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QUALITATIVE AND QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONTENTS OF HYDROALCOHOLIC EXTRACT OF *SALMALIA MALABARICA*

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Abstract

Plant phenolics and proteins have a powerful biological activity, which outlines the necessity of their determination. The aim of present study was to estimate the total phenolic and protein content of hydroalcoholic extract of *Salmalia malabarica.* Phytochemical screening of the plant showed the presence of glycosides, carbohydrates, phenols, proteins and amino acids and saponins. The phenolic content was determined by using Folin-Ciocalteu assay. The protien content was determined by using Lowry method. The total phenolic and protein contents were determined by established methods and were found to be 1.138 mg/100mg, and 129.4 µg/ml in gallic acid and protein equivalents respectively. Relatively high amount of phenolic and protein contents of hydroalcoholic extract of *Salmalia malabarica* make this plant a promising candidate for diseases treatment.

Keywords: *Salmalia malabarica, Hydroalcoholic extract, Phytochemical screening, Total phenolic ontent, Total protein content*.

Introduction

Phenolic compounds are one of the main secondary metabolites derived from pentose phosphate, shikimate and phenyl propanoid pathways in plants [1-3]. They are commonly found in non-edible and edible plants and posses numerological biological effects [4]. They are essential for reproduction and growth of plants. Phenolic compounds posses redox properties, which allows acting as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers and hence they are antioxidants. Phenolic compounds are plant substances which possess in common an aromatic ring bearing one or more hydroxyl groups. There are about 8000 naturally occurring plant phenolics and about half of this number are flavonoids [5]. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression [6]. Phenolics are the largest group of phytochemicals that account for most of the antioxidant activity in plants or plant products [7]. Bovine serum albumin (BSA) is a model globular protein which is widely used in biochemical studies [8, 9]. It is composed of 583 amino acid residues containing several sulfur, oxygen and nitrogen atoms [10]. BSA plays a significant role in many important physiological functions, and its structure and conformation can be easily changed [11].

Different parts of *Salmalia malabarica* [Malvaceae (Bombacaceae)] have been proven traditionally and scientifically for the treatment of several ailments. The gum of *Salmalia malabarica* has been traditionally used mainly in the treatment of haemoptysis of pulmonary tuberculosis, influenza and menorrhagia. It is also known for various other properties such as astringent, antiangiogenic, expectorant, analgesic, emetic, stimulant, anti-inflammatory, anti-hypertensive and antioxidant properties [12]. It has also been used in bladder disorders, calculus, leucorrhoea, tuberculosis and to cure conjunctivitis [13]. *Salmalia malabarica* has been proven effective in the treatment of acne and skin eruption problems [14]. Antibacterial and antifungal characteristics are also reported to be found in Salmalia malabarica. The methanol extract of *Salmalia malabarica* has shown a potent antibacterial activity [15]. Moreover, hypoglycemic and hypolipidemic effects were also studied in an n-hexane extract of sepal of *Salmalia malabarica* [16]. However, qualitative and quantitative determination and other effects of *Salmalia malabarica* still need to be elucidated.

Materials and methods

Plant material collection

The plant *Salmalia malabarica* was collected from local area of Bhopal (M.P.) in the month of Jan, 2018.

Storage

Drying of fresh aerial parts was carried out in sun but under the shade. Dried *Salmalia malabarica* was preserved in plastic bags and closed tightly and powdered as per the requirements.

Defatting and extraction of plant material

Salmalia malabarica was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Dried powdered *Salmalia malabarica* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40 °C.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [17, 18].

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25μg/ml was prepared in methanol. 10 mg of extract dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [19].

Total protein content estimation

The amount of protein was estimated by Lowry's method.

Reagents A. 2% Na_2CO_3 in 0.1 N NaOH
B. 1% NaK Tartrate in H_2O
C. 0.5% CuSO4.5 H_2O in H_2O
D. Reagent I: 48 ml of A, 1 ml of B, 1 ml C
E. Reagent II- 1 part Folin-Phenol [2 N]: 1 part water.

1 ml of each BSA (Bovine serum albumin) working standard 50-250 µg/ml or test in test tubes. The test tube with 1 ml distilled water was serve as blank. Added 4.5 ml of reagent I and incubated for 10 minutes. After incubation added 0.5 ml of reagent II and incubated for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph [20].

