Total Phenolic Contents and Antioxidant Activities of Some Selected Anti-cancer Medicinal Plants from Chhattisgarh State, India.

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Summary

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Botanicals constitute of major part of these traditional medicines. Considerable attention has been focused on anticarcinogenic and antioxidants agents that occur naturally because antioxidants are being identified as anticarcinogens. On this aspect the present study has been designed to explore the antioxidant activity of some medicinal plants traditionally used in treatment of cancer in Chhattisgarh (Herbal state) of India. The ethanolic extracts of five medicinal plants including *Artocarpus heterophyllus, Alangium salvifolium, Buchanania lanzan, Sesbania grandiflora and Wrightia tinctoria* were evaluated for their total phenolic content and in-vitro antioxidant activity by 1, 1'-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, reducing power assay and total antioxidant capacity (Phosphomolybdenum reduction Assay). All five plants show antioxidant activity but Buchanania lanzan found to be most effective antioxidant.

Key words: Antioxidant, anticarcinogenic, *Artocarpus heterophyllus, Alangium salvifolium, Buchanania lanzan, Sesbania grandiflora, Wrightia tinctoria*.

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Introduction

In recent years, phytochemical constituents of plants with varied pharmacological, physiological and biochemical activities have received attention. Plants rich in bioactive Phytochemical reduce the risk of degenerative disorders such as cancer, diabetes, cardiovascular and oxidative dysfunction [1, 2, 3]. A great number of spices and aromatic herbs contain chemical compounds exhibiting antioxidant properties [4]. These properties are attributed to a variety of active phytochemicals including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols and phenolic acids, etc. [5]. Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen have been implicated in degenerative diseases such as cancer, inflammation, atherosclerosis and aging. Free radical-induced oxidative stress has been associated with several toxic cellular processes including oxidative damage to protein and DNA, membrane lipid oxidation, enzyme inactivation, and gene mutation that may lead to carcinogenesis [6].

Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals. These are compounds, when added to lipids and lipid containing foods increases their shelf-life by retarding the process of lipid peroxidation. Also, these have been widely used as food additives to avoid food degradation, and they play an important role in preventing many lifestyle-related diseases and aging, being closely related to the formation of ROS and to lipid peroxidation [7]. Production of ROS during normal cell metabolism is a normal and necessary process that provides important physiological functions. An imbalance between ROS production and antioxidant defenses results in oxidative stress which has been recognized as playing a prominent role in the causation of several age-related and chronic diseases, neurodegenerative and cardiovascular diseases. Intake of sufficient amounts of antioxidants is necessary to prevent free radical-induced oxidative stress. It has been reported that most of the antioxidant capacity of fruits and vegetables may come from total phenolics, anthocyanins, and flavonoids [8, 9].

Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. For example, some studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer, compared with chemotherapy or recent hormonal treatments [10, 11]. Therefore, many plants have been examined to identify new and effective antioxidant and anticancer compounds, as well as to elucidate the mechanisms of cancer prevention and apoptosis [12, 13, 14].

Chhattisgarh is known as the Herbal state of India, is located between 17-23.70N latitude and 80.40-83.380E longitude in the Central Eastern India with extremely rich natural resources and very fertile land stretched across 136.03 thousand sq. km with more than 20 million populations. Chhattisgarh state is divided into three agro-climatic zones namely the Northern hills, Chhattisgarh plains and the Bastar plateau [15].

Use of plants for medicinal remedies is an integral part of the Indian cultural life, and this is unlikely to change in the years to come. Many traditional healers and herbalists in the Chhattisgarh state of India have been treating cancer patients for many years using various medicinal plant species [16, 17]. Hence, an attempt has been made to screen *invitro* antioxidant activity of some medicinal plants used for the prevention and treatment of cancer in Chhattisgarh state, India.

Alcoholic extracts prepared from five medicinal plants, with cancer-related ethnobotanical uses in Chhattisgarh, were tested for their Phytochemical Screening and Antioxidant Activities. The plants studied were: stem bark of *Artocarpus heterophyllus, Alangium salvifolium, Buchanania lanzan, Sesbania grandiflora* and *Wrightia tinctoria*.

