



## PHYTOCHEMICAL EVALUATION AND QUANTIFICATION OF PRIMARY METABOLITES OF *CASSIA PUMILA* LAMK

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### ABSTRACT

The developing countries mostly rely on traditional medicines. The traditional medicines involve the use of different plant extracts or the bioactive constituents. This type of studies provides health applications at affordable costs. Keeping this view in mind, laboratory evolutions were made to assess the phytochemical screening and quantification of primary metabolites in *Cassia pumila* Lamk. It contains higher soluble sugars in pods, starch in stem, lipid in fruits, phenol in pods, ascorbic acid in flowers, proteins in pods as compared to other parts of the plant. Maximum extractive value (11.086%) was found in alcoholic extract among test solvents. All the metabolites were qualitatively present in water and ethanolic extracts.

**Keywords:** Traditional medicines; Phytochemical; Primary metabolites; *Cassia pumila* lamk.

### INTRODUCTION

Plants and plants based medicaments have been employed since the dawn of civilization for prolonging life of man by combating various ailments. Ancient ethnic communities around the world have learnt to utilize their neighborhood herbal wealth for curative purpose. The medicinal values of the plant lie in the bioactive phytochemical constituents that produce definite physiological effects on human body. These natural compounds form the base of modern drugs as we use today<sup>1</sup>. Some of the plants have pharmacological properties while the others are used in indigenous medicine. There are many families of phytochemicals and they help the human body in a variety of ways. Phytochemicals are non nutritive plant chemicals that have protective or disease preventing properties. Hundreds of plants growing in forests are used as source of medicines throughout the world. Primary metabolites are substances widely distributed in nature, occurring in one form or another in virtually all organisms. Primary metabolites, for example; sugars, proteins, lipids, and starch are of prime importance and essentially required for growth of plants<sup>2</sup>.

*Cassia pumila* (Lamk.) belongs to the family Leguminosae, popularly known as "Sarmal/Nelatagache" is a diffuse terrestrial and strout annual herb that is usually found in shades of trees, crevices of rocks and also in the open gravelly substratum, often hidden amongst grasses. The plant species was reported to possess antimicrobial<sup>3</sup> and anti-inflammatory properties<sup>4</sup>. Phytochemically, the plant species have been studied only for spasmolytic anthraquinones<sup>5</sup>.

Phytochemicals are naturally occurring biochemical in plants that give plants their color, flavor, smell and texture. Preliminary phytochemical screening of medicinal plants is a useful method for qualitatively

determination of different metabolite in crude sample. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs.<sup>6,7</sup>

### MATERIALS AND METHODS

#### Collection of plant material

Plant material was collected from the hills of Amer, Jaipur. It was then authenticated by Herbarium, Department of Botany, Rajasthan University, Jaipur, Rajasthan, India.

The stem, leaves, flowers, pods and roots of *Cassia pumila* was properly washed under tap water, shade dried and powdered. The physicochemical parameters like extractive values, florescence characteristics of powdered plant materials and its extract, preliminary phyto-profiling and phytochemical analysis were determined. The powder was treated with acids like 1N HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, Acetic acid and alkaline solutions like 1N NaOH and ammonia.

#### Extraction of plant material

The powdered plant material was subjected to successive solvent extraction taking from non polar to polar solvents like petroleum ether, benzene, acetone, chloroform, ethanol and water. 50gms of powdered plant material was subjected to soxhlet extraction for 24 hrs with the various solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in a cool dry place for the analysis for the presence of preliminary phytochemicals.

#### Analysis of primary metabolites of *cassia pumila*

The extracts obtained were then subjected to preliminary phytochemical screening for the detection of various plant constituents. Each of these extracts was processed



further to evaluate the presence of carbohydrates, proteins, phenols, tannins, flavonoids, phytosterols, sennosides and anthraquinones were analyzed for their presence as per the standard procedures<sup>8</sup>.

