Annals of Biology **38** (2) : 240-244, 2022

Impact Score : 0.32 (Scopus)

Screening and Quantitative Estimation of Bioactive Compounds from *Vitex negundo* and *Ficus carica* Leaf Parts

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(Received : February 02, 2022; Accepted : February 20, 2022)

ABSTRACT

Plants are the pharmaceutical and biochemical living stores, constituting various phytochemical compounds i. e. bioactive compounds such as alkaloids (organic compounds with one nitrogen atom), flavonoids (plant chemicals), saponins (class of bioorganic compounds), phenolic compounds, etc. This paper includes identification, authentication of *Vitex negundo* (*nirgundi*) and *Ficus carica* Linn. plants followed by Soxhlet extraction using solvents with their increasing polarity and quantitative screening of various bioactive metabolites from the obtained phytoextracts. Maximum yield (20.4%) was exhibited by *V. negundo* methanolic phytoextracts followed by (6.1%) obtained by *F. carica* leaves. Ethyl acetate showed intermediate yield followed by the presence of lesser yield in chloroform phytoextracts. Both the plants exhibited the presence of primary and secondary metabolites. However, protein and phenolic content were present in higher amount in plants than the other metabolites.

Key words : Medicinal plants, bioactive compounds, Soxhlet extraction, phytoextracts, phytochemical screening

INTRODUCTION

Plants are the most abundant bio-resource of traditional medicine drugs, nutraceuticals, food supplements, contemporary medicine pharmaceuticals, pharmaceutical intermediates, folk medicine drugs, and chemical derivatives for synthetic drugs. From the ancient time, plants have been playing a big key role for the advancement of people, presenting as an incredible supply of natural medicine. In traditional medical system, medicinal plants have great importance and in use for many applications. Because of their robust pharmacological actions, low toxicity and commercial viability, the therapeutic qualities of plants have also been researched in light of contemporary scientific discoveries around the world (Pohl *et al.*, 2016). Phytochemicals, also known as secondary metabolites, are found in complex combinations that vary by plant organ and development phase. The bioactive ingredients must be understood in order to investigate the plant's true usefulness in medicine (Singh *et al.,* 2016). All plant parts including stem, roots, leaves and flowers consist of various primary and secondary metabolites or the bioactive compounds like phenols, proteins, lipids, saponins, phytosterols, etc. which are otherwise known as the inherent silent tools of self-protection among plants.

Phenols are the simplest representatives of the most diverse groups of secondary metabolites i. e. phenolic compounds. These act as antioxidants (Martins *et al.*, 2016; Sumczynski *et al.,* 2016; Surco-Laos *et al.*, 2016), accumulate on leaves surface and capture 90% of UV- radiation (Verdaguer *et al.,* 2017). Glycosides are the naturally occurring complex organic compounds which play various roles in plants such as : Conversion of toxic materials to non-toxic ones acts as sugar reservoir and helps to regulate certain growth functions. Phytosterols, generally known as plant sterols and stanol esters, play major role in plant's growth and development by acting as signalling molecules (Sonawane *et al.*, 2017).

Natural compounds originating from plants have a lot of promise for drug research and development. Use of synthetic drugs possesses to show quicker effects but their impacts are for short time period and tend to have various side effects on our health. On the other side, herbal drugs or chemicals possess no or lesser side effects. Because synthetic chemicals have harmful effects on human health and the

environment, and because they are more expensive than natural herbs, treatment with natural ingredients is becoming more popular. *Ficus carica*, a deciduous tree, generally known as "Anjeer" belongs to the family Moraceae. The plant has been reported to have numerous bioactive compounds such as arabinose, β-amyrins, β-carotines, glycosides, β-setosterols and xanthotoxol.

Vitex negundo is known by the name as "nirgundi", bears vast applications in traditional medicines as an important ingredient. It is a five-leaved chaste tree, generally evergreen to semi-evergreen species, which contains different class of secondary metabolites, like polyphenolic compounds, terpenoids, glycosidiciridoids and alkaloids (Suganthi and Dubey, 2016).

The leaves consist of butanoic acid, p-hydroxy benzoic acid, oleanolic acid angusid, vitamin C, nishindine, sitosterol. This plant also consists of vetugnoside, negundoside, 5 hydroxy-7, 4'-dimethoxy flavones. Various pharmacological studies such as antioxidant activity (Fatema *et al.*, 2018) and anti-inflammatory activity (Sori *et al.,* 2018) have been reported using *V. negundo* plant extracts. The current study is focused on extraction using Soxhlet unit and quantitative screening of metabolites using *V. negundo* and *F. carica* leaf phytoextracts, which were prepared with three solvents viz., chloroform, ethyl acetate and methanol.

MATERIALS AND METHODS

The fresh leaves of *V. negundo* (nirgundi) and *F. carica* (anjeer) were collected in the month of March from the Neem Vatika Herbal Park, Samargopalpur, Rohtak, Haryana, India (Figs. 1 and 2), Fresh *V. negundo* and *F. carica* leaves were washed with distlled water twice to eliminate unnecessary debris, and then dry shade was applied before coarsely powdering in a mixer grinder and finally stored in an airtight container for further use.

