International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 1229-1231

Received: 01-01-2018
Accepted: 03-02-2018

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Quantitative analysis of phytochemicals in methanolic extract of *Artocarpus heterophyllus*, *Carica papaya* and *Terminalia bellerica* plant leaves

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Abstract
The preliminary phytochemical analysis of *Artocarpus heterophyllus* (Jackfruit), *Carica papaya* (Papaya) and *Terminalia bellerica* (Bhumura) revealed the presence of tannin, saponin, flavonoid, terpenoid, alkaloid, steroid, glycosides and protein etc. in all the three plant leaves. The quantitative analysis of tannin, saponin and flavonoid of *A. heterophyllus* (Jackfruit), *C. papaya* (Papaya) and *T. bellerica* (Bhumura) were analyzed in methanolic extract. Out of the three plants, *T. bellerica* contains highest concentration of saponin (2.67±0.07 mg/100g DW) and Flavanoid (0.825 ±0.00 mg QE/g) of extract. The tannin concentration was highest in *C. papaya* (0.638 ± 0.05%).

Keywords: Phytochemicals, tannin, saponin, flavonoid, secondary metabolites, methanolic extract

1. Introduction
Phytochemicals are the secondary metabolites which are a wide range of organic compounds that are not essential for cell structure and maintenance of life but are often involved in plant protection against biotic or abiotic stresses. Unlike primary metabolites, the absence of secondary metabolites does not result in immediate death, but in the long-term impairment of the organism's survival/fecundity or aesthetics or, perhaps, in no significant change at all. Secondary metabolites are often restricted to a single species or a narrow set of species within a group, whereas primary metabolites are typically found throughout the plant kingdom. Secondary metabolites are involved in a series of ecological roles which include the following protection against herbivores and infection by microorganisms; aiding pollinators and seed-dispersing animals by serving as attractants in smell, colour or taste; and aiding in plant-plant competition (including allelopathy) and in plant-microbe symbiosis [1]. Thus, phytochemicals or the secondary metabolites, while not essential for growth and development, do promote the spread and dominance of plant species in an ecological setting [2].
As a result of this and the reported therapeutic activities associated with different phytochemicals, they are therefore worth the effort in research into the discovery of new drugs or as a viable alternative to the existing drugs.

2. Materials and Method
2.1. Collection of plant sample
The fresh leaves of *Artocarpus heterophyllus, Terminalia bellerica and Carica papaya* were collected from different parts of Assam. The plant materials were washed under running tape water followed by distilled water to remove dust and cut into pieces, dried under shed and pulverized into fine powder in a grinding machine (Bajaj), the powder was kept in small plastic bags away from the light, heat, moisture with proper labeling.

2.2. Preparation of plant extract
Plant extract was prepared by dissolving 100g of the sample in 1litr of methanol and kept for three consecutive days. The plant mixture was stirred two times at an interval of 6-8 hours with a clean glass rod. After third day the mixture was filtered through muslin cloth and filter paper. Filtrate was collected and stored in the refrigerator at 4^0C. Residue was collected and the extraction process was repeated two more times in the similar manner.

Now the pooled filtrate containing methanol was finally extracted using Rotary Vacuum evaporator (EVATOR.Iwp). After that a semi solid plant extract was obtained which was kept in the refrigerator at 4^0C.

3. Qualitative analysis of phytochemicals
Preliminary qualitative phytochemical screening was carried out using standard methods.

4. Quantitative determination of phytochemicals
Phytochemicals viz. tannin, saponin, flavonoids were estimated using methanolic extract of plant leaves.

4.1 Determination of tannin content
The amount of tannin in the methanolic extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5ml of 1N Folin-Ciocalteu reagent, 10 ml of 17% solution of sodium carbonate (Na_2CO_3) and 20 ml of distilled water were added to 1 ml of plant extract in 50 ml volumetric flask. The mixture was made up to the mark with distilled water and was allowed to stand for 20 min when bluish green colouration was developed. Standard tannic acid solution of range 0 -10 ppm were treated similarly as 1 ml of sample above. The absorbance of tannic acid standard solution as well as sample was read after colour development on spectrophotometer at 760 nm.

4.2. Determination of saponin content
The Spectrophotometric method described by Brunner (1984) [3] was used for saponin analysis. One g of finely grounded sample was weighed into a 250 ml beaker and to it 100 ml Isobutyl alcohol was added. The mixture was filtered through Whatman No. 1 filter paper into 100 ml beaker and 20 ml of 40% saturated solution of magnesium was carbonate added. The mixture obtained with saturated magnesium carbonate ($MgCO_3$) was again filtered through a Whatman No 1 filter paper to obtain a clear colourless solution. One ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. 0 -10 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$ solution as done for 1 ml sample above. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectrophotometer at a wavelength of 380 nm. The percentage saponin was calculated.

