Study of phytochemical analysis and antioxidant activity on *Bacopa monnieri*

**ABSTRACT**

The qualitative analysis of primary metabolites of *Bacopa monnieri* was analysed using different solvents such as aqueous, acetone, ethanol, and methanol. Phytochemicals such as amino acid, proteins, and starch were represented, amino acids (1.64 mg/ml), Proteins (197 mg/ml) and Carbohydrates (1.85 mg/ml) were quantitatively analysed in *Bacopa monnieri*. The following phytochemical compounds such as Saponins, Tannins, phenolic compounds, Reducing sugar, Steroids, alkaloids, Flavonoids, Terpenoids and anthroquinone were analysed using different solvents such as aqueous, acetone, ethanol, and methanol for qualitative analysis of Secondary metabolites of *Bacopa monnieri*. Bioactive compounds such as saponins (0.39 mg/gm), Tannins (1.97 mg/gm), Steroids (1.85 mg/gm), alkaloids (1.64 mg/gm), flavonoids (1.62 mg/ml) and Terpenoids (1.60 mg/gm) were quantitatively analysed in *B. monnieri*. The antioxidant activity of *B. monnieri* was analysed using hydrogen peroxide scavenging assay. Among the different Concentration of *B. monnieri*, 1% extract exhibited higher potency of radical scavenging assay (72.8 %) followed by 0.8(66.4 %), 0.6 (58.7 %), 0.4 (54.2 %) and 0.2 (33.6 %). The antioxidant activity of *B. monnieri* was analysed using Reducing power assay. Different concentrations of *B. monnieri* 1% extract showed exhibited higher potency of reducing power assay (70.6%) followed by 0.8% (582%) 0.6% (49.7%), 0.4% (46.3%) and 0.2% (22.5%), respectively.

**Key words:** *Bacopa monnieri*, phytochemical analysis, antioxidant activity, hydrogen peroxide assay, reducing power assay.

**INTRODUCTION**

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous systems of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. This system of medicine caters for the needs of nearly seventy per cent of our population residing in the villages. The Indian subcontinent has a very rich diversity of plant species in a wide range of ecosystems. Ours is a vast country with a wide variation in climate, soil, altitude, and latitude. Nature has bestowed on us a very rich botanical wealth, and a large number of diverse types of plants grow wild in different parts of the country. Increased awareness about the potential of this group of interesting and useful plants has encouraged many innovative and progressive growers and entrepreneurs to take up their cultivation as a commercial enterprise. Apart from health care, this enterprise provides means of livelihood to cores of people (Anbarasan et al., 2010). However, our knowledge of medicinal plants has mostly been inherited traditionally. The use of Plants for various ailments is not confined to the doctors only but known to several households as well. These are many interesting and sometimes astonishing things to learn from collectors of medicinal herbs. Spreading and preserving this knowledge on medicinal plants and their uses have become important for human existence (Anbarashan et al., 2010). Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. Both the general consumer and health-care professionals need up-to-date,
Authoritative information on the safety and efficacy of medicinal plants. But today, many indigenous herbal remedies remain largely undocumented or recognized as potential forms of treatment and consequently continue to be used by only small groups of indigenous populations. The present work was carried out in Rajarajan Manimandapam to explore the medical remedies of some medicinal plants used by the rural people of Thanjavur district in Tamilnadu for the treatment of human ailments (Anbarashan et al., 2010).

Documentation of the indigenous knowledge through ethno-botanical studies is important for the conservation and utilization of biological resources (Muthu et al., 2006; Macia et al., 2005). Therefore, determining the local names and indigenous uses of plants has significant potential social benefits (Kargioglu et al., 2008; Jayakumar, 2013b). The present study investigated the plants traditionally used in the treatment of specific diseases, and the local names of these plants.

