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Quantitative phytochemical screening of *Filicium decipiens* Wight & Arn. leaves

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Abstract: *The quantitative phytochemical analysis was conducted on the leaves of Filicium decipiens for the content of secondary metabolites like alkaloids, flavonoids, glycosides, saponin, phenol and tannin by gravimetric and spectrophotometric methods. It is revealed that saponin (78 mg/g) and flavonoid (64 mg/g) content was present in maximum amount than the other secondary metabolites in the studied plant sample. The results of the present study support the therapeutic usage of the secondary compounds that can be used in new drugs for the treatment of various diseases. Hence it is confirmed the presence of phytochemicals and their respective quantities in the leaves of F. decipiens and further, the appropriate medicinal value is to be checked with relevant experiments and animal models.*

Keywords: *Filicium decipiens, Extracts, Quantitative analysis, Secondary metabolites, therapeutic usage*

I. Introduction

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans and animals against diseases. A number of phytochemical are known, some of which include: alkaloids, flavonoids, glycosides, saponins, tannins, anthraquinones, steroids and terpenoids. They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more [1].

In recent years, secondary plant metabolites (Phytochemical and phytotherapy) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents [2]. Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defence mechanisms against predation by microorganisms, insects and herbivores [3]. Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activities. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings. The evaluation of all the drugs is based on phytochemical and pharmacological approaches which lead to the drug discovery referred as natural products screening [4], [5].

There are large number of medicinal plants whose scientific importance and their phytochemicals has not been explored. All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine, particularly in India. The study plant *Filicium decipiens* White & Arn. belongs to the family sapindaceae were analyzed their phytochemicals quantitatively based on gravimetric and spectrophotometric methods. Hence, the highlight of the present study is there are no reports have been studied previously for quantitative phytochemicals of the species of *F. decipiens* were selected.

II. Methodology

A. Collection of Plant Materials

The healthy and matured leaves of *Filicium decipiens* White & Arn. were collected from our college campus, Pollachi, Coimbatore district and Tamilnadu state in India, during the month of December/January.

B. Preparation of the powder sample

The leaves were washed thoroughly 2 - 3 times with running tap water, leaf material was then air dried under shade. After complete shade drying the plant material was grinded in the mixer, the powder was kept in small plastic bags with proper label.

C. Preparation of Extract - Soxhlet Extraction

Crude plant extract was prepared by Soxhlet extraction method. About 50gm of powdered plant material was packed into a thimble and extracted with 500ml of methanol. The process of extraction was carried out at 55 - 85° C till the solvent become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30 - 40^0 C till all excess solvent got evaporated. Dried extract was kept in refrigerator at 4° C for future use.

D. Quantitative Determination of Phytochemical

i) Determination of alkaloids [6], [7]

Fifty gram of powdered sample was dispersed in 500 ml of 10 % acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 hours before it was filtered. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre - weight filter paper was used to filter off the precipitate and it was washed with 1 % ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60° C for 30 min. and transferred to desiccators to dry and cool and then weighed repeatedly until a constant value was obtained. The weight of the alkaloid was determined by deducting the filter paper weight. Triplicates were maintained.

ii) Determination of total flavonoids [8]

One gram of plant samples was repeatedly extracted with 100 ml of 80 % aqueous methanol at room temperature. The mixture was filtered through a Whatman No. 1 filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into water bath and allowed to evaporate to dryness and weighed.

iii) Determination of total saponins [9]

Fifty gram of powder sample was put into a conical flask and 500 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55° C. The mixture was filtered and the residue was re-extracted with another 500 ml of 20 % ethanol. The combined extracts were reduced to 100 ml by heating over water bath at about 90° C. The concentrate was transferred to a 1000 ml separating funnel and 50 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. To this, 150 ml of n-butanol was added. The combined n-butanol extract were washed twice with 25 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the oven to a constant weight, the saponin content was calculated and expressed in percentage.

iv) Determination of total glycosides [10]

Fifty gram of powdered dry samples of leaves was taken and added to 800 ml of alcohol (20 %) shaken for 6 hours and filtered. The residue was again extracted two times with 800 ml alcohol (20 %) and with chloroform (200 ml x 5) in a separating funnel. All the chloroform extracts (lower layer) were combined and transferred to a conical flask and dried over anhydrous Na_2so_4. The chloroform layer was decanted in the R.B. flask. Na_2So_4 was washed with chloroform (15 ml x 3) and the washings are added to the main chloroform bulk and it was evaporated to dryness. The dried residue is dissolved in a mixture of chloroform and methanol (1: 1) and filtered. The filtered was evaporated to dryness over a water bath on a weighted watch glass and the residue weighted as the glycosides content.

v) Determination of phenol [11]

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2 N of the folin-ciocalteu's reagent and 1.5 ml 20 % of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed

by vigorous shaking and finally allow to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

vi) Determination of tannin [12]

The methanolic extract (1 ml) was mixed with folin-ciocalteu's reagent (0.5 ml) followed by the addition of saturated Na_2Co_3 solution (1 ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant was obtained by centrifuge and absorbance was recorded at 725 nm using VU - visible spectrophotometer. Tannins content was calculated as mg tannic acid equivalent from a linear regression equation obtained from a calibration curve.

vii) Detection of Oil (Gravimetric method)

3 g of dry powder and added 10ml of methanol and chloroform (1 : 2) mixture. After centrifuged and collect the supernatant carefully. Add two drops of 0.005 KCL on the supernatant, it was thoroughly shaken and allowed to stand up for 5 min. The bottom lipid layer was collected in a pre - weighted beaker. Then the beaker kept in a water bath to evaporate the methanol and chloroform content. After cooling the beaker were reweighed using an electric balance.

viii) Data analysis

The methodology was repeated for triplicate value. Further the values are converted to mg/g. Results were averaged, and given as mean ± standard deviation, calculated by using the Microsoft excel.

