



RESEARCH ARTICLE

PHYTOCHEMICAL SCREENINGS OF THE ETHANOL ON *BORRERIA ARTICULARIS*, *ICHNOCARPUS FRUTESCENS* AND *ZINGIBER OFFICINALE* LEAVES

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ABSTRACT

The Phytochemical activity of the ethanol extract of leafy part of the plants of *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale* was studied to fix the parameters for pharmacognostical standards. These created an interest to test the possible phytochemical activity of the plant. In the screening process of selected plants indicate the presence of Protein, Total Carbohydrates, Total Free Amino acids, Proline, Phenols, β -carotene, Ascorbic acid, Thiamine, Calcium, Sodium and Potassium. This phytochemical study was performed by using standard procedure. The ethanolic extract of the leafy part of selected plants showed anti-gallstone activity in Albino wistar rats.

INTRODUCTION

Naturally occurring substances are of plants, animals and mineral origin. They are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development. Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc., are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques (Hafiza, 2002). The phytoconstituents such as phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants. Saponins have been reported to exhibit haemolytic and foaming activity, antifungal, anti-inflammatory (Takagi *et al.*, 1985), fungistatic (Zehavi *et al.*, 1986) and molluscidal. Plants are now occupying important position in allopathic medicine, herbal medicine, homeopathy and aromatherapy.

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Medicinal plants are the sources of many important drugs of the modern world.

Borreria articularis

It has been found all over Bangladesh in fields and fallow lands as well as pastures. The plant contains sitosterol and ursolic acid; and d-mannitol. Seeds contain isorhamnetin (Abdul Ghani, 1998). The chloroform extract of the aerial parts and roots of *Borreria articularis* yielded a new triterpene, 3- α acetoxo-oleana-12-en-29-oic acid along with β -amyryn. The structures were established by means of spectral as well as chemical studies (Mukharjee, 2004). *Borreria articularis* contains two compounds, identified as ursolic acid and stigmasterol. *Borreria articularis* has been claimed to be useful in treating fever, bladder stones, sores, wounds, headache and constipation.

Ichnocarpus frutescens

Ichnocarpus frutescens (Family-Apocynaceae) is an evergreen plant, and this plant is used in traditional Indian medicine for centuries to treat several illnesses. This plant is also known as Dudhi; 'Shyamalata' in Bengali, 'Black creeper' in English and 'Ananta', 'Sariva' in Sanskrit. This plant is grown wild in the hilly areas of Tripura. *Ichnocarpus frutescens* leaf, stem and root were investigated for its physicochemical and

phytochemical screening (Mishra *et al.*, 2009). Various parts of this plant are used as a cure for fever, dyspepsia, skin troubles and headache. Laboratory studies have demonstrated that extracts of the plant inhibit tumors, protect liver cells from acetaminophen induced damage and in ameliorating hyperlipidemia in diabetic rats. It also has analgesic and anti-inflammatory properties, reduces fever. The plant flowers fasting blood glucose and improves glucose tolerance in diabetes (Singh *et al.*, 2012). Studies on chemical constituents of the plant revealed the presence of phenylpropanoids, phenolic acids, coumarines, flavonoids, sterols and pentacyclic triterpenoids (Khan *et al.*, 1995; Lakshmi *et al.*, 1985). Pharmacological investigations have demonstrated that *I. frutescens* possess hepatoprotective and antioxidant activity (Dash *et al.*, 2007).

Zingiber officinale

Ginger, the rhizomes of the plant *Zingiber officinale* Roscoe (Family Zingiberaceae), is arguably one of the most widely used culinary agent and spice in the world (Baliga *et al.*, 2011; Baliga *et al.*, 2012). In addition to its culinary use, ginger also possess medicinal properties, and has been used since antiquity to treat ailments like cold, headaches, nausea, stomach upset, diarrhea, digestive, gastrointestinal disturbances, rheumatic complaints, nausea, asthma, parasitic infections, arthritis and muscular discomfort in the various alternative and folk systems of medicine in the world (Baliga *et al.*, 2011; Baliga *et al.*, 2012; Chrubasik *et al.*, 2005; Ali *et al.*, 2008; Palatty *et al.*, 2013; Haniadka *et al.*, 2012; Haniadka *et al.*, 2013). Scientific studies carried out in accordance to the principles of modern system of medicine have convincingly shown that ginger possesses numerous health benefits like antimicrobial, antiviral, gastroprotective, antidiabetic, anti-hypertensive, cardioprotective, anticancer, chemopreventive and immunomodulatory effects (Baliga *et al.*, 2011; Ali *et al.*, 2008). In the present study an attempt has been made to evaluate the phytochemical activity of *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale* plants extract.