Sacred groves are special forest area imbued with super natural powers and commonly comprise sites for ritual, initiation and other special functions and ceremonies. Wherever they occur, access to sacred groves is highly restricted, resulting in a hands-off approach regarding people's interaction with their biological wealth. Taboo associations attached to sacred groves have played an important role in their protection by indigenous and local people. Their continued survival has effect offered protection to the biological life there in turning into biodiversity reservoirs. Sacred groves act as an abode for many rare endemic, endangered species and economically important plants of fruit bearing and medicinal properties.

Medicine properties are found in the roots, stems, barks, leaves, and fruits to treat ailments including tuberculosis, meningitis and diarrohea (Uhegbu et al., 2009). Roasting or soaking the seed has nutritional benefits as it increases the content and properties of certain nutrients (Uhegbu et al., 2009). Majority of West African populace consumes Detarium microcarpum using different processing methods, without adequate information on the effect of the processing methods on the proximate and phytochemical composition of the seed. Therefore, this study is designed to ascertain the effect of soaking and boiling time on the proximate and phytochemical compositions of the seed D. microcarpum.

According to World Health Organization (WHO), medicinal plants are those plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs. The wild edible medicinal leafy vegetables occupy an important place among food crops as these provide adequate amount of crude fiber, fats, carbohydrates, proteins, water and mineral elements such as Ca, Na, K, Fe, Mg, Mn, Cu, Zn etc, in addition to vitamins, and certain hormone precursors studies have shown that vegetarians are less susceptible to disease, they live longer, healthier and have stronger immunity. Most of the medicinal plants have potential to provide nutrients present in them to the consumers and utilization of these plants can provide a solution to the problem of malnutrition to a great extent. In nature, there are many underutilized green leafy vegetables of promising nutritive value, which can nourish the ever increasing human population.

Phytochemicals constituents in plant materials are biologically active chemical compounds which are essential for the regular metabolic activities. Plants are rich source of biologically active components. Natural antioxidants such as flavonoids, tannins and phenols are essential for healthy body functions. Phytochemicals are products of the secondary metabolism in plants, mainly belong to classes such as terpenes and derivatives, phenylpropanoids, isothiocyanates, Sulphur compounds and are often present as glycosides (Arirhan et al., 2012b and a; Murali et al., 2020; Tamizhazhagan et al., 2017).

Herbal medicine denotes therapeutic uses of plants. Plants are one of the major sources of drugs due to its secondary derivative bioactive chemical constituents which play vital role to cure many ailments. The plant is distributed in tropical forests of central and southern India and plant also grows in parts of African continent. The plant is mostly used in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma, hypertension and also recommended for fast relief of ailment such as malaria fever (Ozaslan et al., 2016).

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. They often play an important role in plant defense against herbivores and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, and recreational drugs. A primary metabolite has an important ecological function. Examples include antibiotics and pigments.

Bacopa monnieri (L.) Wetst is an elite nootropic perennial herb found throughout the Indian subcontinent. The herb is reported to have an extensive range of pharmacological properties viz. antioxidant, anti inflammatory, cardiotonic and cognitive enhancing which are attributed due to the presence of triterpenoid saponins and bacosides (Jain et al., 1993; Rastogi et al., 1994; Tiwari et al., 2012). Researchers have developed many techniques for conservation of this threatened species (Sharma et al., 2007; Tanveer et al., 2010) through in vitro regeneration using varied types of explants (Kumari et al., 2010; Mehta et al., 2012; Mohapatra et al., 2008). Extensive work has been reported on the effect of media manipulations and elicitation for secondary metabolite production (Sarkar et al., 2012; Tanveer et al., 2010), these reports have used a single accession of the plant species.

In the last few decades, there is an exponential growth in the field of herbal medicine. It is getting popularize in
developing countries owing to its natural origin and lesser side effects. Nowadays, herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality of raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters etc (Patil et al., 2011). For pharmacological or pharmaceutical use, scrutiny of a crude drug for its botanical identity is required (Poornima et al., 2009). Generally, herbal formulations are prepared from fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained (Modi et al., 2010).