Materials and methods

Chemicals and reagents

Potassium ferricyanide, ferrous chloride, ferric chloride, Folin–Ciocalteu's reagent (FCR), methanol, sulphuric acid, Potassium chloride, Sodium phosphate, ammonium molybdate and trichloroacetic acid (TCA) were purchased from E. Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Butylated Hydroxyl Toluene (BHT), Ascorbic acid (AA) and gallic acid were purchased from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

Plant material and extraction

The bark of five plants was collected from the tribal belt of Chhattisgarh during the months of November and December. The plants were identified by Dr. H. B. Singh, Scientist, National Institute Scientific Communication and Research, New Delhi (India). The voucher specimen has been stored in herbarium of SLT institute of Pharmaceutical Sciences, Bilaspur India (specimen No. 01/ HSAH, 02/ HSAS, 03/ HSBL, 04/HSSG and 05/ HSWT).The dried bark of the plants were cleaned of dirt and ground to powder, using a commercial mill. Dried powder was defatted with light petrol (60–80^{0}C) and filtered. The residue was extracted with 90% Ethyl Alcohol by using Soxhlet extraction apparatus. Then solvent was completely removed under reduced pressure and stored in vacuum desiccators. The percentage yield of the extracts was calculated.

Preliminary phytochemical screening

All extracts were subjected to qualitative chemical screening for the identification of the various major classes of active chemical constituents. Test for flavonoids: 2 ml of the extract was filtered and 1 ml of the filtrate was mixed with dilute NaOH, golden yellow precipitate confirmed the presence of flavonoids. Test for phenols: 2ml of the extract was mixed with 3ml 5% ferric chloride and five drops of potassium ferricyanide, dark green precipitate confirmed the presence of phenols. Test for steroids/saponins: 1 g of the extract was mixed with 10 ml of warm distilled water, frothing persistent indicated the presence of saponins. An additional test was performed by Liebermann– Burchard test. To 100mg of extract, 2 ml of acetic anhydride was added; the mixture was thoroughly stirred, heated for 2 min on a water bath and allowed to stand at room temperature. When 2 ml of sulfuric acid was gently added to 0.7 ml of a supernatant acetic anhydride layer, the upper layer gave a blue to green colour confirming the presence of steroidal saponins [18].

Determination of total phenolics

Content of total phenolics was determined according to methods of folin-ciocalteu reaction [19]. Different concentrations of gallic acid (10 µg/ml-50 µg/ml) were prepared for standard curve. 0.1 ml of these different concentrations of Gallic acid was taken in different test-tubes and to it 0.5 ml undiluted Folin-Ciocalteu reagent was added. After 1 min, 1.5 ml 20 % (w/v) anhydrous sodium carbonate was added and volume was made up to 10 ml with water. After 1 hour incubation at 25^{0}C, the absorbance was measured at 760 nm. 0.1 ml of extracts (1mg/ml in methanol) was taken and followed as above. Absorbance of this sample was taken and gallic acid equivalents (GAE) were determined by extrapolation of the standard curve. The results were expressed as µgGAE/mg.

DPPH radical scavenging activity

The DPPH free radical scavenging ability of the compound was assessed by the method described by Gulçin *et al.* [20]. 0.1mM DPPH solution (4mg/100ml) prepared in methanol. Samples were prepared to get concentration of 1mg/ml in methanol, various concentrations of sample solution is further diluted with methanol to 2ml than added 1ml of DPPH solution incubated at room temperature for 10 min absorbance was measured at 515 nm against blank. The free radical scavenging ability was calculated using following equation.

Scavenging effect (%) = $[(A_{C\ 515\ nm} - A_{S\ 515\ nm} / A_{C\ 515\ nm}) \times 100]$

Where A_c is the initial absorbance of stable $DPPH^{\cdot}$ radical without test compound and A_s is the absorbance of $DPPH^{\cdot}$ radical in presence of sample. DPPH radical scavenging activity of extract was expressed as IC50 value and compared with Ascorbic acid.

Measurement of reducing power

The reducing power of the samples was determined by the method described by Elmastas *et al*. [21] with minor modifications. 0.5 ml of different concentrations of sample was taken than 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (0.5 ml, 1%W/V) was added. Reaction mixture was incubated at 50^0 C for 20 min. After cooling, 1.5 ml of trichloroacetic acid solution (10% W/V) was added to terminate the reaction. 0.5 ml ferric chloride (0.1% W/V) was added and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

Phosphomolybdenum antioxidant assay

Total antioxidant activity of plants extract was evaluated by Phosphomolybdenum antioxidant assay method described by Prieto *et al.* [22]. Total antioxidant capacity was measured by spectrophotometric method. 0.1ml of the extract (10 mg/ml) dissolved in water was combined in eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95^0C for 90 min. After cooling to room temperature; the absorbance of the aqueous solution of each was measured at 695 nm against blank. The antioxidant activity was expressed as the number of gram equivalents of BHT.