MATERIALS AND METHODS

Collection of plant materials

Based on the literature, the plants were collected and extracts were prepared using ethanol as a solvents extract for *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale*.

Phytochemical activity

Borreria articularis, *Ichnocarpus frutescens* and *Zingiber officinale* seeds were procured from Tamil Nadu Agricultural University, Coimbatore. The seeds were cold treated (10°C) for 3 days to break dormancy and synchronize germination. Seeds were germinated in roll towels and germinating seedlings of similar size were sown in the control and experimental pots and watered. After four weeks the plants were harvested and the leaves of *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale* were collected and air dried. The plant materials were extracted using ethanol as a solvent in soxhlet extractor. Various plant parameters were

analyzed as follows. Protein estimation by Lowry's method (Lowry *et al.*, 1951). Determination of Total Carbohydrates by Anthrone method (Hedge and Hofreiter, 1962). Estimation of Total Free Amino acids, Proline, Phenols (Mc Donald *et al.*, 2001). β -carotein, Ascorbic acid, Thiamine, calcium, Sodium and Potassium, Estimation of free fatty acids as per the method of Sadasivam and Manikam (1996) were carried out.

Statistical analysis

All the data were analyzed and expressed as mean of six individual observations. Standard Error and Students't' test, were calculated as per the method of Pillai and Sinha (1968).

RESULTS AND DISCUSSION

Recently, interest has been raised in many countries on the commercial extraction of medicine from plants that contribute to cures for major diseases such as cancer and AIDS. The WHO estimates that a minimum of 20,000 plant taxa has recorded medicinal uses. It is estimated that up to 70,000 plant species are used in folk medicine and a majority of these species are found in the Asia-Pacific region. However, the use of medicinal plants is faced with many constraints. Some of these constraints include: plants with medicinal values not fully identified, invented and characterized, information and knowledge not being adequately documented and disseminated. Many issues are not addressed and resolved (i.e. equity and sustainability) and the alarming commercial over-exploitation and consequent genetic erosion of medicinal plants. Studies have pointed out that many drugs that are used in market have come from folk-use and use of plants by indigenous cultures (Anon, 1993).



Plate 1. *Borreria articularis*



Plate 2. *Ichnocarpus frutescens*



Plate 3. *Zingiber officinale*

Studies to date have demonstrated that phytochemicals can have complementary and overlapping mechanisms of action including scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, antibacterial and antiviral effects (Waladkhani and Clemens, 1998). In the present research work three tropical medicinal plant species *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale* due to their high antioxidant properties they were chosen and analysis for the phytochemicals are discussed below. Table 1-6 represent the phytochemical analysis in the leafy parts of the plant *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale* during its growth from 10 days to 30 days from the days of plantation of the sapling in the controlled condition. The results indicate that all the factors were high with the increase in the period showing the healthy growth of the plant. Table 1, 3 and 5 represent the phytochemical factors such as Carbohydrate, Protein, fat, chlorophyll and total amino acid g. Table- 2, 4 and 6 represent the phytochemical analysis on Vitamins and mineral factors such as total phenolis, β -carotene, ascorbic acid, thiamine, flavonoids, potassium, sodium, iron and calcium were analyzed.

Table 1. Phytochemical analysis on leafy parts of the *Borreria articularis*

Factors	Leafy parts		
	10 Days	20Days	30 Days
Carbohydrate g/100g	4.8 \pm 1.04	6.8 \pm 2.05	8.5 \pm 1.04
Protein g/100g	4.5 \pm 1.04	6.5 \pm 2.05	8.2 \pm 1.04
Fat g/100g	0.56 \pm 1.13	0.74 \pm 2.05	0.86 \pm 1.04
Chlorophyll μ g/100g	7.25 \pm 1.121	16.54 \pm 1.21	25.0 \pm 1.13
Total amino acid g/100g	2.74 \pm 1.13	3.26 \pm 2.05	4.25 \pm 1.13