The leaf portion of both the plants was collected and further processed to drying. For which the leaves were placed between the newspapers and on frequent alternate days the newspapers were changed and some weight was put upon them for the proper drying of the samples.

The dried samples of the plants were then mounted upon wooden cardboard of standard size i. e. 28 cm (breadth) x 42 cm (length) followed by proper tagging of the label card which constituted the information about the sample plant such as habitat, collection date, collection time, family, etc.

The plant species were identified based on

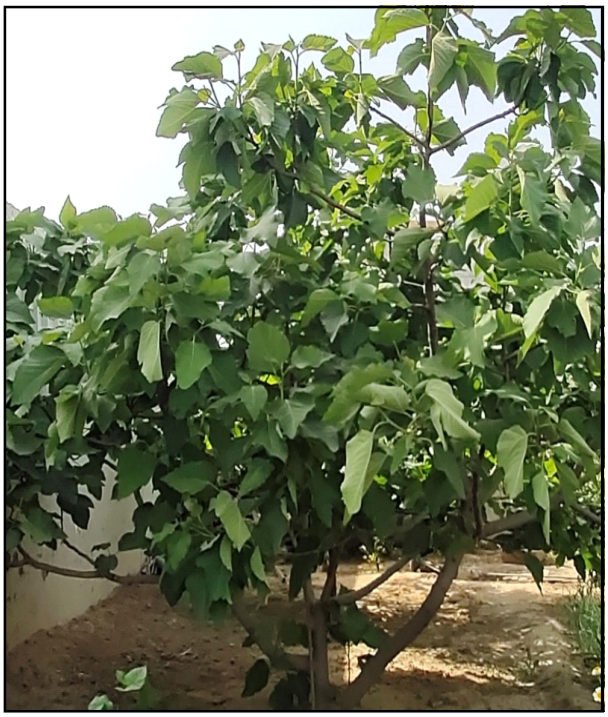

Fig. 1. *Ficus carica* tree.

Fig. 2. *Vitex negundo* tree.

their morphological appearance and their anatomical classification and further authenticated. A voucher number/Herbarium number was then allotted for both the individual plants :

***Ficus carica*–APRF-H-51**
***Vitex negundo*–APRF-H-52**

50 g of dried and coarsely powdered plant leaf samples were extracted with solvents chloroform followed by ethyl acetate and methanol in order of their increasing polarity for the sequential extraction using Soxhlet apparatus using three solvents of increasing polarity.

The procured yield of the phytoextracts (%) was calculated as :

$$[B - A] \times 100$$

Where,

A represents pre-weight (in grams) and
B represents after weight (in grams)

The Soxhlet extracts of leaves of *V. negundo* and *F. carica* plants were further screened for the detection of various primary and secondary metabolites such as proteins, saponins, phenols, lipids and phytosterol contents.

50 mg test samples each were homogenized in 10 ml of cold 10% trichloroacetic acid (TCA) for 30 min and then kept at 40°C for 24 h. After centrifugation, the supernatants from each of these combinations were removed. Each residue was resuspended in 10 ml of 5% TCA and incubated for 30 min on a water bath at 80°C. The samples were refrigerated before being centrifuged and the supernatants were eliminated. The residue was then washed with distilled water, diluted in 10 ml 1N NaOH, and allowed to remain at room temperature overnight.

The total protein content of each of the preceding samples (1 ml) was evaluated by spectrophotometer using the Lowry *et al.* technique. BSA was prepared as a stock solution in 1N sodium hydroxide (Sigma Chem. Co., St. Louis, USA) (1 mg/l). Eight concentrations (ranging from 0.1 to 0.8 mg/l) were measured in a test tube, and each sample's volume was raised to 1 ml by adding distilled water. Each received 5 ml of newly prepared alkaline solution, which was kept at room temperature for 10 min (prepared by combining 50 ml of 2% sodium carbonate in 0.1 N sodium hydroxide and 1 ml of 0.5% $CuSO_4.5H_2O$ (Copper (II) sulfate in 1% sodium potassium tartarate). After 30 min, the optical density of each sample was measured at 750 nm using a spectrophotometer against a blank using 0.5 ml of Folin-Ciocalteau reagent (commercially available reagent diluted with equal volume of distilled water shortly before use).

Separately, the test materials (200 mg each) were macerated for 2 h in 10 ml of 80% ethanol and then kept at room temperature overnight. The mixtures were centrifuged, and the supernatants were separated and stored in 80% ethanol up to 40 ml. Each sample's total phenol content was therefore assessed.

One hundred mg of the test samples were thoroughly mixed in 10 ml distilled water before being transferred to a conical flask containing 30 ml chloroform and methanol. The mixture was thoroughly mixed and left at room temperature in the dark overnight for maximum extraction. After that, 20 ml chloroform and 2 ml water were used to centrifuge the mixture. The lower layer of chloroform, which included all of the lipids, was carefully collected in pre-weighed glass vials, while the colored aqueous layer of methanol, which contained all of the water-soluble compounds and the thick pasty outer face layer, was discarded. After being evaporated to dryness, the chloroform layers were weighed. After three replications, the mean values of each treatment were obtained.

