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Research Article

**QUALITATIVE AND QUANTITATIVE ESTIMATION OF
TOTAL PHENOLICS AND TOTAL FLAVONOIDS IN
LEAVES EXTRACT OF SARACA ASOCA (Roxb).****Dr. Garima Bartariya¹, Abhishek Kumar^{2*}, Basant Kumar³**¹Department of Life Science, ITM University, Gwalior, Madhya Pradesh
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E-mail- basantsingh31071994@gmail.com**Abstract:**

Saraca asoca is a evergreen plant, belongs to the family *Caesalpiniaceae*. This plant is commonly known as Ashoka or Sita ashoka. This plant has many pharmacological activities. In ancient time asoca was used as medicinal plant to cure various diseases such as uterine fibroids, piles diabetes and many others. In the present study leaf extract of asoca in five different solvent was used for both qualitative and quantitative estimation. Qualitative analysis was done to trace the presence of various compounds where as the quantitative analysis was done to evaluate the concentration of them. Phenols are important compound for the plant because it plays important role in nutrient uptake, photosynthesis, protein synthesis and binds to the other substance such as cellulose, protein and others. Flavonoids have many biological activities and pharmacological activities such as anti-microbial, anti-inflammatory, anti-tumor and many others. Flavonoids occur frequently in nature and found in vegetables, fruits, tea, coffee and fruit drinks. Results of qualitative study showed that phenols were present in chloroform and methanol extract whereas flavonoids were present in methanol and aqueous extract. Results of quantitative analysis exhibited that methanolic extract contain higher phenolic level and chloroform contains lower phenolic level whereas flavanoids level was higher in distilled water and lower in petroleum ether extract. The concentration of phenolic and flavonoids remained in between the range of 0.03429 to 0.173477 mg GA/g and 10.175 to 16.3875 mg RU/g respectively.

Keywords: *Saraca asoca*, phytochemicals, phenols, flavanoids, Quantitative analysis, Qualitative analysis.**Corresponding author:****Abhishek Kumar,**

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INTRODUCTION:

Saraca asoca (Roxb.) is a evergreen plant belongs to family caesalpiniaceae. It is commonly known as Ashoka. This plant is well known for its medicinal value [1]. The plant contains peripinnate, oblong and lanceolate leaves. Bark of this plant is dark brown and black in colour with watery surface and flowers fragmented, polygamous apetalous, bract small, deciduous and calyx petaloid [2, 3, 4]. *Saraca asoca* contains many medicinal compounds and oftenly used in all parts of India in the treatment of uterine fibroids, leucorrhoea, piles, dysentery, wound healing [5].

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which have many health benefits as macronutrients and micronutrients [6]. These compounds are known as secondary metabolites and have various biological properties such as anti-oxidant, anti-microbial, anticancer activity and helps in stimulation of immune system [7]. Phytochemicals are known to protect plants but recent researches shows that they also protect humans from diseases [7]. Phytochemicals accumulate in different parts of plant like roots, stem, leaves, flowers, fruits and seeds [8]. Phytochemicals are very useful for the maintaining of human health.

Phenols and flavonoids are widely distributed phytochemicals of plant which protect plants from various diseases. Phenols are most important secondary metabolites for the plants because it helps in photosynthesis, protein synthesis as well as nutrients binding [9, 10]. Phenols have several beneficial effects on human health such as used as antiseptic, used to treat sore throat and pharyngitis [11, 12]. Phenols are also used as preservative for some vaccines [13].

Flavonoids are low molecular weight compounds and contains "15" carbon atoms. It is densely distributed phytochemical in plant and participate in many function of plant. They are divided into two groups such as athocyanins and anthoxanthins [14]. Flavonoids are most important pigment of plant and responsible for colring of flower, providing yellow or red/blue color to petal to attract pollinator animals. It is frequently found in nature and occurs in vegetables, fruits and tea [15]. Flavonoids have many pharmacological activities such as antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumor activities [16].

MATERIALS AND METHODS:

The experiments were conducted in ITM University Gwalior Madhya Pradesh. The study was based on the evaluation of *Saraca asoca* significance in medicinal field. Fresh and disease free leaves of *Saraca asoca* were collected from the botanical garden. Collected leaves were washed with distilled water and shed dried at room temperature. The shed dried leaves were ground

and stored in air-tight container and used for solvent extraction. Five different solvents such as petroleum ether, chloroform, ethanol, methanol and distilled water were used for foliar extraction by soxhlet method.

Qualitative Analysis:

Qualitative analysis for phenol was done by following the methodology of Kalakotla *et al.* 2014, Nayak Sarojini *et al.* 2011 and Jayaramu *et al.* 2016 [1,17,18]. For this two ml of test sample was mixed with 2 ml of 5% ferric chloride solution in a test tube. Formation of blue colour indicated the presence of phenol.