Values mean \pm SD of 6 individual observations. Values are significant at $P \leq 0.001$

Table 2. Vitamins and Minerals Factors leafy parts of the *Borreria articularis*

Factors	Leafy parts		
	10 Days	20Days	30 Days
Total Phenolics mg/g	454 \pm 2.03	654 \pm 2.03	858 \pm 2.03
β - Carotene μ g/100g	26.54 \pm 2.03	37.25 \pm 2.03	48.54 \pm 2.03
Ascorbic acid μ g/100g (Vitamins C)	175.24 \pm 2.03	329.42 \pm 2.03	485.28 \pm 2.03
Thiamine μ g/100g	0.12 \pm 1.14	0.19 \pm 1.14	0.26 \pm 1.14
Flavonoids mg/g	16.56 \pm 1.14	25.46 \pm 1.14	34.24 \pm 1.14
K mg/100g Potassium	186.28 \pm 1.1	264.25 \pm 1.1	342.54 \pm 1.1
Na mg/100g Sodium	24.62 \pm 1.2	36.54 \pm 1.2	48.26 \pm 1.2
Fe mg/100g Iron	32.54 \pm 1.23	45.36 \pm 1.23	58.24 \pm 1.23
Ca mg/100g Calcium	147.26 \pm 1.23	291.42 \pm 1.23	235.28 \pm 1.23

Values mean \pm SD of 6 individual observations. Values are significant at $P \leq 0.001$

Table-1 indicated that among the tested plants leaf extract *Borreria articularis* has been found to be effective during its 10 to 30 days. On its 30th days 8.5 g/100g Carbohydrate, 8.2 g/100g Protein, 0.86 g/100g Fat, 25.0 μ g/100g chlorophyll and 4.25 g/100g total amino acid is present. The total amino acid content was high 4.25 g/100g showing the presence of essential amino acid production in the plant. Cysteine is one of the key amino acids present in all living things. Cysteine plays a key role in stabilizing extracellular proteins. Cysteine strengthens the protective lining of the stomach and intestines which may help prevent damage caused by aspirin and similar drugs. In addition, cysteine may play an important role in the communication between immune system cells. Cysteine is one of the few amino acids that contains sulfur. This allows cysteine to bond in a special way and maintain the structure of proteins in the body. Cysteine is a component of the antioxidant. The body also uses cysteine to produce taurine another amino acid. Cysteine may possibly help to reduce the effects of aging on the skin, assist in healing after surgery or burns and help protect the skin from radiation injury (Salim, 1993). Chlorophyll absorbs most in the red and blue portions of the electromagnetic spectrum thus its intense green color. Chlorophyll has anti-inflammatory, antioxidant and wound-healing properties. Chlorophyll and chlorophyllin are able to form tight molecular complexes with certain chemicals known or suspected to cause cancer (Kamat *et al.*, 2000). The amount of chlorophyll in the *Borreria articularis* found to be remarkably high (25.0 μ g/100g).

Table 3. Phytochemical analysis on leafy parts of the plant *Ichnocarpus frutescens*

Factors	Leafy parts		
	10 Days	20Days	30 Days
Carbohydrate g/100g	4.5 \pm 1.04	6.4 \pm 2.05	8.2 \pm 1.04
Protein g/100g	4.2 \pm 1.04	6.3 \pm 2.05	7.95 \pm 1.04
Fat g/100g	0.53 \pm 1.13	0.72 \pm 2.05	0.83 \pm 1.04
Chlorophyll μ g/100g	6.54 \pm 1.121	15.64 \pm 1.21	24.75 \pm 1.13
Total amino acid g/100g	2.54 \pm 1.13	3.36 \pm 2.05	4.18 \pm 1.13

Values mean \pm SD of 6 individual observations. Values are significant at $P \leq 0.001$.

