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PHYTOCHEMICAL SCREENING OF *CATHARANTHUS ROSEUS* AND *FICUS RACEMOSA* LEAVES EXTRACTS: A STATISTICAL INFERENCE

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Abstract: This study was aimed at conferring the comparative abundances of ten different phytochemicals (alkaloids, polyphenols, flavonoids, tannins, saponins, sterols, vitamin-C, coumarins, terpenoids, and cardiac glycosides) from leaves of *Catharanthus roseus* and *Ficus racemosa*. The color strength or the precipitate formation was used as analytical answers to these tests. Seven phytochemicals viz. alkaloids, polyphenols, tannins, saponins, sterols, terpenoids, and cardiac glycosides in *Catharanthus roseus* leave {X^2 (df); p-value: 6.2 (2); 0.04} whereas saponins in *Ficus racemosa* leave {X^2 (df); p-value: 3.8 (2); 0.15} were also acknowledged as highest concentration category in this study. It is anticipated that the vital phytochemical properties documented in our study in the native medicinal plants of *Catharanthus roseus* and *Ficus racemosa* will be beneficial for explanation and groundwork of Pharmacognosy profiling of medicinal plants.

Key words: Cassava, medicinal plants, statistical inference, phytochemicals

INTRODUCTION

Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago [1]. From that point the development of traditional medical systems incorporating plants as a means of therapy can be traced back only as far as recorded documents of their likeness. However, the value of these systems is much more than a significant anthropologic or archeological fact. Their value is as a methodology of medicinal agents, which, according to The World Health Organization [2], almost 65% of the world's population has incorporated into their primary modality of health care [3].

Kokate CK (2009) has acknowledged in his test book that Periwinkle or *Catharanthus roseus (L.) G. Don* (Family Apocyanaceae), commonly known as "Nayantara" is an erect bushy perennial herb and evergreen shrub. The species was formerly known as Vinca rosea. The native of "Periwinkle" is mainly Madagaskar. This plant is grown commercially for its medicinal uses in Australia, Africa, India and Southern Europe. Except the highly alkaline or water logged soil, "Periwinkle" does not require any special conditions of soil. It favorably grows in light sandy soil, rich in humus. The rainfall of about 100 cm is most suitable for it. The leaf is simple, opposite, estipulate, petiolate and PP Joy (1998) has enlisted phytochemical compositions of *Catharanthus roseus (L.) G. Don* resulting more than 100 alkaloids and related compounds have so far been isolated and characterized from this plant [4, 5]. *Ficus racemosa* is distributed throughout Bangladesh particularly in evergreen forests and moist localities. Root, bark, leaves, fruit and galls are part of tree used for therapeutic activity. Bark, leaves and unripe fruit are carminative, astringent, stomachic and vermicide. *Ficus racemosa* fitting to family Moraceae is known to possess astringent activity. It is used in dental preparations [6]. All parts of *Ficus racemosa* are medicinally important in traditional system of medicine in India and have been used extensively in biliary disorders, jaundice, dysentery, diabetes, and diarrhea and in inflammatory conditions [7, 8]. The general objective of this study was to screen the ten selected phytochemicals such as Alkaloids, Flavonoids, Polyphenols, Saponins, Steroids, Coumarins, Terpenoids, vitamin-C, Tannins, and Cardiac glycosides in the two selected plants leaves (*Catharanthus roseus* and *Ficus racemosa*) and the specific objective was to statistical inference for the distribution of ten selected phytochemicals within and/or among nominated plants leaves.

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MATERIALS AND METHODS

Plant materials

The present study encompassed plant species which were *Catharanthus roseus* and *Ficus racemosa*.

Chemicals

80% Methanol, H_2So_4, Wagner's reagent (Iodine in potassium iodide), NaOH, HCl, FeCl3, [K3 (Fe (CN) 6], Acetic anhydride or Acetyl acetate, Chloroform, NH4OH, Ether and Indophenols.

Sample collection

Two medicinal plants were collected locally from the farm lands in Bangladesh. *Catharanthus roseus* and *Ficus racemosa* leaves were collected from Tongi (Gazipur) and Chadpur local area in Bangladesh respectively. The plants collected were identified by local agriculture officer. Fresh and tender leaves of selected plants were used for phytochemical analysis.

Preparation of plant extract and phytochemical screening

The leaves of the selected plants were collected from the plants and then washed off under running tap water to eradicate dust. The plant samples were then air dried for few days and the leaves were grinded off into powder and kept in polythene bags for future uses. The extracts of selected sample powder were prepared by soaking 50gm of dried powder in 100 ml 80% methanol and shaken well. The solution then filtered with the help of filter paper and filtered extracts of the selected plant sample were taken and used for further phytochemical analysis. Phytochemicals screening of the selected plant extracts were done by using standard methods [9, 10, 11, and 12].

