OPTIMIZING CONDITIONS FOR GALLIC ACID EXTRACTION FROM *CAESALPINIA DECAPETALA* WOOD

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ABSTRACT

Caesalpinia decapetala is a wild plant found in the Sub-Himalayan tract and planted in hedges throughout in India. The bark of *Caesalpinia decapetala* is a rich source of tannins. It has been used in treatment of jaundice, stomach disorders and biliousness. The leaves and root are used as purgative and emmenagogue. The process described in this paper outline the extraction of gallic acid from *Caesalpinia decapetala* which is used as antioxidant and anti-inflammatory. Optimization of various solvent extraction parameters was performed to assess maximum yield of gallic acid from *Caesalpinia decapetala* wood. The extraction parameters optimized are solvent, temperature and time for extraction. Optimization was carried out by performing different sets of experiments. The most suitable conditions for extraction of gallic acid were found to be extraction at temperature (65-70^{o}C), extraction time 48 hours and solvent composition Ethanol: Water (70:30). At these optimum extraction parameters the maximum yield of gallic acid obtained is 17.85%.

Keywords: *Caesalpinia decapetala*, gallic acid, extraction, optimization.

INTRODUCTION

Caesalpinia decapetala is a thorny climber or shrub up to 25 m in height, commonly found wild in the sub-Himalayan tract and planted in hedges throughout India. It is planted in gardens for its large racemes of bright yellow flowers. It is an excellent hedge-plant. A bath with decoction of the plant is useful in treatment of jaundice. The leaves are used to treat the burns, biliousness and stomach disorders (The Wealth of India, 1988). It is used as laxative, tonic, carminative and antipyretic (Kirtikar and Basu, 1984). Leaves and root of *Caesalpinia decapetala* act as a purgative and emmenagogue (Guha Bakshi and Sensarma, 1999). The leaves of *Caesalpinia decapetala* contain cassane diterpenoid, caesaldecan, spathulenol, 4, 5-epoxy-8(14)-caryophyllene, squalene, lupeol, *resveratrol*, quercetin, astragalin and stigmasterol (Kiem et *al*., 2005). In Maharashtra and South India, the bark is used for tanning (The Wealth of India, 1988). The objective of this study was to optimize the extraction parameters of gallic acid from the wood of *Caesalpinia decapetala*. This method optimizes the effect of solvent composition, temperature and time for extraction of gallic acid from wood of *Caesalpinia decapetala.* Since single solvent is not used, this may be deleted. The interaction between the factors influencing extraction of gallic acid was established and the effect of the factors on extraction of gallic acid from *Caesalpinia decapetala* was also described.

MATERIAL AND METHOD

Plant material

The plant wood of *Caesalpinia decapetala* was collected from Nashik region in the month of October-2007 and identified at Department of Botany, KTHM College of Art, Commerce and Science, Nashik (MS). The dried wood was milled with the help of pulveriser, sieved with 60 mesh and stored in air-tight container at 25^{o}C.

Extraction procedure

Dried wood powder was extracted by using mixture of different solvents. The major process parameters of extraction were optimized. These include solvents (Acetone: Water 70:30, 80:20 and Ethanol: Water 70:30, 80:20); temperature (25-30^{o}C, 50-55^{o}C, 65-70^{o}C) and time (12 hr, 24 hr, 48 hr, 60 hr). Gallic acid is soluble in ethanol, acetone and water therefore these solvents are selected for extraction. *Caesalpinia decapetala* wood powder was put into 100 ml conical flask and Acetone-Water; Ethanol-Water mixture were added in it, then kept aside for selected temperature (25-30^{o}C, 50-55^{o}C, 65-70^{o}C) for different periods of time (12 hr, 24 hr, 48 hr, 60 hr). Five gram of wood sample with 25 ml solvent was used for each treatment. After extraction, the extracts were concentrated, vacuum evaporated and were kept in desicator.

