Chapter II
Phytochemical and pharmacognostic studies on Portulaca quadrifida Linn.
2.1 Introduction

Phytochemistry or plant chemistry is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function (Harborne, 1998).

A key component of this research is the ability to accurately identify and quantitate the specific phytochemicals. Today analytical equipments are available that can rapidly separate, identify and accurately quantify the phytochemicals from plant materials literally in a matter of minutes.

Natural product analysis requires three basic steps, a way to separate the various phytochemicals in the extracts, quantification and a method of identifying the phytochemicals. For this, pure standards are essential. Despite thousands of phytochemicals that have been identified and reported in the literature, commercially available phytochemical standards are still rarely available and are usually quite expensive.

Fortunately a great deal of progress has been made in improving chromatographic techniques over the past decade. So purification of chemicals is more efficient and large segments of the purification process can be automated to yield sufficient quantities of purified compounds, in days or even hours.

Pharmacognosy is the study of medicines derived from natural sources. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources."
The human being appears to be afflicted with more diseases than any other animal species. There can be little doubt then he, very early, sought to alleviate his sufferings from injury and diseases by taking advantage of plants growing around him. In the past, almost all the medicines used were from the plants growing around him. It has been estimated that two-third of the world’s population seek health care from sources other than conventional biomedicine. While many of these individuals undoubtedly self-medicate, most of them seek care from learned practitioners of traditional, indigenous systems of medicine, viz., Ayurveda, Kampo, Native American Medicine, Traditional Chinese Medicine, Traditional Hawaiian Medicine, Unani, Latin American folk systems, etc. Despite diverse cultures, languages, geographic locations, world views and health beliefs, the medical systems originated through them have common characteristics which includes: (i) The use of complex interventions often involving multiple botanical products, (ii) Individualized diagnosis and treatment, (iii) An emphasis on disease prevention versus disease treatment, (iv) Maximizing the body’s inherent healing ability and (v) Treatment of the patient (physical, mental and spiritual) versus a single pathology.

In olden times, vaidyas used to treat patients on individual basis and prepared drugs according to the requirement of the patients (Ali et al., 2005). Today, several medicinal plants and their products are still in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market (WHO). However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines (Dahanurkar et al., 2000). Therefore it has become extremely important to make an effort towards standardization of the plant material to be used as medicine. The process
of standardization can be achieved by stepwise pharmacognostic studies (Ozarkar, 2005). These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy (Thomas et al., 2008). Present work was carried out to study the preliminary phytochemical and pharmacognostical aspects of *P. quadrifida* Linn.
2.2 Materials and Methods

2.2.1 Collection and identification of plant material

Fresh whole plant material of *P. quadrifida* Linn. were collected from the local fields of Gulbarga. The plant specimen was identified and authenticated by Prof. Y.N. Seetaram Dept. of Botany, Gulbarga University, Gulbarga. A voucher specimen (No. HGUG -906) is preserved in the herbarium of Dept. of Botany, Gulbarga University, Gulbarga.

2.2.2 Preparation of plant extracts

The whole plant material was dried in the shade upto 10 weeks. The dried plant material was powdered in a mixer grinder. The powder was then subjected to successive sequential soxhlet extraction with various organic solvents ranging from highly non-polar to polar solvent systems such as petroleum ether (40-60 ºC), chloroform, ethanol and distilled water. Each extraction was exhaustively carried out for at least 10 hours. The solvent was removed from the different extracts by evaporation under reduced pressure. The various extracts were designated as follows- Petroleum ether extract of *P. quadrifida* (PEEPQ), Chloroform extract of *P. quadrifida* (CEPQ), Ethanol extract of *P. quadrifida* (EEPQ) and Aqueous extract of *P. quadrifida* (AEPQ). Cold maceration was also done using ethanol and water. The extracts were filtered individually, evaporated to dryness and then color and percent yields of all the extracts were determined.

2.2.3 Preliminary phytochemical analysis

The dried semisolid extracts of *P. quadrifida* Linn. viz., PEEPQ, CEPQ, EEPQ and AEPQ were subjected for detection of various phytochemical constituents such as alkaloids,
flavonoids, saponins, tannins, glycosides, carbohydrates, amino acids and triterpenoids (Harborne, 1998). PEEPQ, CEPQ and EEPQ were not soluble in water and hence these extracts were dissolved in Di Methyl Formamide (DMF) for phytochemical investigation.

2.2.4 Quantitative estimation of primary metabolites

2.2.4a Proteins

The total protein content in the powdered plant material was carried out using Folin-Ciocalteu’s reagent method (Lowry et al., 1951). 500 mg of the plant material was taken in a clean pestle and mortar and homogenated with 5ml of Tris-buffer (ph 6.0) and centrifuged at 2000 rpm for 1 min. The supernatant was used for the quantitative estimation of proteins.