Qualitative analysis for flavonoids was done by following the methodology of Kalakotla *et al.* 2014, Nayak Sarojini *et al.* 2011 and Jayaramu *et al.* 2016 [1,17,18]. For this flavonoids were tested by performing Alkaline reagent test. Few drops of sodium hydroxides solution i.e NaOH was added in 2 mili-litter of test extract. Acute yellow colour formed which turned into colour less solution on addition of few drops of dilute sulphuric acid (H_2SO_4), which indicated that extract possess flavonoids in it.

Quantitative Estimation:

Quantitative estimation was done by using the method of Senguttuvan *et al.* 2014 and Jaradat *et al.* 2015 [19, 20].

Phenols:

Total concentration of phenol was determined by using standard method of Senguttuvan *et al.* 2014 and Jaradat *et al.* 2015 [19,20]. Hundred microlitter of test sample was mixed with 105 mili-litter of 5% of Folin-Ciocalteu reagent and 1 ml of 20 % of sodium carbonate (Na_2CO_3). The solution was mixed properly and incubated for twenty minutes at room temperature. The absorbance was taken at 730nm by spectroscopy (PerkinElmer). Gallic acid was used as standard compound.

Flavonoids:

The total flavonoids content was determined by using standard method of Senguttuvan *et al.* 2014 and Jaradat *et al.* 2015 [19,20]. For testing of flavonoids 0.1 ml of sample extract were mixed with 0.3 ml of distilled water followed by addition of 0.03 ml of 5% sodium nitrate ($NaNO_2$). This mixture was incubated for 5 minutes at room temperature and 0.03 ml of 10% almunium chloride ($AlCl_3$) was added in it. After the addition of Almunium chloride is again incubated for 5 minutes at room temperature followed by addition of 0.2 ml of 0.1 M sodium chloride (NaCl). Absorbance was measured at 510 nm by spectroscopy (PerkinElmer). Distilled water was used as reference and Rutin as standard compound.

RESULTS AND DISCUSSION:**Qualitative Analysis:**

Phytochemicals, also known as secondary metabolites, are biologically active, which have many health benefits. Phenols are widely distributed secondary metabolites of plants and it has significant effect on plants like they acts as flower pigments and protect plants from invading agents. Flavanoids have strong history in ayurvedic medicine and also have various uses in skin protection, normal brain function, maintaining blood sugar level and blood pressure regulation. Results of qualitative analysis showed that phenols were present in foliar extract of methanol as well as chloroform solvent. This result was supported by findings of Nisha *et al.* 2016 [21] whereas flavonoids were present in methanol as well as aqueous extract. This is supported by the findings of Saha *et al.* 2012, Sarojini *et al.* 2011 and Pradhan *et al.* 2010 [17, 22, 23].

Quantitative Analysis:**Phenol:**

Phenols have many beneficial effects on human health in addition to this phenol derivatives are also used to prepare cosmetics such as sunscreens, hair colouring and etc. Result of quantitative study for phenol revealed that methanol extract contains higher phenol level. The concentration of phenol remains in between the range of 0.03429 to 0.173477 mg GA/g. It is lower in chloroform but higher in methanol. The order of the phenol content was chloroform < petroleum ether < distilled water < ethanol < methanol i.e 0.03429, 0.05326, 0.0880204, 0.124388, 0.173477 mg GA/g respectively. Methanol is a good solvent for the extraction of phenol and this result was supported by Ghatak *et al.* 2015, Jayaram *et al.* 2016 and Formagio *et al.* 2014 [9, 18, 24].

Table 1- Phenols and Flavonoids in Different Extract of Leaves of *Saraca asoca*

Phytoconstituents	Petroleum ether	Chloroform	Ethanol	Methanol	Distilled water
Phenol	-	+	-	+	-
Flavonoids	-	-	-	+	+

Table 2: Absorbance of Standard Compound (Gallic acid) at $\lambda = 730\text{nm}$

Gallic acid concentration (mg/ml)	Absorbance (mean) at 730nm
0.0312	0.236
0.0468	0.356
0.0625	0.401
0.125	0.909
0.25	2.036

