic acid.

Free radical scavenging activity of different extracts of *P. brunonis* at five different concentrations of each extracts (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml) was compared with the positive control ascorbic acid. It was found that at 100µg/ml PBU (**65.04±0.58**) possess significant DPPH radical scavenging activity. PCH at 200 (**79.38±0.54**) and 300µg/ml (**86.62±0.34**) showed maximum scavenging activity. But at 400 (**93.73±1.11**) and 500 µg/ml (**96.26±0.93**) PBU again showed maximum scavenging power. It is evident that maximum free radical scavenging activity was found in butanol extract.

## CONCLUSION

The results of present study revealed that all the extracts exhibited different extent of biological activity. Comparatively butanolic extract showed significant antioxidant activity and total flavonoid contents. The present study would certainly help to ascertain the potency of the crude butanol extract of aerial parts of *P. brunonis* as a potential source of natural antioxidants. However, further research is required to identify individual components forming anti oxidative system and develop their application for pharmaceutical and food industries.

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