Results and discussion

Preliminary phytochemical screening and total phenolics content

Preliminary Phytochemical screening of different extracts shows there is presence of flavonoids, phenolics, steroids/ saponins etc. The percentages of yielded alcoholic extract from and total phenolic content in all the plants are listed in Table 1. Total phenolic content in ethanolic extract of different plants as estimated by Folin-ciocalteu Reagent method in the present study shows that different plants show difference in gallic acid equivalent (GAE). This is due to vary in nature of active ingredients in various samples (Table-1). The highest total phenolic content was observed in *Buchanania lazan* (30.13 ±0.22 µg GAE/mg) followed by *Sesbania grandiflora* (21.12 ±0.15µg GAE/mg), *Wrightia tinctoria* (19.53 ±0.17 µg GAE/mg), *Alangium salvifolium* (17.41 ±0.17 µg GAE/mg) and *Artocarpus heterophyllus* (4. 75 ±0.09 µg GAE/mg), respectively.

Table – 1. Yield of Alcoholic extracts and Total Phenolic Content.

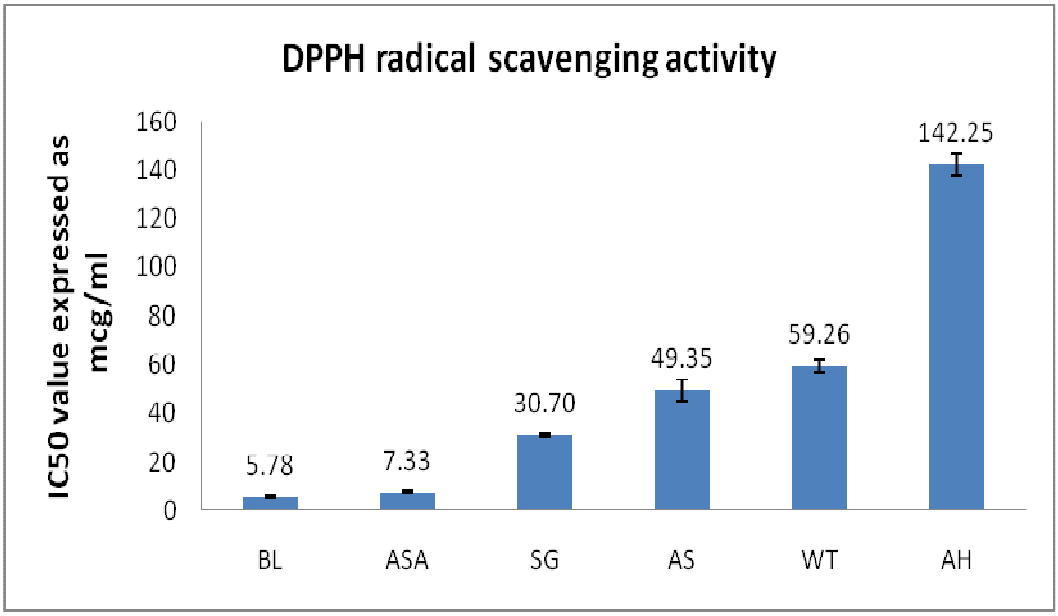
S. No.	Sample	% yield	Total phenolic content (µg GAE /mg of extract)
1	*Artocarpus heterophyllus*	6.2 ± 0.4	4. 75 ±0.09
2	*Alangium salvifolium*	8.7 ± 0.7	17.41 ±0.17
3	*Buchanania lazan*	18.5 ± 1.1	30.13 ±0.22
4	*Sesbania grandiflora*	11.6 ± 0.6	21.12 ±0.15
5	*Wrightia tinctoria*	16.6 ± 0.8	19.53 ±0.17

Values expressed in mean ± S.D. (n=3) GAE, Gallic Acid Equivalent.

DPPH radical scavenging activity

Free radicals are known to be a major factor in biological damages and DPPH has been used to evaluate the free radical-scavenging activity of natural antioxidants. DPPH, which is a radical itself with a purple color, changes into a stable compound with a yellow color by reacting with an antioxidant and the extent of the reaction, depends on the hydrogen donating ability of the antioxidant. Figure-1 shows the DPPH radical scavenging activity of different extracts which is expressed in terms of IC50 value with respect to ascorbic acid as control. Lower the IC50 value shows more antioxidant potential. *Buchanania lazan* exhibited the strongest antioxidant activity through all concentration of DPPH assay. At lower concentration less difference in DPPH scavenging activities was observed between standard and samples. However, as concentration increases the difference in scavenging activities between ascorbic acid and different samples become more significant.