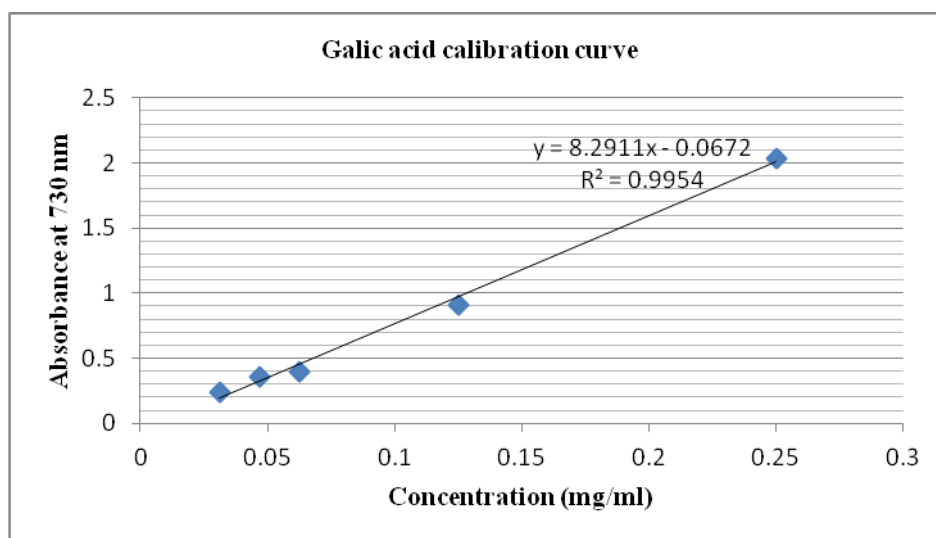
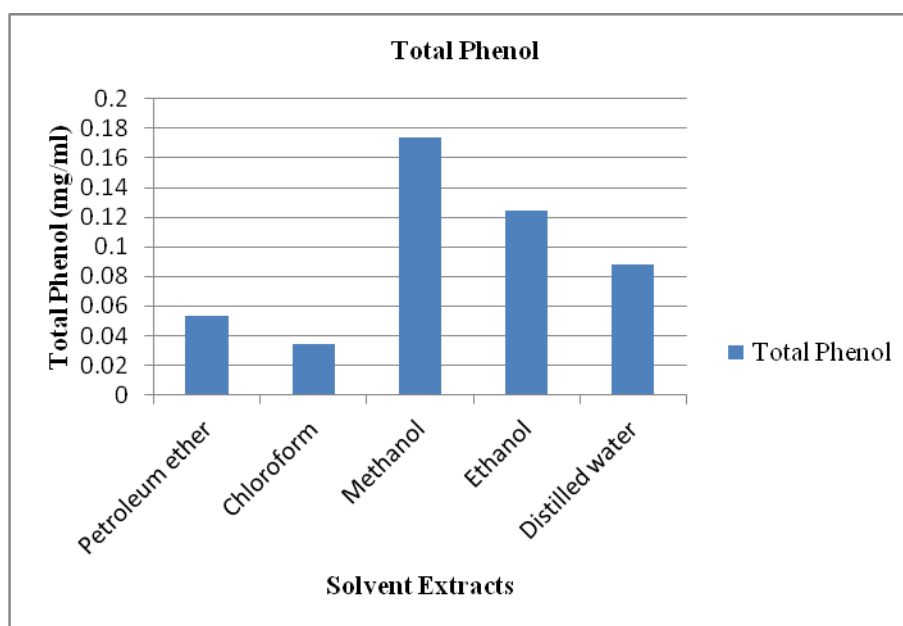
**Fig 1: Standard Calibration Curve of Gallic acid**

Table 3: Total Phenolic Content in different Extract of *Saraca asoca*

Solvents	Total Phenolic Content (mg GA/g) \pm SD
Petroleum ether	0.05326 \pm SD 0.010
Chloroform	0.03429 \pm SD 0.02
Methanol	0.173477 \pm SD 0.05
Ethanol	0.124388 \pm SD 0.0180
Distilled water	0.088204 \pm SD 0.43

**Fig 2: Total phenol Content in different Extract of *Saraca asoca*.****Flavonoids:**

Flavonoids have many pharmacological activities such as antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumor activities. Flavonoids was frequently used phytoconstituents in ayurvedic medicine for skin protection, brain function, blood pressure regulation so it has a strong history in ayurvedic medicine. Result of quantitative study for flavonoids showed that

distilled water extract contains higher flavonoids content in it which is similar to the result of Ghatak et al. 2015^[9]. The order of flavonoids content was distilled water> ethanol> methanol> chloroform> petroleum ether^[9]. The concentration of flavonoids remains between the range of 10.175mg RU/g (in petroleum ether) to 16.3875 mg RU/g (in distilled water).