Anatomical structure is very important for studying biological specimens for the purpose of classification, pathological changes and the physiological significance of certain cell organelles or structures in relation to the habitat (Coopoosamy and Naidoo, 2011). Microscopy allows the identification of herbal drugs and the detection of individual components of a mixture. It is important to ensure quality and purity of herbal medicines in order to maximize the efficacy and minimize adverse side effects. Adulteration and misidentification of herbal drug can cause serious health problems to consumers (Serrano et al., 2010).

Several medicinal plants have been used as a source of many potent and powerful drugs. Medicinal property of a plant is due the presence of chemical entities (phytochemical constituents) which are extracted from the plant or the plant parts. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, ultraviolet (UV) exposure and pathogenic attack are referred to as phytochemicals (Koche et al., 2016). Medicinally important plants contain diverse group of phytochemicals with great variations in solubility and stability. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals. Aim for the phytochemical analysis is the characterization of an active principle responsible for some toxic or beneficial effect shown by a crude plant extract when tested against a living system. Quantification of the different phytoconstituents present in the plants is of equal importance. Phytochemicals are divided into two groups (according to their functions in plant metabolism); these are primary and secondary metabolites. Primary metabolites such as carbohydrates, proteins and lipids are essential for the plant metabolism. Secondary metabolites, which are not essential for the plant metabolism, are formed as byproducts in the biochemical pathways. These include very interesting and useful classes of compounds such as alkaloids, terpenoids, anthocyanins, saponins phenolic compounds like flavonoids, tannins, etc. These secondary metabolites can be utilized for the identification of plant material (Krishnaiah et al., 2009). Antioxidants function as reducing agents, ultimately eliminating free radical intermediates and inhibiting further oxidation (Phang et al., 2013).

*Bacopa monnieri* (L.) Wettst. is an important medicinal plant belonging to the family Scrophulariaceae and is used in the traditional systems of medicines as memory booster (Gohil and Patel, 2010). It is also referred to as, *Herpestis monniera* L. Kunth. *B. monnieri* (L.) Wettst. is locally known as water hyssop, brahmi or Jalanimba in India. The name brahmi is derived from the word 'Brahma', the mythical 'creator' in the Hindu pantheon. As the brain is the center for creative activity, any compound that improves the brain health is called brahmi, which also means 'bringing knowledge of the supreme reality (Prasad et al., 2010). Brahmi is a small, smooth, hairless, somewhat fleshy and creeping herb. The plant is a short duration annual herb, frequent in moist habitat and water edges throughout tropical and subtropical India. It grows best near flowing water and wet lands in plain and foothills and is particularly abundant in monsoon. Brahmi can grow in a variety of soil types if the habitat provides wet and semi shade conditions. Near-neutral, clayey loam to clayey soils are best suited for the growth of *B. monnieri*. In North India, it can grow in a wide range of temperatures 150 - 400°C and soil pH 5.0 - 7.5. However, it can even grow well in soils with pH 7.5 or even more. It becomes dormant during the winter months except when grown near running water. Flowers and fruits appear in summer (Ayush, 2008).

**MATERIALS AND METHODS**

**Collection of plant sample**

The plant materials stem of *B. monnieri* were collected from the Senpagapuram of Thanjavur District, Tamil Nadu.

**Preparation of plant stems powder**

The collected plant samples were air dried. Thereafter, the sample was ground in grinding machine made for the laboratory. Exposure to direct sunlight was avoided to prevent the loss of active components. These powdered materials were used for further analysis.

**Preparation of stem extract**

Chemical test were carried using acetone, aqueous, ethanol, and methanol extract and for the powdered specimens standard procedures were used to analyse the phytochemical constituents described by Sofowara (1993),

*Academia Journal of Microbiology Research; Gayathri et al. 023*
Qualitative phytochemical analysis

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) and Evans (1996).