III. Results

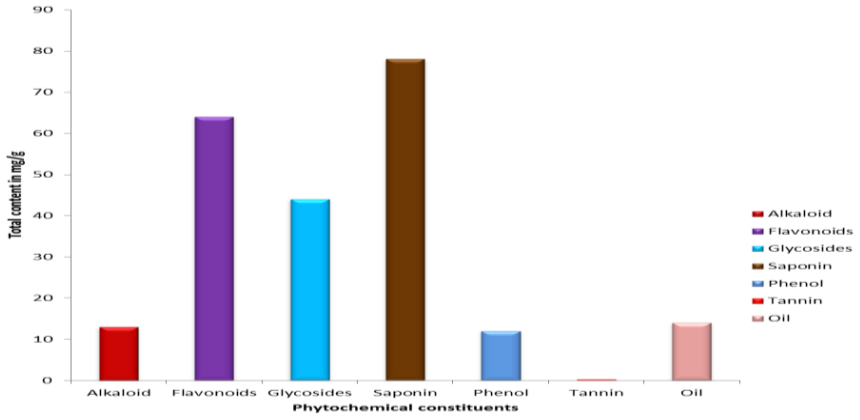
The result of the gravimetric and spectrophotometric studies are revealed that the quantity of major secondary metabolites (alkaloids, flavonoids, glycosides, saponins, phenol, tannins and oil) in the leaves of *Filicium decipiens* White & Arn. are showed in Table 1 and Figure 1. The present study was observed that the content of the following secondary compounds viz. alkaloids 13 mg/g, flavonoids 64 mg/g, glycosides 44 mg/g, saponins 78 mg/g, phenolic content 12 mg/g, tannins 0.3 mg/g and essential oil 14 mg/g of the extract. The overall results revealed that the saponins and flavonoids are present in high amount and the alkaloid and tannin are present in lower amount of the leaves of *F. decipiens* (Table 1).

Table 1. Quantitative analysis of leaves of *Filicium decipiens.*

S.No	Phyto constituents	Total content in mg/g
1	Alkaloid	13 ± 1.53
2	Flavonoids	64 ± 2.52
3	Glycosides	44 ± 1.00
4	Saponin	78 ± 1.53
5	Phenol	12 ± 0.58
6	Tannin	0.3 ± 0.08
7	Oil	14 ± 1.53

N = 3 Values are expressed as mean ± Standard Deviation (SD)

Figure 1. Quantitative phytochemical content variations of leaves of *Filicium decipiens*



IV. Discussion

Many studies have illustrated the differences in composition and concentration of phytochemical among plant extracts of the different species. Our results are agreed with the phytochemical studies of *Cardiospermum halicacabum* revealed that the various plant parts like leaf, seed coat and stem contain a broad spectrum of secondary metabolites such as flavonoids, terpenoids and cardiac glycosides are quantitatively rich in three different solvent extract [13]. Similarly the content of certain secondary metabolites of medicinal importance in leaves of the plant, *Acacia caesia s*howed that the compounds such as alkaloids (3.50 mg/g), flavanoids (11.67 mg/g), glycosides (3.00 mg/g) and saponins (3.00 mg/g) were estimated by gravimetric and spectrophotometric methods [14]. [15] Pragada *et al*. (2011) carried out preliminary phytochemical analysis and quantification of total phenols, and antibacterial activities of the hydro alcoholic (70 % ethanol) extract of *Acalypha indica*.

Alkaloids are the most significant compounds play a metabolic role in the living systems and are involved in the protective function in animals. Steroidal alkaloids are medicinally evolved. Flavonoids have been used against the cancer causing tumors and it inhibits the promotion of growth and progression of tumors [16]. Phenols when mixed with the flavonoids compounds in plants are reported to show multiple activities like antioxidant, anti-carcinogenic, anti-inflammatory etc. [17]. Tannins inhibit the pathogenic fungi and antimicrobial activity of extracts showed better activity by the presence of tannins.

Generally all the sapindaceae members are recorded as saponin rich family similarly the saponins are also rich in the study species *F. decipiens* (78 mg/g). Saponins are steroid and triterpenoid glycosides that display diverse biological activities [18]. Based on various authors saponins have been studied for their wide range of properties, including beneficial and detrimental effects on human health, pesticidal, insecticidal, molluscicidal and fungicidal activity, bitterness and sweetness and other industrial applications such as foaming and surface active agents [19] to [26].

V. Conclusion

The study confirmed the presence of phytochemicals and their respective quantities in the leaves of *F. decipiens* and further, the medicinal value is to be checked with relevant experiments and animal models.

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