Table 4. Vitamins and Minerals Factors leafy parts of the plant *Ichnocarpus frutescens*

Factors	Leafy parts		
	10 Days	20Days	30 Days
Total Phenolics mg/g	446.24 \pm 2.01	645.36 \pm 2.01	846.18 \pm 2.01
β - Carotene μ g/100g	24.34 \pm 2.01	35.42 \pm 2.01	45.28 \pm 2.01
Ascorbic acid μ g/100g (Vitamins C)	168.26 \pm 2.01	324.42 \pm 2.01	480.74 \pm 2.01
Thiamine μ g/100g	0.14 \pm 1.11	0.18 \pm 1.11	0.24 \pm 1.11
Flavonoids mg/g	14.58 \pm 1.11	23.56 \pm 1.11	32.54 \pm 1.11
K mg/100g Potassium	175.36 \pm 1.4	250.42 \pm 1.4	325.34 \pm 1.4
Na mg/100g Sodium	22.42 \pm 1.6	34.28 \pm 1.6	46.34 \pm 1.6
Fe mg/100g Iron	29.54 \pm 1.21	42.26 \pm 1.21	55.45 \pm 1.21
Ca mg/100g Calcium	142.54 \pm 1.21	283.45 \pm 1.21	425.36 \pm 1.21

Values mean \pm SD of 6 individual observations. Values are significant at $P \leq 0.001$

Table-2 represent the phytochemical analysis on vitamins and minerals factors in the leaf extract of *Borreria articularis* during its growth from 10 days to 30 days from the day of plantation. It showed that 858, 48.54, 485.28, 0.26, 34.24, 342.54, 48.26, 58.24 and 235.28 in 30th day. Where analyzed the leafy extract growth for 10th, 20th and 30th days the leafy

were found to be increased in the growth period. The quantity of ascorbic acid (Vitamin – C) was very high (485.28 µg) showing its high therapeutic leafy extract of *Borreria articularis* against inflammation, oxidative stress and so on. As a powerful antioxidant, vitamin C may help to fight cancer by protecting healthy cells from free-radical damage and inhibiting the proliferation of cancerous cells. The body does not produce vitamin C. Foods containing the highest sources of vitamin C include green peppers, citrus fruits and juices, strawberries, tomatoes, broccoli, turnip greens and other leafy greens, sweet and white potatoes and cantaloupe (Boothby and Doering, 2005). Consuming foods rich in β-carotene appears to protect the body from damaging molecules called free radicals. Beta-carotene's antioxidant actions make it valuable in protecting against and in some cases even reversing, precancerous conditions affecting the breast, mucous membranes, throat, mouth, stomach, prostate, colon, cervix, and bladder (Krinsky, 1992).

Table 5. Phytochemical analysis on leafy parts of the plant *Zingiber officinale*

Factors	Leafy parts		
	10 Days	20Days	30 Days
Carbohydrate g/100g	4.6 ±1.21	6.5 ±1.21	8.4 ±1.21
Protein g/100g	4.3 ±1.21	6.2 ±1.21	8.0 ±1.21
Fat g/100g	0.54 ±1.02	0.69 ±1.02	0.85 ±1.02
Chlorophyll µg/100g	6.25±2.06	15.47±2.06	24.70±2.06
Total amino acid g/100g	2.65±2.04	3.46 ±2.04	4.20 ±2.04

Values mean ± SD of 6 individual observations. Values are significant at $P \leq 0.001$

Table 6. Vitamins and Minerals Factors Leafy parts of the plant *Zingiber officinale*

Factors	Leafy parts		
	10 Days	20Days	30 Days
Total Phenolics mg/g	425.46±2.05	624.54±2.05	828.65±2.05
β- Carotene µg/100g	25.46±2.05	36.28±2.05	46.56±2.05
Ascorbic acid µg /100g (Vitamins C)	173.54±3.01	325.42±3.01	478.26±3.01
Thiamine µg/100g	0.12±1.02	0.17±1.02	0.25±1.02
Flavonoids mg/g	15.24±1.12	23.41±1.02	31.58±1.02
K mg/100g Potassium	182.54 ±1.06	255.26±1.04	328.46±1.06
Na mg/100g Sodium	25.54±1.04	36.46±1.06	47.28±1.05
Fe mg/100g Iron	31.26±1.16	43.68±1.16	56.64±1.31
Ca mg/100g Calcium	135.28±1.16	276.86±1.16	418.54±1.31

Values mean ± SD of 6 individual observations. Values are significant at $P \leq 0.001$