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer’s test

Filtrates were treated with Mayer’s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Detection of flavonoids

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of steroids

Two ml of acetic anhydride was added to five mg of the plant extracts, each with two ml of H₂SO₄. The colour was changed from violet to blue or green in some samples, indicating the presence of steroids.

Detection of terpenoids

Salkowski’s test

Five mg of the extract of the stems was mixed with 2 ml of chloroform and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face indicates the presence of terpenoids.

Detection of anthroquinones

Borntrager’s test

About 5 mg of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drops of 10% NH₃ were added to the mixture and heated. Formation of pink colour indicates the presence anthroquinones.

Detection of phenols

Ferric chloride test

10 mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test

10 mg extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of saponins

About 0.5 mg of the plant extract was shaken with 5 ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Detection of tannins

A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates the presence of tannins.

Detection of carbohydrates

The plant extracts of 0.5 mg was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

Detection of protein and amino acids

Biuret test

To 0.5 mg of extract, equal volume of 40% NaoH solution and two drops of one percent copper sulphate solution were added. The appearance of violet colour indicates the presence of protein.

Detection of oils and resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.
Quantitative phytochemical analysis

Estimation of alkaloids

Alkaloid was determined using Harborne (1973) method. One gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and it's covered and allowed to stand for 4 h. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH₄OH was added by drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH₄OH and then filtered. The residue is the alkaloids which was dried and weighed.

Estimation of flavonoids

One gram of plant sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No1 filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed (Krishnaiah et al., 2009).

Estimation of total phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of NH₄OH solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was read at 505 nm.

Estimation of carbohydrate

100 mg of sample was hydrolyzed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 h. It was cooled at room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml using distilled water. From this, 0.2 ml of sample was pipetted out and made up the volume to one ml with distilled water. Then one ml of phenol reagent was added, followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30°C for 20 min. The absorbance was read at 490 nm (Krishnaveni et al., 1984).

Antioxidant activity

DPPH free radical scavenging activity (Yordabov and Christova, 1997)

The antioxidant activity of the plant extracts were performed on the basis of the scavenging effect on the stable DPPH free radical activity. The stock solution of 0.1 mM DPPH was prepared freshly in methanol and kept in the dark at 4°C. 3 ml of 1 mg concentration of plant extracts was added to 1 ml DPPH stock solution and incubated at ambient temperature in the dark for 20 min. After incubation, the absorbance was recorded against blank at 517 nm. Butylated hydroxy toluene (BHT) was used as the standard for antioxidant activity. All analysis was performed in triplicate. Inhibition of DPPH radical in term of percentage (%) was calculated using the formula:

\[
\text{DPPH Scavenged} (\%) = \frac{(A_{\text{control}} - A_{\text{test}})}{(A_{\text{control}})} \times 100
\]

Where A control is the absorbance of the control (L-Ascorbic acid) and A test is the absorbance of reaction mixture samples (in the presence of sample). All test were run in triplicates (n=3), and average values were calculated.

Thiobarbituric acid (TBA) method (Sawarka et al., 2009)

TBA method was used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100°C), malondialdehyde binds with TBA to form a red complex that can be measured at 532 nm. 2 ml of 20% Trichloroacetic acid and 2 ml of 0.67%TBA solutions were added to 2 ml of the mixtures containing the sample (0.2 – 0.8 microgram) prepared using the FTC (Ferric thiocyanate) method. This mixture was kept in water bath (100°C) for 10 min, cooled at room temperature, and centrifuged at 3000 rpm for 20 min. Antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay. Malondialdehyde was used as a standard (concentration of 20 µg). The percentage of antioxidant activity was calculated using the following formula:

\[
\text{Percentage of activity} = \frac{\text{Absorbance of (Control –Test)}}{\text{Absorbance Control}} \times 100
\]

Scavenging activity of H₂O₂ radical activity (Ruch et al., 1989)