Root, stem, pods, flowers and leaves of *Cassia pumila* were evaluated quantitatively to estimate the total levels of soluble sugars, starch, proteins, lipids, phenols, amino acid and ascorbic acid following the established methods for the sugars, starch<sup>9</sup>, protein<sup>10</sup>, lipids<sup>11</sup>, phenols<sup>12</sup>, amino acid<sup>13</sup>, ascorbic acid<sup>14</sup>.

## RESULTS

**Physico chemical evaluation:** Shade dried and coarsely powdered plant material was subjected to sequential solvent extraction in Soxhlet extractor successively using petroleum ether, benzene, acetone, chloroform, alcohol and distilled water. The maximum yield was found in alcohol extract i.e 11.086. Total extractive values are shown in table 1.

Preliminary phytochemical investigation revealed that petroleum ether extract contain phytosterols and sennosides, benzene extract contains carbohydrates, phytosterols and sennosides, acetone extract contains carbohydrates, flavonoids and sennosides, chloroform extract contains carbohydrates, phytosterols, flavonoids and sennosides, alcohol extract contains carbohydrates, proteins, tannins, phytosterols, flavonoids and sennosides, aqueous extract contains carbohydrates, lipids, proteins, phenols, flavonoids and anthraquinones (Table 2).

Primary metabolites viz soluble sugar, starch, proteins, lipid, ascorbic acid, total phenolic, DNA and RNA contents are quantified in different plant parts (root, stem, pods, flowers and leaves) and shown in table 3.

The powdered material of *Cassia pumila* treated with different acids, bases and other chemicals. After treatment powder observed and fluorescence were tabulated in table 4.

**Table 1:** Successive solvent extraction of air dried plant material of *Cassia pumila*

S.No	Solvent	Color and Consistency	Extractive value
1.	Petroleum ether	Greenish Yellow sticky	3.092
2.	Benzene	Pale yellow oily	1.365
3.	Acetone	Brown	0.367
4.	Chloroform	Yellow brown oily	0.768
5.	Alcohol	Reddish orange sticky	11.086
6.	Aqueous	Dark brown viscous	4.030

**Table 2:** Preliminary Phytochemical Test for Different Extracts of *Cassia pumila* Lamk (Obtained by Successive Solvent Extraction)

S.No	Test	Petroleum ether	Benzene	Acetone	Chloroform	Alcohol	Aqueous
1.	Carbohydrates	-	+	+	++	+++	+++
2.	Lipids	-	-	-	-	-	-
3.	Proteins	-	-	-	-	++	++
4.	Phenols	-	-	-	-	-	+
5.	Tannins	-	-	-	-	++	-
6.	Flavonoids	-	-	+	+	+	++
7.	Phytosterols	++	++	-	+++	++	-
8.	Sennosides	+	+	+	++	+	-
9.	Anthraquinones	-	-	-	-	-	+

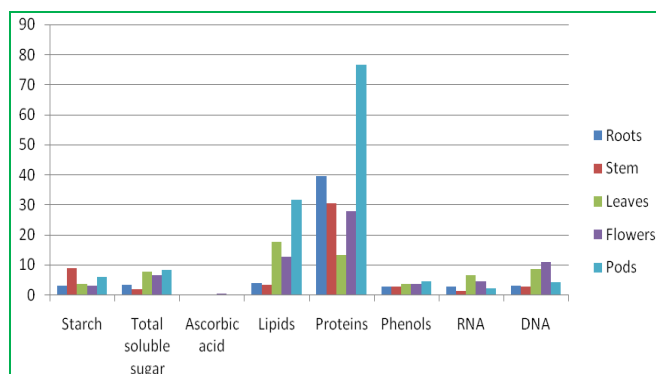
+ Relative intensities - No reaction

**Table 3:** Concentration of primary metabolites in *Cassia pumila* (mg/gdw)\*

	Roots	Stem	Leaves	Flowers	Pods
Starch	3.08	9.04	3.84	2.86	5.84
Total soluble sugar	3.34	2.03	7.86	6.48	8.39
Ascorbic acid	0.09	0.26	0.245	0.326	0.096
Lipids	3.89	3.48	17.67	12.68	31.68
Proteins	39.40	30.43	13.33	27.68	76.46
Phenols	2.68	2.74	3.67	3.64	4.56
RNA	2.74	1.46	6.60	4.48	2.06
DNA	2.89	2.78	8.67	10.78	4.08