Results and discussion

Phytochemical screening of the plant showed the presence of glycosides, carbohydrates, phenols, proteins and amino acids and saponins Table 1. The total phenolic (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X+0.002, R^2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

The phenolic content was determined by using Folin-Ciocalteu assay. The protien content was determined by using Lowry method. The total phenolic and protein contents were determined by established methods and were found to be 1.138 mg/100mg, and 129.4 µg/ml in gallic acid and protein equivalents respectively Table 2-4 and Fig. 1 & 2. In recent years, the use of herbal products in disease treatment has received increasing attentions due to their diverse phytometabolic contents with various chemical structures and biological activities. Plants possess high amounts of polyphenols and protein leading therefore to various defensive and disease fighting properties. Phenolic compounds are plants secondary metabolites considered as very important plant constituents due to the presence of one or more hydroxyl groups on their aromatic ring. Those phenolic compounds being non harmful to human's health, there is an increase of the use of plants with high phenolics amount in the food industry aiming to improve the quality of foods [21].

Conclusion

Phenolic compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavour and also in providing health-beneficial effects. The general assessment of the analytical results for the plant extract definitely shows the individual specificity of each sample and a rich diverse spectrum of phenolic compounds. The plant merits further investigation to isolate its active constituents and to establish the activity in animal models. Therefore, present study can be useful to get a higher concentration of these substances since this species has a long history of commercial use.

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Table 1: Phytochemical screening of hydroalcoholic extract of *Salmalia malabarica*

S. No.	Test	*Salmalia malabarica*
1.	Detection of alkaloids: a) Hager's Test: b) Dragendroff's Test:	 -ve -ve
2.	Detection of carbohydrates: a) Fehling's Test:	 +ve
3.	Detection of glycosides: a) Legal's Test:	 +ve
4.	Detection of saponins a) Froth Test:	 +ve
5.	Detection of phenols a) Ferric Chloride Test:	 +ve
6.	Detection of flavonoids a) Alkaline Reagent Test: b) Lead acetate Test:	 -ve -ve
7.	Detection of proteins and aminoacids a) Xanthoproteic Test:	 +ve
8.	Detection of diterpenes a) Copper acetate Test:	 -ve

+ve = present, -ve = absent

Table 2: Calibration curve of gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

Table 3: Calibration curve of BSA

S. No.	Concentration	Absorbance
0	0	0
1	50	0.055
2	100	0.111
3	150	0.163
4	200	0.215
5	250	0.268

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Table 4: Total phenolic and protein content

S. No.	Extracts	Total Phenol (mg/100mg)	Total protein (µg/ml)
1.	*Hydroalcoholic extract*	1.138	129.4

Figure 1: Graph of estimation of total phenolic content

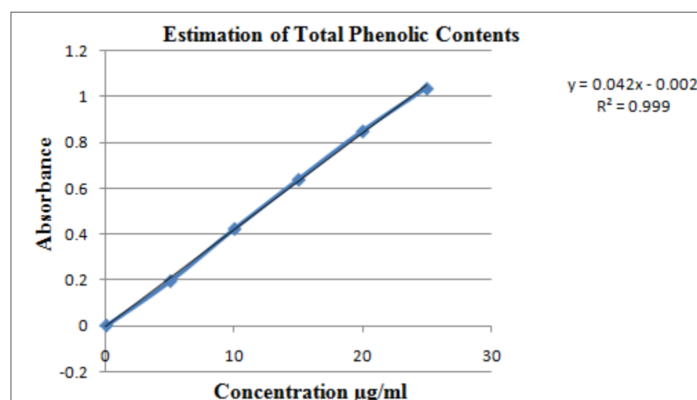
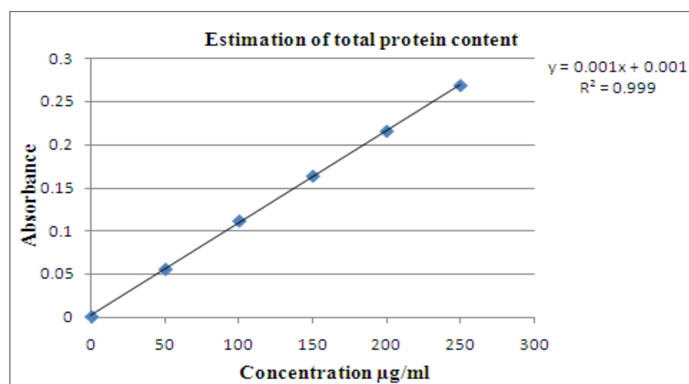


Figure 2: Graph of Estimation of Total Protein content



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