The H₂O₂ scavenging of the fish samples was determined according to this method. A solution of H₂O₂ (40 mM) was prepared in PBS (pH 7.4) and concentration was determined spectrophotometrically (Gene Quant 1300 UV-Vis) at 230 nm. The leaf extracts (0.2 – 0.8 microgram) were added to a H₂O₂ solution (0.6 ml, 40 mM) and incubated at room temperature for 10 min, the absorbance of H₂O₂ at 230 nm was determined after 10 min against a blank solution containing the leaf extracts without H₂O₂. Ascorbic acid was used as standard (concentration 20 µg). The percentage scavenging of H₂O₂ was calculated using the following equation:
Table 1: Qualitative analysis of primary metabolites of *Bacopa monnieri*.

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Aqueous</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fehling’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Molisch’s test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benedict’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(+): Present, (-): Absent.

Table 2: Quantitative analysis of primary metabolites of *Bacopa monnieri*.

<table>
<thead>
<tr>
<th>Primary compounds</th>
<th>Quantity (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>1.64</td>
</tr>
<tr>
<td>Proteins</td>
<td>1.97</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Scavenging activity of $\text{H}_2\text{O}_2$ radical activity = \[
\frac{(\text{Abs std} - \text{Abs sample})}{\text{Abs std}} \times 100
\]

Reducing power (Oyaizu, 1986)

The reducing power of the aqueous extract was determined according to this method. One ml of the leaf extract containing (0.2 – 0.8 µg) in 1 ml of the deionized water was mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferrocyanide (1%). The mixture was incubated at 50°C for 20 min. 2.5ml of TCA (10%) and centrifuged at 3000 rpm. The upper layer of the solution was mixed with 2.5ml distilled water and FeCl$_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power. The absorbance was compared with the standard ascorbic acid (concentration 20 µg).

The percent increase in reducing power was calculated using the following equation:

\[
\text{Increase in reducing power (}) \times 100
\]

Scavenging activity of superoxide dismutase (Yen and Chen, 1995)

The scavenging activity of superoxide dismutase was determined by this method. The reaction mixture consists of 1 ml of extract (0.2 – 0.8 µg), 1 ml of PMS (phenazinemethosulphate) (60 µm) prepared in phosphate buffer (0.1 M pH 7.4) and 1 ml of NADH (phosphate buffer) was incubated at 25°C for 5 min, the absorbance was read at 560 nm against blank samples. The absorbance was compared with the standard xanthine oxidase (concentration 20 µg):

\[
\text{% of scavenging activity of Superoxide dismutase} = \frac{A_{\text{test}} - A_{\text{std}}}{A_{\text{std}}} \times 100
\]

RESULTS

Qualitative analysis of primary metabolites of *Bacopa monnieri*

Qualitative analysis of primary metabolites of *B. monnieri* was analysed using different solvents such as aqueous, acetone, ethanol and methanol. Presence and absence of phytochemicals such as aminoacid, proteins, starch, Fehling’s test, molischis test and Benedicts test were represented (Table 1).

Quantitative analysis of primary metabolites of *Bacopa monnieri*

The bioactive compounds such as aminoacids (1.64 mg/ml), Proteins (197 mg/ml) and Carbohydrates (1.85 mg/ml) were quantitatively analysed in *B. monnieri* (Table 2).

Qualitative analysis of secondary metabolites of *Bacopa monnieri*

The phytochemical compounds such as Saponins, Tannins, phenolic compounds, Reducing sugar, Steroids, alkaloids, Flavonoids, Terpenoids and anthroquinone were analysed...
using different solvents like aqueous, acetone, ethanol, and methanol. The presence and absence of phytochemicals of Bacopa monnieri are shown in Table 3.

**Table 3: Qualitative analysis of secondary metabolites of Bacopa monnieri.**

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Aqueous</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthroquinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+): Present, (-): Absent.

**Quantitative analysis of secondary metabolites of Bacopa monnieri**

Bioactive compounds such as saponins (0.39 mg / gm), Tannins (1.97 mg / gm), Steroids (1.85 mg / gm), alkaloids (1.64 mg / gm), flavonoids (1.62 mg /ml) and Terpenoids (1.60 mg / gm) were quantitatively analysed in B. monnieri. The results are shown in Table 4.