Statistical analysis

The screening of ten selected phytochemicals among the nominated plants *Catharanthus roseus* and *Ficus racemosa* leaves was statistically evaluated. Pearson Chi-square test was used to find out correlation of phytochemicals distribution in selected plants leaves extracts. This analysis was carried out using SPSS version 21.

RESULTS

This study has exposed the presence of phytochemicals considered as active medicinal chemical constituents. The all selected plants show the ten selected phytochemicals with different concentrations, as shown in table 1. Graphical presentation of ten selected phytochemicals viz. alkaloids, flavonoids, polyphenols, saponins, steroids, coumarins, terpenoids, vit C, tannins, and cardiac glycosides are exhibited in figure 1.

Table 2 shows the relative frequencies of phytochemicals among two selected plants. 7 (70%) out of ten phytochemicals are highest concentration in *Catharanthus roseus* whereas 1 (10%) selected phytochemicals are found in *Ficus racemosa* leaves.

In *Catharanthus roseus* leaves, alkaloids, polyphenols, saponins, steroids, terpenoids, tannins, and cardiac glycosides were in highest concentration category while coumarins in medium and flavonoids as well as vit-C in lowest concentration categories. The phytochemicals distributions in Catharanthus roseus leaves were statistically significant ($p < 0.05$). On the other hands; statistically non-significant phytochemical distributions were found in *Ficus racemosa* ($p > 0.05$).

Table 1: Phytochemicals screening of *Catharanthus roseus* and *Ficus racemosa* leaves extracts

Name of phytochemicals	Methods of chemical reactions	Leaves extracts	
		Catharanthus roseus	*Ficus racemosa*
Alkaloids	Wagner's test	+++	-
Flavonoids	Pew's Tests	+	+
Polyphenols	Ferric chloride test	+++	++
Saponins	Foam Test	+++	+++
Steroids	Salkowski test	+++	++
Coumarins	Alkaline test	++	+
Terpenoids	Salkowski test	+++	++
Vit-C	Indophenol reaction	+	-
Tannins	Ferric chloride test	+++	+
Cardiac glycosides	Keller-killiani test	+++	-

Legends: ["+++" = high amount after added of reagent immediately;" ++" = moderate amount after 5 minutes of reagent added; "+ "= low amount after 10 minutes of reagent added and "-" = absence]

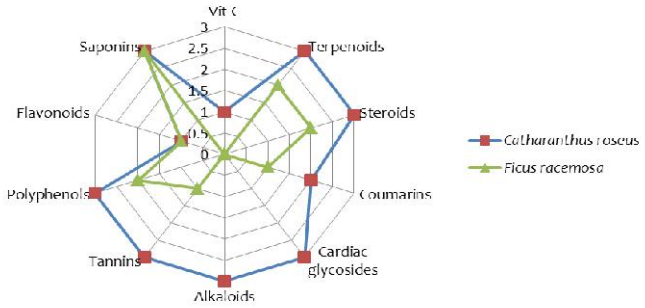

Figure 1: Cosmological representation of ten selected phytochemicals of *Catharanthus roseus* and *Ficus racemosa* leaves extracts. [Parenthesis; 1 = "+", 2 = "++", and 3 = "+++"]

The flavonoids, tannins, and coumarins were recorded as lowest concentration category while polyphenol, steroids, and terpenoids as medium concentration category as well as saponins with highest concentration category in *Ficus racemosa*. The alkaloids, vit-C and cardiac glycosides were absent in *Ficus racemosa* leaves extracts

Table 2: Frequency of selected ten phytochemicals screened in *Catharanthus roseus* and *Ficus racemosa* leaves extracts

Qualitative features	Leaves extracts	
	Catharanthus roseus N (%)	*Ficus racemosa* N (%)
Highest (+++)	7 (70%)	1 (10%)
Medium (++)	1 (10%)	3 (30%)
Lowest (+)	2 (20%)	3 (30%)
Absence (-)	0	3 (30%)
Total (N%)	10 (100%)	10 (100%)
X^2 (df);p value	6.2 (2); 0.04*	3.8 (2); 0.15

*statistically significant

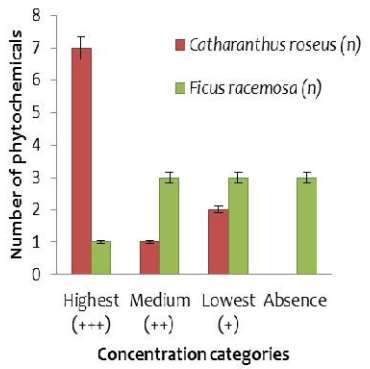

Figure 2: Graphical presentation of ten selected phytochemicals with their concentration categories specifications. The column (X-axis) represents the concentration categories of selected plants with two color stratified and the row (Y-axis) with frequency of phytochemicals. Error bars are generated with 5% value from selected data chart.