Table 4: Absorbance of Standard Compound (Rutin) at $\lambda= 510$ nm

Rutin concentration (μ g/ml)	Absorbance (mean) at 510nm
0.3	0.126
0.6	0.176
1.2	0.201
2.4	0.368
6	0.588

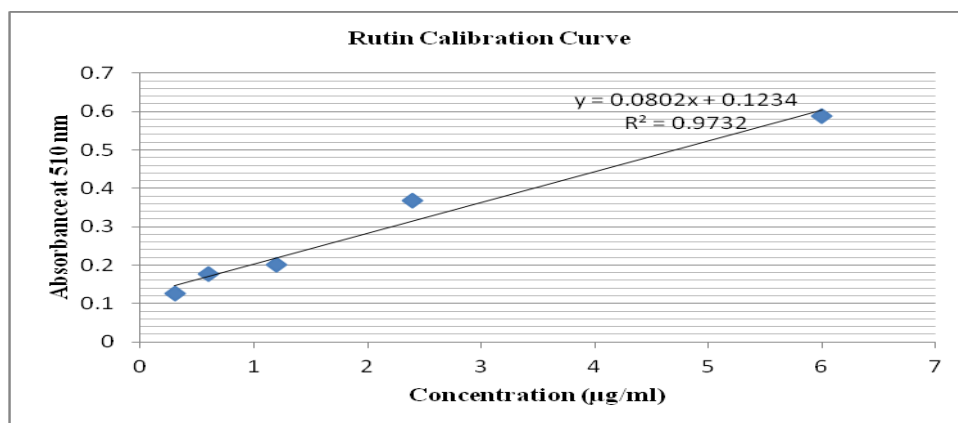
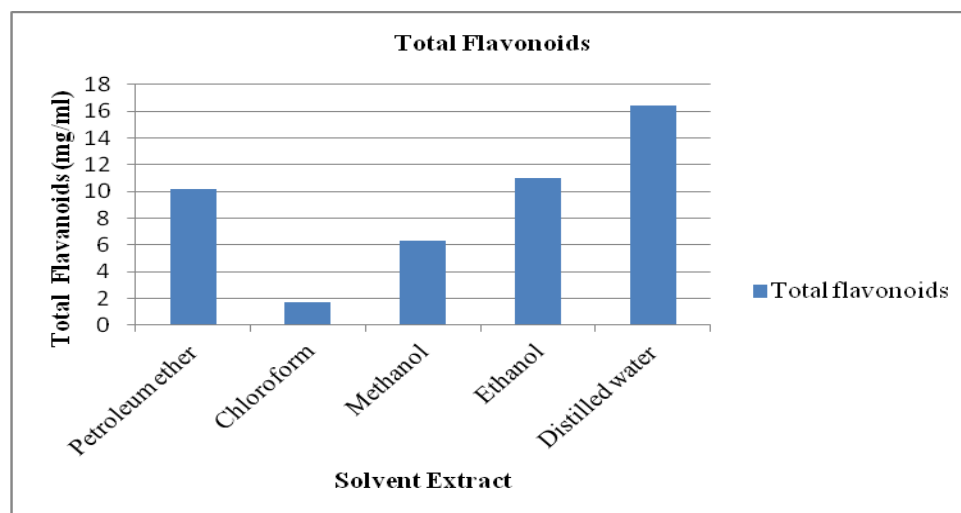


Fig 3: Standard Calibration Curve of Rutin

Table 5: Total Flavonoids Content in different Extract of *Saraca asoca*

Solvents	Total Flavonoid Content (mg RU/g) ± SD
Petroleum ether	10.175 ±SD 4.32
Chloroform	1.675 ±SD 0.42
Methanol	6.275 ±SD 1.80
Ethanol	10.9875 ±SD 1.98
Distilled water	16.3875 ±SD 4.34

Fig 4: Total Flavonoids Content in different Extract of *Saraca asoca***CONCLUSION:**

Saraca asoca has many pharmacological activities because it contains many medicinal compounds, which is often used in all parts of India as medicine in the treatment of uterine fibroids, leucorrhoea, piles, dysentery, wound healing. All parts of this plant contain a large number of phytochemicals. Phytochemicals are known as secondary metabolites and have various biological properties such as anti-oxidant, anti-microbial, anticancer activity and helps in stimulation of immune system. Phytochemicals are known to protect plants but recent researches show that they also protect humans from diseases and are very useful

for the maintaining of human health. Phenols and flavonoids are widely distributed phytochemicals of plants which protect plants from various diseases. Phenols have a significant effect on plants as well as human health like in plants, they act as flower pigments and protect plants from invading agents and in human health phenols act as antiseptic, preservative for some vaccines. Flavonoids have a long history in ayurvedic medicine and also have various uses in skin protection, normal brain function, maintaining blood sugar level and blood pressure regulation. Flavonoids have many pharmacological activities such as antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumor activities. Results of qualitative analysis

showed that phenols were present in foliar extract of methanol as well as chloroform solvent whereas flavonoids were present in methanol as well as aqueous extract. Result of quantitative study revealed that methanol extract contains higher phenol level whereas distilled water extract contains higher flavonoids level in it.

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