**Antioxidant activity of Bacopa monnieri**

**Hydrogen peroxide scavenging assay**

The antioxidant activity of B. monnieri was analysed using hydrogen peroxide scavenging assay. Among the different concentrations of B. monnieri, 1% extract exhibited higher potency of radical scavenging assay (72.8 %), followed by 0.8(66.4 %) 0.6 (58.7%) 0.4 (54.2%) and 0.2 (33.6%), respectively (Table 5 and Figure 1).

**Reducing power assay**

The antioxidant activity of B. monnieri was analysed using Reducing power assay. Different concentrations of B. monnieri 1% extract exhibited higher potency of reducing power assay (70.6%), followed by 0.8% (582%) 0.6 (49.7%), 0.4% (46.3%) and 0.2% (22.5%), respectively (Table 6 and Figure 2).

**DISCUSSION**

In previous survey on medicinal plants from senpagapuram, Thanjavur district, Tamil Nadu, India, 50 medicinal plants were observed and listed. This is the first survey on medicinal plants of senpagapuram in Thanjavur district. Being an entertainment tourist spot, trees are cultivated in large numbers through mankind. According to the plants habit, 19 were Trees (38%), 17 Herbs (34%), 8 Shrubs (16%) and 6 climbers (12%). In the present study, Herbs had higher medicinal property than others. Herbs such as Abutilon indicum, Acalypha indica, Achyranthus aspera, Alternanthera sessilis, Andrographis paniculata, Catharanthus roseus, Centella asiatica, Eclipta prostrata etc. were used as a whole plant to cure various diseases. The common uses of herbaceous medicinal plants have also been reported in other parts of world (Tariq et al., 2013). In previous study, Malvaceae with 3 species, Euphorbiaceae with 3 species, Myrtaceae with 3 species and Fabaceae with 2 species, were recorded. The remaining families had only one species each. Among our observation, the three families were more frequently used by the village people in the study area. Among the genera, Ficus with 2 species had highest number of species and the remaining genera had only one species each. In contrast to the above studies, it has been reported that the family Acanthaceae ranked top having 5 ethnomedicinal plant species, followed by Amaranthaceae (4sp), Caesalpiniaace (4sp), Euphorbiaceae (2sp), Lamiaceae (2sp), Areceaceae (2sp) and Liliaceae (2sp) species each. The remaining families had only one species each. The most extensively used plant part in the preparation of medicine for various ailments is the leaf (50%), followed by entire plant (26%), root (26%), seed (26%), fruit (18%), flower (24%), bark (14%), terminal bud (2%) and rhizome (2%). Leaves remain green and available in plenty for the most months of the years. Extensive phytochemical analysis in relation to functional genomics and systems biology within an organism, tissue or cell under a given set of conditions at specific time is dubbed as metabolomics (Sindelar and Patti, 2020).

The antibacterial activity was determined by measuring the diameter of zone of inhibition. Methanol extracts were found to be the most potent antimicrobial agent as compared with other extracts. Aqueous extracts showed no activity against any of the microorganisms. Hexane and
petroleum ether extracts showed similar antimicrobial activity but less significant in comparison to methanol extracts. The results are in accordance with previous studies conducted on this aspect. The MIC of the methanol extracts was found to be lowest, that is, 0.039 mg/ml for *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Saccharomyces cerevisae*. The experiments were performed in triplicates and the mean of diameter of zone of inhibition were observed.

The screening test revealed the presence of many phytochemicals in either one or both extracts. After the qualitative analysis, UV-Spectrophotometer was used to quantify phenols, flavonoids, saponins and alkaloids where higher phytochemical concentrations were recorded in the pulp followed by whole fruit and then the seeds. This study therefore supports the use of *T. tetraperta* in traditional systems of medicine owing to its active chemical compounds, and has given many vital insights on which part of *T. tetraperta* fruit to consume as concentrations of these compounds varies in the pulp, seeds and whole fruit.