The richest sources of beta-carotene are yellow, orange, and green leafy fruits and vegetables. In the leaf extract of *Borreria articularis* beta carotene were found to be high (48.54µg) showing its contribution to high antioxidant effect of the leafy extract of the plant. Flavonoids are natural polyphenolic molecules common to most flowering plants. They include flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Although not considered vitamins and flavonoids have a number of nutritional functions have been described as biological response modifiers. Most of them act as antioxidants and some have anti-inflammatory properties (Kuhnau, 1976). Minerals by themselves are inactive chemical elements like the iron or calcium in a rock. But in the body, mineral nutrients are required to build tissues. Minerals such as potassium, sodium, iron and calcium were analysed in the *Borreria articularis* leaves on it's for 10, 20

and 30 days. The leaves were found to effective on the plant. This may be due to the uptake of these minerals by the plant from the soil through roots. Usually leafy vegetables are considered as the rich source of minerals particularly iron, calcium and zinc. The present study reveals that the minerals such as calcium, potassium and sodium are found in moderate levels when compared to plants which act as the rich sources of minerals like spinach (Whitney and Hamilton, 1984). Table-3 represents the phytochemical analysis on biochemical factors in the leaf extracts of *Ichnocarpus frutescens* showed 4.5 g/100g of carbohydrates, 4.2 g/100g of protein, 0.53 g/100g of fat, 6.54 µg/100g of chlorophyll and 2.54 g/100g of total amino acids is present on 10 days leaf. On 20th day leaf 6.4, 6.3, 0.72, 15.64 and 3.36 g/100g of carbohydrate, protein, fat, chlorophyll and total amino acids is present. On its 30th day it is resulted that 8.2 g/100g of carbohydrate, 7.95 g/100g of protein, 0.83 g/100g of fat, 24.75 µg/100g of chlorophyll and 4.18 g/100g of total amino acids is present.

Table-4 represents the phytochemical analysis on vitamins and minerals factors in the leafy parts of the plant *Ichnocarpus frutescens* during its growth from 10 days to 30 days from the day of plantation. It showed that 446.24 mg/g of total phenolics, 24.34 µg/100g of β-carotene, 168.26 µg/100g of ascorbic acid, 0.14 µg/100g of thiamine, 14.58 mg/g of flavonoids, 175.36 mg/100g of potassium, 22.42 mg/100g of sodium, 29.54 mg/100g of iron and 142.54 mg/100g of calcium on its 10th day. 645.36, 35.42, 324.54, 0.18, 23.56, 250.42, 34.28, 42.26 and 283.45 on its 20th day. 846.18, 45.28, 480.74, 0.24, 32.54, 325.34, 46.34, 55.45 and 425.36 on its 30th day respectively.

Table-5 represents the phytochemical analysis on biochemical factors in the leaf extracts of *Zingiber officinale* showed 4.6 g/100g of carbohydrates, 4.3 g/100g of protein, 0.54 g/100g of fat, 6.25 µg/100g of chlorophyll and 2.65 g/100g of total amino acids is present on its 10 days leaf. On 20th day leaf 6.5, 6.2, 0.69, 15.47 and 3.46 g/100g of carbohydrate, protein, fat, chlorophyll and total amino acids is present. On its 30th day it is resulted that 8.4 g/100g of carbohydrate, 8.0 g/100g of protein, 0.85 g/100g of fat, 24.70 µg/100g of chlorophyll and 4.20 g/100g of total amino acids is present. Table-6 represents the phytochemical analysis on vitamins and minerals factors in the leafy parts of the plant *Zingiber officinale* during its growth from 10 days to 30 days from the day of plantation. It showed that 425.46 mg/g of total phenolics, 25.46 µg/100g of β-carotene, 173.54 µg/100g of ascorbic acid, 0.12 µg/100g of thiamine, 15.24 mg/g of flavonoids, 182.54 mg/100g of potassium, 25.54 mg/100g of sodium, 31.26 mg/100g of iron and 135.28 mg/100g of calcium on its 10th day. 624.54, 36.24, 325.42, 0.17, 23.41, 255.26, 36.46, 43.68 and 276.86 on its 20th day. 828.65, 46.56, 478.26, 0.25, 31.58, 328.46, 47.28, 56.64, and 418.54 on its 30th day respectively. From the results it is evident that the phytochemical factors of *Borreria articularis*, *Ichnocarpus frutescens* and *Zingiber officinale* increased with the period of growth, showing the healthy growth of the plants. All the phytochemical factors such as total carbohydrate, protein, fat, total amino acids, vitamins and minerals factors are indicative of the healthy state of leafy extract in the plant.

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