2.2.4b Carbohydrates

The total carbohydrates content in the whole powdered Plant material was estimated by Anthrone method (Hedge and Hofreiter, 1962).

2.2.4c Amino acids

The total amino acids were estimated using Ninhydrin method (Moore and Stein, 1948). 500 mg of the plant material was ground in a clean pestle and mortar, with small amount of acid washed sand. To this, 5 to 10 ml of 80% (v/v) ethanol was added and centrifuged. Further, the resultant supernatant was used for the quantitative estimation of amino acids.
2.2.5 Quantitative estimation of secondary metabolites

2.2.5a Determination of total phenols by spectrophotometric method:

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 taken into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide 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 (Edeogal et al., 2005). This was measured at 505 nm using gallic acid curve. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/l solutions of gallic acid in methanol: water (50:50, v/v).

2.2.5b Alkaloids

The alkaloids content was estimated by Ikan’s method (Ikan, 1981). 10 g of powdered plant material was macerated with methanol (analytical grade) in pestle and mortar and centrifuged. The supernatant was condensed to \( \frac{1}{4} \) th volume and dilute acetic acid was added in a separating funnel. The acid layer was collected to which 25 ml of \( n \)-hexane and chloroform (1:1) mixture was added and shaken well. The chloroform layer was collected and washed with distilled water and pH was adjusted to 11-12 by the addition of \( \text{NH}_4\text{OH} \) the chloroform layer was separated and filtered using Whatman filter paper No.1. The filtrate was finally transferred to a clean and pre-weighed beaker and dried under reduced pressure at 40 °C for 6 h. The amount of alkaloids was calculated using the following formula.

Total alkaloids = Weight of the alkaloid residue (x)/weight of the plant material x 100

Where , weight of the alkaloid residue (X) = Z – Y
Y = Wt of the evaporating dish

Z = Wt of the alkaloid containing dish

2.2.5c Tannins

The tannin content was estimated according to Folin-Denis method (Schanderi, 1970). 500mg of powdered plant material was taken in to a clean test tube and 7.5 ml distilled water was added and boiled for 30 min. the contents were centrifuged at 2000 rpm for 20 min. and the supernatant collected.

2.2.5d Triterpenoids

The triterpenoid content was estimated using Sanchez method (Sanchez et al., 1972). 500 mg of the dried powdered plant material was refluxed with 25 ml of 3N hydrochloric acid at 60ºC for 4 h. The solid matter was filtered through Whatman filter paper No.1 and washed with dilute ammonia until the washings were neutral (ph 6.8-7.0). The triterpenoids were extracted from the residue in a Soxhlet extractor using chloroform.

2.2.5e Saponins

The method used was that of Obadoni and Ochuko (2001). 20 g of sample powder was put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55ºC. The mixture was filtered and the residue reextracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.
60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated.

2.2.5f Flavonoids

The flavonoids were quantitatively estimated by Swain and Hill’s method (1959). 500 mg of powdered plant material was homogenated with 10 ml of methanol in a pestle and mortar. The extract was centrifuged (remi Modl-24, India) at 3000 rpm for 10 min. and supernatant was evaporated to dryness keeping in a hot water bath (80º). Thus, the residue obtained was redissolved in 5 ml of distilled water and used for quantitative estimation of flavonoids.

2.2.6 Study of physicochemical parameters

2.2.6a Determination of total ash value

One gram of powdered plant material was taken in a previously weighed sintered silica crucible and incinerated by gradually increasing the heat until free from carbon and cooled, then kept in a desiccator for 15-20 minutes and the ash was weighed by using electronic balance and percent total ash was calculated with reference to the air dried plant material (Raghunathan, 1976).

2.2.6b Determination of acid insoluble ash

The total ash obtained was boiled for 15 minutes with 25 ml of 25% hydrochloric acid. This was filtered and the insoluble matter was collected on ashless Whatman filter paper, ignited in a previously weighed sintered crucible at a temperature not exceeding 450 ºC and then kept in
a desiccator for 15 minutes. The residue was weighed in electronic balance and the percent acid insoluble ash was calculated with reference to the air dried plant material (Raghunathan, 1976).

2.2.6c Determination of water soluble ash

The total ash obtained was boiled with 25 ml of water for 15 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450 °C and then kept in a dessicator for 15 minutes. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powdered material (Raghunathan, 1976).

2.2.6d Study of Organoleptic characteristics

Determination of moisture content

About 1.5 gm of the powdered plant material was taken into a weighed flat and thin porcelain dish. Dried in the oven at 100 °C. Cool in a desiccator and observed. The loss in weight was recorded as moisture. The percent moisture content was calculated with reference to the air dried powdered plant material.