The crude methanolic extract of the roots for the presence of bioactive components was done adopting Harborne
Table 6: Antioxidant activity of *Bacopa monnieri* Reducing power assay.

<table>
<thead>
<tr>
<th><em>Bacopa monnieri</em> extract concentration (%)</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>22.5</td>
</tr>
<tr>
<td>0.4</td>
<td>46.3</td>
</tr>
<tr>
<td>0.6</td>
<td>49.7</td>
</tr>
<tr>
<td>0.8</td>
<td>58.2</td>
</tr>
<tr>
<td>1.0</td>
<td>70.6</td>
</tr>
</tbody>
</table>

Figure 2: Antioxidant activity of *Bacopa monnieri* reducing power assay.

Ethno-medical practices are preferred largely because medicinal plants are less expensive, readily available and reliable, and they are considered to have fewer side effects than modern medicines. This study assessed the survey of ethno-medical plant species used by Aduthurai, Thanjavur district of Tamil Nadu (Jayakumar, 2013a).

In the present investigation, the ethno-medical study was carried out in Pudukkottai and Tanjavur district Tamil Nadu South India. The ethno-medical uses of 143 plant species spread over 51 families are described under this study. The village peoples have used the plants for many ailments. The medicinal plants used by them are arranged alphabetically: botanical name, Vernacular name, Family name, Part of medicinal use and Mode of administration.

The similar documented was made for Eighty-nine plants belonging to 51 families identified as traditional folklore medicinally used species (Munisamy et al., 2011). Gastro intestinal problems like digestive problems, diarhrea, dysentery, stomach ache and constipation were treated using specific herbal prescriptions by the rural peoples (Sundaresan et al., 2013).

Medicinal plants are also used for respiratory problem such as cough, cold, bronchitis and asthma. Swellings, Leucorrhoea, Skin problem, joint paints, urinary diseases, diuretic, fever, diabetes, treatment of boils and stomach ache problems are also treated using herbal medicines by the rural peoples in the groves site. This study assessed the survey of ethno-medical plant species used by Aduthurai, Thanjavur district of Tamil Nadu (Rameshkumar et al., 2013). Effectiveness of the herbal drug was connected to nature of the disease and dose response. Doses differ from patient to patient from time based on the cause and effectiveness of the drug.

The proximate result showed that there is significant differences between the moisture content of the control (8.90%) and the other values of the other samples at different time interval at p< 0.05 level of significance. The sample soaked for 30 h had high moisture content than those treated at other different time interval. The moisture content of the seeds increases as the treatment time increases. The high rate of moisture in food substances increases its susceptibility to microorganism spoilage. There was significant difference (P>0.05) between the Ash, fibre, fat and protein of the seeds of *Detarium microcarpum*, with the control having the highest value in the four classes of food. The ash content of the treatment seeds was lower to the values reported for *Detarium microcarpum* and other
related seeds: *Detarium microcarpum* (3.09%), *Muluna sloanei* (3.46%), *Brachystegia nigerica* (4.07) and *Afeeli africana* (13.51%) as reported by (Igwenyi and Azoro, 2014). The fibre content at different treatments was significant different (P>0.05) from one another, with the sample heated for a longer time had the lowest fibre content. The fibre value was lower than the value reported by Igwenyi and Azoro (2014) for *Afzelia africana* (3.25%), and similar to the values for *Brachystegia nigerica* (2.63%), *Mucana shoanei* (2.99%) and *Detarium microcarpum* (2.63%); ash content measurement could be a measure of the food quality. The level of ash is an indication of adulteration. Adulteration is the contamination of food product due to inorganic substances present in the food being analyzed (Pearson, 1976). The crude fibre, as an inorganic residue left after the defatted food materials, is treated with boiling dilute hydrochloric acid, diluted sulphuric acid, boiling dilute sodium hydroxide, alcohol and ether. Fibre shortens the transit time of food through the gastrointestinal tracts, reduces low density lipoprotein and hence, keeps the gut healthy. Fibre supplements or fibre-rich foods may function as normal dietary agents by modulating the digestive absorptive process. They are very important in promoting a range of physiological effects, including increased fetal bulk, water holding capacity, absorption of organic molecules such as bile acids, cholesterol and toxic components (reduced bile acid and plasma-cholesterol levels), reduction of minerals and electrolytes (Igwenyi, 2008). Recent advances in phytochemical analysis have allowed the accumulation of data for crop researchers due to its capacity to footprint and distinguish metabolites that are present with in an organisms, tissues or cells (Ishak et al., 2021).