Estimation of total phenolic (Harbone, 1998; Jadhav *et al*., 2006)

The total phenolic content of Ethanol: Water extracts and Acetone: Water extract of *Caesalpinia decapetala* were determined by using the Folin – Ciocalteu assay. A stock solution (1 mg/ml) of the extracts was prepared in methanol. From the stock solution, 1 ml of the extracts of different concentrations ranging from 20 to 100 µg/ml was taken into a 25 ml volumetric flask and 10 ml of water and 1.5 ml of Folin – Ciocalteau reagent were added to it. The mixture was kept for 5 min, and then 4 ml of 20% sodium carbonate solution was added and made up to 25 ml with double distilled water. The absorbance

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Table: Effect of various parameters on the yield of gallic acid rich extract

A	Effect of solvent on the yield of gallic acid rich extract*		
	Solvent	Solvent ratio	% yield of gallic acid
	Acetone : Water	70:30	9.10
	Acetone : Water	80:20	12.07
	Ethanol : Water	70:30	17.85
	Ethanol : Water	80:20	10.19
B	Effect of temperature on the yield of gallic acid rich extract**		
	Temperature (^oC)	% yield of gallic acid	
	25-30	2.47	
	50-55	2.76	
	65-70	17.86	
C	Effect of time on the yield of gallic acid rich extract***		
	Time (hours)	% yield of gallic acid	
	12	5.02	
	24	7.09	
	48	17.85	
	60	17.85	

Weight of plant material: 50 gm
*No. of extraction, 4; Temperature, 65-70^oC (maceration); Time of extraction, 48 hours.
**No. of extraction, 4; Solvent/ Solvent composition, ethanol: water (70:30); Time of extraction, 48 hours.
***Solvent/Solvent composition, ethanol: water (70:30); Temperature, 65-70^oC (maceration); Time of extraction, 48 hours; No. of extraction 4.

was recorded at 765 nm after 30 min. Percentage of total phenolics was calculated from calibration curve of gallic acid plotted by using the above procedure, and total phenolics were expressed as % gallic acid.

Measurement of gallic acid (Banik and Pandey, unpublished results)
The amount of gallic acid was determined by using High Performance Thin Layer Chromatography (HPTLC). Standard gallic acid and the samples were spotted on precoated silica gel 60 F_{254} aluminium coated plate (E-Merck grade) as narrow, 10 mm wide band at a constant rate 10 μls^{-1} using a Camage Linomat IV model applicator under nitrogen atmosphere. A mixture of Toluene: Ethyl acetate: Formic acid (2.5:5:0.5) was used as mobile phase. The spots were quantified using Camage TLC Scanner with Wincats 4 software at 272nm.

RESULTS AND DISCUSSION

Caesalpinia decapetala is one of the widely used drugs in traditional medicines. In the present study, preliminary phytochemical testing showed the presence of phenolics and tannins along with flavonoids. The amount of total phenolics in Ethanol: Water and Acetone: Water extracts of *Caesalpinia decapetala* wood was found to be 1.32% (w/w) and 1.039% (w/w) respectively.

A total of three variables were analyzed with regard to their effects on gallic acid yield. In this study various process parameters (Solvent, temperature, time of extraction) for the extraction of gallic acid from *Caesalpinia decapetala* were optimized. Overall material balance of the process (in one experiment) was 50g *Caesalpinia decapetala* wood powder in 250 ml solvents.

Optimization was carried out by varying one parameter at a time, and keeping the other parameters at a constant, during each set of experiment. The results of these experiments are reported in table. It shows that by changing the solvent from Acetone: Water to Ethanol: Water and the solvent ratio from 70:30 to 80:20 the yield of the gallic acid rich extract increases up to 17.85%. Therefore the optimum solvent is Ethanol: Water (70:30). Similarly the change in yield of gallic acid rich extract at different temperature and time of extraction is given in table. It shows that extraction at 65-70^oC for 48 hours gives the yield up to 17.85%. Further increase in the time

of extraction from 48 hr to 60 hr does not produce changes in yield of gallic acid. Hence the optimum condition for extraction in this case is: Solvent: Ethanol: Water (70:30); Temperature of extraction: at 65-70^{o}C; Time of extraction: 48 hours (Koul and Koul, 2007).

CONCLUSION

The optimized parameters were validated by repeating these parameters in a large number of experiments and the results of all these experiments were consistent. The maximum yield of gallic acid is 17.85% (Patel *et al*., 2008).

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