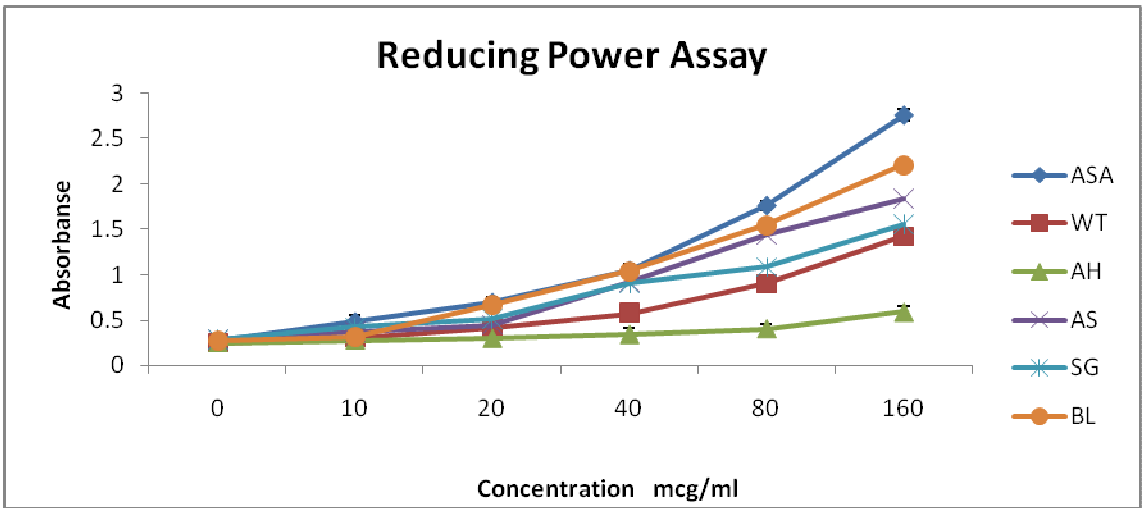
Figure -1. DPPH radical scavenging IC50 values of all extracts and ascorbic acid.



Measurement of reducing power

Reducing power method for the determination of antioxidant activity determines the capacity of reducing the oxidation potential of oxidants. The reducing powers of the samples due to the hydrogen donating abilities. In this assay ascorbic acid was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. In the present study for the determination of reducing power of different extracts as well as standard shows, with respect to increase in concentration there is increase in absorbance. Out of five plants studied for antioxidant activity *Buchanania lazan* shows good antioxidant activity, which is comparable to that of ascorbic acid (Figure-2).

Figure -2. Free radical reducing power of all extracts and ascorbic acid.



ASA = Ascorbic acid, AH= *Artocarpus heterophyllus,* AS= *Alangium salvifolium,* BL= *Buchanania lanzan,* SG= *Sesbania grandiflora* and WT= *Wrightia tinctoria.*
Values expressed in mean ± S.D. (n=3).

Phosphomolybdenum antioxidant assay

The different plant extracts were also used to determine their total antioxidant capacities by the formation of green phosphomolybdenum complex. The formation of the complex was measured by the intensity of absorbance in extracts at the concentration of 5mg/ml for each extract. The results indicate that under these conditions *Buchanania lazan* is found to be powerful antioxidant. However, the differences in the degree of molybdenum reduction were observed. This is due to vary in content of total phenolics as established in total phenolic compound estimation (Table-2).

Table -2. Total anti-oxidant activity in terms of Equivalents of BHT.

S. No.	Extracts	BHT equivalent (mg/mg)
1	*Artocarpus heterophyllus*	0.383 ± 0.006
2	*Alangium salvifolium*	0.466 ± 0.009
3	*Buchanania lazan*	0.502 ± 0.014
4	*Sesbania grandiflora*	0.498 ± 0.007
5	*Wrightia tinctoria*	0.443 ± 0.004

Values expressed in mean ± S.D. (n=3)

Conclusion

The results obtained from this study show that multiple *in vitro* methods targeting different radical species are important for testing antioxidant potential of a standardized herbal extract. Employment of more than one test method specific to a radical species gives a better estimate of comparative antioxidant potential of a test compound.

In our study, *Buchanania lazan* was comparatively stronger amongst the extracts from the five barks screened. Incidentally this extract also showed the highest phenolic content. Since it was reported that the antioxidant activity of a plant extract often originates from phenolic compounds [23 & 24]. The results suggest that there was a correlation between the total phenolic content and antioxidant activity in the plant samples.

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