DISCUSSION

The ten selected phytochemicals distribution among the two selected plants has been correlated statistically in this study. Generally, phytochemicals acts as poisonous agents and protect the plants against insects and herbivores. Some acts as regulatory growth factors of growing plants [13]. Hesse *et al.,* (2002) documented that the alkaloids showed stimulant to CNS, anti-microbial activities, sympathomimetic, vasodilator, antihypertensive, antipyretics, antimalarial [14] and some psychoactive drugs namely methamphetamine (Yaba), amphetamine and so on produced from isolated alkaloids [15]. The plant *Catharanthus roseus* with highest alkaloids bestows high medicinal values.

Saponin was in highest concentration categories among two selected plants and has the property of precipitating and coagulating red blood cells [16]. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and hemolytic activity, and bitterness [17]. Several epidemiological studies have shown beneficial effects of polyphenol in cancer, cardiovascular, and neurological diseases. The health benefits associated with polyphenol containing preparation consumption have also been corroborated in animal studies of cancer chemoprevention, hypercholesterolemia, atherosclerosis, parkinson's disease, alzheimer's disease, and other aging-related disorders [18]. The existence of phenolic compounds in this plant signpost that these selected plants may be used as a medicine. The tannin content of the herb is guessed to account for the pragmatic anti-inflammatory activity of the plant material. It employs anti-inflammatory effects probably by inhibiting the release, synthesis and /or production of inflammatory cytokines and mediators, including prostaglandins, histamine, polypeptide kinins and so on [19]. Tannins have astringent properties, accelerate the healing of wounds and inflamed mucous membranes. Tannins are also testified to have various physiological effects like anti-parasitic anti-irritant, anti-scretolytic and anti-microbial activities. Plants containing tannin are used to treat non-specific diarrhea and inflammation of the mouth [20].

In our study, flavonoids contents of the two selected plants were also reported and Havsteen BH(2002) acknowledged that the flavonoids control plant growth by reticence of the exocytosis of the auxin indolyl acetic acid, as well as by stimulation of gene expression, and they stimulus other biological cells in numerous ways. Flavonoids inhibit or kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. Yet, their toxicity to animal cells is low. Flavonoids are major functional components of many herbal and insect preparations for medical use, e.g., propolis (bee's glue) and honey, which have been used since ancient times. Modern authorised physicians are increasing their use of pure flavonoids to extravagance many important common diseases, due to their proven ability to inhibit specific enzymes, to simulate some hormones and neurotransmitters, and to scavenge free radicals [21].

The methanolic extracts of *Catharanthus roseus* and *Ficus racemosa* leaves were also reported that the steroids, coumarins, terpenoids, and vit-C were present at different concentration categories and these phytochemicals exhibited the medicine values. Liu H (2011) and Farinola N (2005) reported that coumarins used in the treatment of asthma and lymphedema as well as this medication was a blood thinner used to keep blood flowing smoothly and prevent the formation of blood clots [22, 23].

Terpenoids, the largest group of phytochemicals, traditionally used for medicinal purposes in India and China, are currently being explored as anticancer agents in clinical trials. Terpenoids (also called "isoprenoids") are secondary metabolites occurring in most organisms, particularly plants. More than 40 000 individual terpenoids are known to exist in nature with new compounds being discovered every year. A large number of terpenoids exhibit cytotoxicity against a variety of tumor cells and cancer preventive as well as anticancer efficacy in preclinical animal models [24].

Cardiac glycosides encompass a large family of naturally resulting compounds, the central structures of which contain a steroid nucleus with a five-membered lactone ring (cardenolides) or a six-membered lactone ring (bufadienolides) and sugar moieties [25]. A few widely documented examples of cardiac glycosides are digoxin, digitoxin, ouabain, and oleandrin. Their positive inotropic effects help conquer the active counter-transportation of Na^+ and K^+ across the cell membrane, leading to an increase in the intracellular Na^+ concentration, a decrease in the intracellular K^+ concentration, and a consequent increase in cardiac contraction [26]. Epidemiologic indication suggests that breast cancer patients who were treated with digitalis have a significantly lower mortality rate, and their cancer cells had more benign characteristics than those from patients not treated with digitalis [27]. Interestingly, the concentrations of cardiac glycosides used for cancer treatment are extremely close to those found in the plasma of cardiac patients treated with the same drugs, suggesting that the anticancer effects of these drugs are exerted at non-toxic concentrations [28].

The selected two medicinal plants are the source of the phytochemicals play a vital role in preventing various diseases. The phytochemical analysis of the medicinal plants is also important and has commercial attention in both research institutes and pharmaceutical companies for new drugs manufacturing. Thus we hope that the important phytochemical properties acknowledged by our study in the local plant of *Catharanthus roseus* and *Ficus racemosa* leaves will be helpful in the managing different diseases. So, the need to discover and develop *Catharanthus roseus* and *Ficus racemosa* plant is crucial, especially in the view of the rapidly growing need for improvement in the medicine. More so, the plant, if developed can be of immense use to both pharmaceutical and cosmetic industries, since it contains bioactive compounds.

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