In a conical flask, 20 g of medication powder was dissolved in 100 ml of 20% aqueous ethanol. The solution was heated for 4 h with steady stirring at 55°C. The solution was filtered, and 200 ml of 20% ethanol was used to remove mark. After that, both extracts were combined and the solvent was evaporated until the extract had reached a volume of 40 ml. In a separating funnel, 20 ml of diethyl ether was used to extract the concentrate. The aqueous layer was kept, whereas the ether layer was thrown away. By adding 60 ml n-butanol to the aqueous extracts, they were purified. It was also rinsed twice with 10 ml of 5% aqueous NaCl. The saponin content was calculated as a percentage after the solution was dried.

On a water bath, dry and powdered plant material was defatted for 24 h in petroleum

ether (60-80°C). The defatted material was air-dried before being hydrolyzed for 4 h in 30% HCl (v/v). Each hydrolyzed sample was rinsed in distilled water until it reached pH 7, then dried. Benzene was used to extract the dry product for another 24 h. By employing a rotary evaporator, the extract was filtered and dried.

RESULTS AND DISCUSSION

The extracted yield by chloroform, methanol and ethyl acetate extracts of the plants *V. negundo* and *F. carica* was calculated. From both these plants, *V. negundo* showed that the methanol extracts registered higher percentage of yield. It may be due to high polarity of methanolic solvent, which can draw high variety of plant constituents than the other solvents. The per cent yield of crude phytoextracts (chloroform, ethyl acetate and methanol) from *V. negundo* and *F. carica* leaves is shown in Table 1. Methanol crude extracts of *V. negundo* leaves resulted highest yield (20.4%), followed by *F. carica* leaves (6.1%). Ethyl acetate leaf extracts of *F. carica* showed intermediate yield of 11.0%, followed by 2.0% yield of *V. negundo* leaf extracts. Chloroform extracts showed lowest yield in comparison to ethyl acetate and methanol.

Table 1. Phytoextracts yield (%) obtained by Soxhlet

Plant sample	Solvent	Yield (%)
Ficus carica	Chloroform	2.1
	Methanol	6.1
	Ethyl acetate	11.0
Vitex negundo	Chloroform	5.1
	Methanol	20.4
	Ethyl acetate	2.0

Using methanol, chloroform, water and acetone as solvents, Singh *et al.* (2016) found that methanolic extracts of *V. negundo* leaves resulted the highest extraction yield, which was then followed by bioactive screening, with the presence of glycosides, phenols, proteins, saponin and tannins. Pawar and Kamble (2017) concluded that the aqueous extract (6.75%) had the highest soluble extractive percentage of *V. negundo* leaves, followed by methanolic extract (4.35%) and acetone extract (1.8%).

The present study revealed that chloroform, ethyl acetate and methanol phytoextracts of leaves part of the plants *V. negundo* and *F. carica* exhibited the presence of various primary and secondary metabolites such as proteins phenols, lipids, saponins and phytosterols (Table 2). In solvent phytoextracts of *V. negundo* leaves, phenolic content of 2.70 mg/g wt. was found to be present in higher amount followed by proteins (2.49 mg/g wt.), phytosterols (1.1 mg/g wt.), lipids (0.5% w/w) and saponins (0.41% w/w). Whereas in solvent phytoextracts of *F. carica* leaves, protein content was found to exhibit higher amount of 1.39 mg/g wt. followed by phenols (1.22 mg/g wt.), phytosterols (1.03 mg/g wt.), lipids (0.35% w/w) and saponins (0.33% w/w).

Table 2. Metabolite screening of *Vitex negundo* and *Ficus carica*

S. No.	Metabolites	Concentration content	
		Vitex negundo leaves	*Ficus carica* leaves
1.	Proteins	2.49 mg/g wt.	1.39 mg/g wt.
2.	Phenols	2.70 mg/g wt.	1.22 mg/g wt.
3.	Phytosterols	1.1 mg/g wt.	1.03 mg/g wt.
4.	Lipids	0.5% w/w	0.35% w/w
5.	Saponins	0.41% w/w	0.33% w/w

In this study, it was found that in *V. negundo* leaf phytoextracts, phenols were present in higher amount than the other metabolites, whereas in *F. carica* leaf phytoextracts, proteins were present in higher amount. Similarly, Bameta *et al.* (2019) revealed the presence of bioactive compounds such as alkaloid, flavonoid, carbohydrates, glycosides, proteins and amino acids using the leaf extract of *V. negundo.*

CONCLUSION

Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. *V. negundo* and *F. carica* exhibited the presence of various primary and secondary metabolites. Methanolic phytoextracts of leaves of *V. negundo* yielded more than other used solvents i. e. ethyl acetate and chloroform. Thus, the leaves phytoextracts of the plant can be used as a potential alternate to synthetic chemicals. The preliminary findings pave the door for further research employing phytoextracts derived from methanol, chloroform and ethyl acetate for the development of innovative powerful medicines and phytomedicine.

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