Foreign organic matter, crude fibre content and extractive values were also determined using standard protocols (Raghunathan, 1976).
2.3 Results and Discussion

2.3.1 Preliminary phytochemical analysis

Phytochemical analysis of *P. quadrifida* Linn. revealed the presence of various secondary metabolites such as alkaloids, flavonoids, saponins, tannins and glycosides.

2.3.2 Quantitative estimation of primary and secondary metabolites

The quantitative estimation of powdered plant material of *P. quadrifida* Linn. was carried out for various primary and secondary metabolites. Powdered plant material contained significant amount of primary and secondary metabolite contents (Table-2.1). The pharmacological effects of any plant depends on its secondary metabolite content. Thus this plant can be further studied for its various pharmacological properties.

2.3.3 Determination of physico-chemical properties

2.3.3a Ash values

The residue remaining left after incineration represents the inorganic salts naturally occurring in the drug and adhering to it. It varies within definite limits according to the soils. It may also include inorganic matter deliberately added for the purpose of adulteration. Hence, an ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash values are helpful in determining the quality and purity of drug and these values are also important quantitative standards (Kokate, 2006). The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash that is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash is imposed, especially in cases when the calcium oxalate content of the drug is higher.
Results of the ash values are tabulated in Table-2.2. The total ash value, acid insoluble ash value and water soluble ash value were found to be 9.76, 0.94 and 5.8 % respectively. The total ash value was relatively high which may be due to high content of carbonates, phosphates, silicates and silica.

2.3.3b Organoleptic characteristics

Percent weight loss on drying or moisture content is the amount of water and volatile matters in a sample when the sample is dried under specific conditions. The moisture content of powdered plant material of *P. quadrifida* Linn. was found to be 8.75 % (Table-2.2). The less moisture content of the plant material could prevent bacterial, fungal or yeast growth (African pharmacopoeia, 1986). Foreign organic matter in the powdered plant material was found to be 8.6 %, this may be contributed to the wildness of the plant leading to its contamination in the course of its collection.

Crude fibre is the residue remaining after sequential digestion with sulfuric acid and sodium hydroxide solutions. The compounds removed are predominantly protein, sugar, starch, lipids and portions of both the structural carbohydrates and lignin. These residues (containing cellulose, hemicellulose, lignin, ash and tannins) are indigestible substances, often called structural carbohydrates, and are characterized by low or no nutritional value. Crude fibre content of the plant material of *P. quadrifida* Linn. was found to be 32 %.

The information obtained from the present study can be used to identify and decide the authenticity of this drug in herbal industry/trade and can be included as microscopic standards in Indian Herbal Pharmacopeia. The present investigation not only adds to the existing knowledge
of *P. quadrifida* Linn. but also and will be quite useful for development of a formulation in health care system.

### 2.4 Conclusion

The quantitative analysis of various secondary metabolites of *P. quadrifida* Linn. revealed that the plant contains significant quantities of secondary metabolites and can be studied further for various pharmacological effects.
Table-2.1-Quantitative estimation of primary and secondary metabolites of *P. quadrifida*

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Powdered plant material (mg/100g)</th>
</tr>
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<tbody>
<tr>
<td>Proteins</td>
<td>66.92±0.16</td>
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<tr>
<td>Carbohydrates</td>
<td>46.12±0.09</td>
</tr>
<tr>
<td>Amino acids</td>
<td>1.02±0.01</td>
</tr>
<tr>
<td>Total phenols</td>
<td>35.72±0.12</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>0.50±0.026</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.45±0.01</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>0.25±0.023</td>
</tr>
<tr>
<td>Saponins</td>
<td>1.86±0.023</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>12.90±0.031</td>
</tr>
</tbody>
</table>
Table-2.2- Physicochemical properties of *P. quadrifida* Linn.

<table>
<thead>
<tr>
<th>S No.</th>
<th>Parameters</th>
<th>Values obtained(% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total ash value</td>
<td>9.76</td>
</tr>
<tr>
<td>2</td>
<td>Acid insoluble ash</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>Water soluble ash</td>
<td>5.8</td>
</tr>
<tr>
<td>4</td>
<td>Moisture content</td>
<td>8.75</td>
</tr>
<tr>
<td>5</td>
<td>Foreign organic matter</td>
<td>8.6</td>
</tr>
<tr>
<td>6</td>
<td>Crude fibre content</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>Alcohol soluble extractive</td>
<td>6.4</td>
</tr>
<tr>
<td>8</td>
<td>Water soluble extractive</td>
<td>10.33</td>
</tr>
</tbody>
</table>