\*mg/gdw- milligram per gram dry weight





**Figure 1:** Concentration of primary metabolites in *Cassia pumila* (mg/gdw)

**Table 4:** Fluorescence Analysis of Drug Powder of *Cassia pumila* Lamk

Treatment	Colour after treatment in daylight
<b>Powder treated with</b>	
Conc. H <sub>2</sub> SO <sub>4</sub>	GD-BN
Acetic acid	GN-YW
1N HNO <sub>3</sub>	OL-YW
5% I <sub>2</sub> solution	OL
5% FeCl <sub>3</sub> solution	PL-GN
Few drops of dil. NH <sub>3</sub> + K <sub>4</sub> Fe(CN) <sub>6</sub> solution	BR-RD
10% NaOH followed by a drop of CuSO <sub>4</sub>	OR-YW YW-OR
40% NaOH + few drops of 10% lead acetic solution	YW
Acetic acid Conc. H <sub>2</sub> SO <sub>4</sub>	DS-RD
Conc. HNO <sub>3</sub> + excess of NH <sub>3</sub>	OR-YW
Acetic acid + trace of FeCl <sub>3</sub> and transferred to the surface of Conc. H <sub>2</sub> SO <sub>4</sub>	YW

Abbreviations: BN= Brown, BL = Blood, DR= Dark, DT=Dusty, GN=Green, LF=Leafy, MA=Magenta, OL=Olive, OR=Orange, PL=Pale, RD= Red, Yw=Yellow

Quantitative estimation of sugar shows that content of starch is more in stems i.e. 9.04 mg/gdw and minimum in flowers i.e. 2.86 mg/gdw. The maximum amount of sugar was found in pods 8.39 mg/gdw and minimum amount was observed in stems 2.03 mg/gdw. Maximum amount of ascorbic acid was observed in flowers 0.326 mg/gdw and minimum amount was observed in roots 0.09 mg/gdw. Total levels of lipids were found to be higher in fruit 31.68 mg/gdw and minimum in stem 3.48 mg/gdw. The maximum amount of protein was found in pods 76.46 mg/gdw and minimum in leaves 13.33 mg/gdw. The maximum of phenol was found in pods 4.56 mg/gdw and minimum in roots 2.68 mg/gdw. Total levels of RNA were found to be higher in leaves 6.60mg/gdw and minimum in stem 1.46mg/gdw. The maximum level of DNA was present in flowers 10.78 mg/gdw and minimum in stem 2.78 mg/gdw.

## DISCUSSION

Preliminary phytochemical screening of plant is very useful for determination of the active constituents in different solvents and their yields. Most of the active principles are found in alcoholic and aqueous extracts. Our results were in agreement of previous reported results. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds. Plant synthesizes primary metabolites (lipid, protein, starch, sugars, phenol etc.) for the normal growth and development of itself. Many polysaccharides purified from Chinese medicinal herbs and phenols are bioactive and possess immunomodulating, anti-tumor and antibacterial activities<sup>15</sup>.

## CONCLUSION

In present investigation we found maximum extractive value (11.086%) found in water extract among test solvents. All the metabolites were qualitatively present in water and ethanolic extracts. The highest amount of sugar (8.39mg/gdw), protein (76.46mg/gdw), lipid (31.68mg/gdw) and phenol (4.56mg/gdw) was observed in fruits, ascorbic acid (0.326mg/gdw) and DNA (10.78mg/gdw) was observed in flowers, starch(9.04mg/gdw) in stem and RNA (6.60mg/gdw) was observed in leaves as compared to other parts of the plant. This study shows that plant parts having rich primary metabolites could be used industrially as raw materials for the synthesis of bioactive compounds. Therefore, economic use depends partially on the quantitative and qualitative aspects of their organic reserves, specially carbohydrates, proteins, phenols and lipids. These primary metabolites further used for biosynthesis of secondary metabolites or bioactive compounds.

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