4.3 Determination of flavonoid content
Estimation of flavonoid content in plant extract was carried out using the method of Orden *et al.* (2006) [4]. According to this method, 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample. After one hour at room temperature, the absorbance was measured at 420 nm using UV- vis Spectrophotometer. A yellow colour indicated the presence of flavonoid. Extracts were evaluated at a final concentration of 0.1mg /ml. Total flavonoid content was calculated as the quercetin equivalent (mg/g) from the calibration curve.

5. Result and Discussion
5.1 Qualitative analysis of phytochemicals
The preliminary phytochemical analysis of *A. heterophyllus* (Jackfruit), *C. papaya* (Papaya) and *T. bellerica* (Bhumura) revealed the presence of tannin, saponin, flavonoid, terpenoid, alkaloid, steroid, glycosides and protein etc

5.2 Quantitative determination of tannin, saponin and flavonoid
Tannin, saponin and flavonoids are the plant secondary metabolites. Tannins are the phenolic compounds found in the cell vacuole surface of plant. Tannins bind to proteins nonspecifically either by hydrogen or covalent bond to amino acid group of proteins. Low to moderate amount of tannin in diet is beneficial for ruminant animals as tannins binds to the protein making it unavailable for microbial degradation in rumen. Thus, increases the intestinal absorption of protein which is reflected in terms of growth performance of the animals [5]. Saponins are naturally occurring surface acting agents and have the ability to form froth in aqueous solution. They consist of aglycon and sugar moeity. It has expectorant, diueretic activities [6]. Flavonoids are the classes of polyphenolic compounds which have antioxidant properties. Flavanoids have been reported to multiple biological functions including antimicrobial, cytotoxicity, anti-inflammatory. The quantitative analysis of tannin, saponin and flavonoid of *Artocarpus heterophyllus* (Jackfruit), *Carica papaya* (Papaya) and *Terminalia bellerica* (Bhumura) were analyzed in methanolic extract and the results are depicted in Table 1.

Table 1: Tannin, saponin and flavonoid content in methanolic extract of *Artocarpus heterophyllus, Carica papaya and Terminalia bellerica*

Sl. No.	Name of the Plant	Tannin content (%)	Saponin content mg/100g	Flavonoid content mgQE/g
1	*Artocarpus heterophyllus*	0.253_A±0.06	2.61±0.06	0.797_A±0.00
2	*Carica papaya*	0.638_B±0.05	2.64±0.05	0.769_B±0.00
3	*Terminalia bellerica*	0.562_{BC}±0.09	2.67±0.07	0.825_C±0.00
Overall (Mean ± SE)		0.485±0.06	2.64±0.03	0.797±0.01

$_{A,B,C}$ Means with different subscripts in a column differ significantly in groups (P<0.01)

Statistical analysis revealed that there is significant (P<0.01) difference among the three plant leaves i.e. *Artocarpus heterophyllus, Carica papaya and Terminalia bellerica* in terms of tannin and saponin concentration. The highest concentration of tannin (%) was found in *Carica papaya* (0.638 ± 0.05) followed by *Terminalia bellerica* (0.562 ± 0.09) and *Artocarpus heterophyllus* (0.253 ± 0.06). However, the flavonoid concentration (mg QE/g) was highest in *Terminalia bellerica* (0.825 ±0.00). But there is no significant (P<0.01) difference in saponin concentration among all the three plant leaves and the highest concentration of saponin (mg/100g) was found in *Terminalia bellerica***.** Ayodele and Olabode, 2015 [7] reported a flavonoid concentration of 0.275 ± 0.0015 mg QE/g in fresh leaves and 0.615 ± 0.025 in dry leaves of *C. papaya.* Similar findings were also reported in *Artocarpus heterophyllus* by Shanmugapriya *et al.*, 2011 [8] who have reported a flavonoid (mg QE/g) concentration of 4.05 ± 0.01 for ethanolic, 2.21 ± 0.02 for acetone, 2.67 ± 0.01 for ethyl acetate and 0.86 ± 0.01 for aqueous fraction of seed extract. The flavonoid concentration in *T. bellerica* was

recorded as 16.15 ± 2.42 mg QE/g of extract in an experiment [9]. Sharma *et al.,* 2015 **[10]** reported the tannin and saponin content of *A. heterophyllus* as 1.45 ± 0.29 g/ 100 g DW and 2.57 ± 0.17 g/100 g DW. On the other hand Gupta *et al.,* 2011 [11] recorded saponin concentration in jackfruit as 6.32 ± 0.098 g/100 g seed. Thus, from all the three medicinal plants *A. heterophyllus, C. papaya* and *T. bellerica* are rich source of phytochemicals and protects the individuals from oxidative damage.

6. Conclusions

The present study indicates that leaves of *Artocarpus heterophyllus, Terminalia bellerica and Carica papaya are rich source of phytochemicals.* Out of all the three medicinal plant leaves, *Terminalia bellerica* contained highest amount of saponin and flavonoid, while, *Carica papaya* was rich in tannin and thus protects the individuals from oxidative damage.

7. Acknowledgement

We are highly thankful to Department of Biotechnology (DBT), Govt. of India, New Delhi for their financial support and help during the research work.

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