The antioxidant analysis proved that maximum inhibitory potential of leaf extract was obtained in *Lumnitzera racemosa* and minimum scavenging activity was found in *Suaeda nudiflora*. The yellow color spots on TLC sheet represent the presence of bioactive compounds and antioxidants. Hanna et al. (2008) reported that the yellow colour spots indicate presence of carotenoids and other phenolic compounds. The phenolic compounds from plants are known to be good anti-oxidants (Eswareiah et al., 2019). The present study aims to address the morphological, biochemical, phytochemical and regenerative potential of five accessions of *Bacopa monnieri*. A new LC-ESI-MS method through single ion monitoring (SIM) was developed for the simultaneous identification and quantification of Bacoside A3, Bacoside II, Bacopasaponin C and Bacoside X in crude extracts of shoot and root samples of the herb. The main challenge in assessing antioxidant activity in date extracts is their complex chemical environment. The presence of a wide variety of phenolic compounds appears to affect their reactivity with the assay reagents. For example, the formation of hydrogen-bonded complexes or covalently-bonded dimers can deactivate some phenolic functions.

Samad et al. (2016) and Alam et al. (2021) stated that besides the total phenolic compounds, the antioxidant activity also depends on the degree of polymerization as well as the hydroxylation of phenolic compounds. Antioxidant properties of polysaccharides are due to the electron donor or hydrogen donor functional groups attached to the polysaccharide chains (Yu et al., 2020). These proper-ties vary from one functional group to the other functional groups and are easily assayed by performing in-vitro antioxidant assays.

Functional attributes including the antioxidant behavior of plants-based polysaccharides are much important due to their meaningful transfer of value-added properties in different practical commodities. This review is aimed to attract the attention of researchers to boost the antioxidant activities of plants-based polysaccharides using different chemical modification methods. In this review, different modification methods of plants-based polysaccharides were particularized to make ease to select the competent modification protocols and to analyze the resulted antioxidant potential of native and modified polysaccharides. The antioxidant activities of plants-based polysaccharides were increased significantly after chemical modification. These significant improvements in antioxidant activities of plants-based polysaccharides suggested employing them in food, pharmaceutical and cosmetic products to improve their healthy life (Ahmad, 2021).

**SUMMARY AND CONCLUSION**

The effect of biological activity of “Neer Bhirami” plant has been well formulated and designed in all aspects. The estimation of primary metabolites from the plants showed maximum amino acid, protein, starch and carbohydrate. The results also showed activity observed with regards to quantitative test of amino acid, protein and carbohydrate with extraordinary production. All the compounds have medicinal values. For quantitative secondary metabolites, maximum quantity was observed for saponins (0.39 mg/g), tannins (1.64 mg/g), steroids (1.85 mg/g), alkaloids(1.64 mg/g), flavanoids (1.62 mg/g), and terpenoids (1.60 mg/g), respectively with regards to antioxidant properties of *B. monnieri* as hydrogen peroxide scavenging assay showed excellent performance than the higher reducing power assay method. At higher concentration of (1%) Neer Bhirami extract greater antioxidant activity was observed, but at low concentration, moderate antioxidant activity was observed.

**REFERENCES**


