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PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY IN THE BARK EXTRACTS OF FAGRAEA CEILANICA THUNB. (LOGANIACEAE), AN EPIPHYTIC SPECIES OF WESTERN GHATS

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ABSTRACT

The genus *Fagraea* belongs to the family loganiaceae, and comprises a diverse group of trees, shrubs, scrambling lianas, and epiphytic stragglers. The preliminary phytochemical screening from the bark extracts of *Fagraea ceilanica* Thunb., revealed the presence of alkaloids, saponins, reducing sugars, tannins, flavonoids, steroids and glycosides in hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extracts. The total phenolic content evaluated in the solvent extracts of bark showed high phenolic content in methanolic and ethanolic extracts than the other extracts. The evaluation of antioxidant activity by various methods viz., DPPH radical scavenging assay, total antioxidant power by FRAP method and determination of reducing power was carried out. The hexane extract revealed high radical scavenging activity by DPPH evaluation. The methanolic extract showed high total antioxidant power by FRAP assay and also in the reduction power followed by other extracts. Results presented here reveal the presence of important phytochemicals present and also showes *F. ceilanica* to be an important medicinal plant, as the bark showed good antioxidant activity.

KEY WORDS: *Fagraea ceilanica*, Phytochemicals, Antioxidant activity, Phenolic content, Reducing power assay, DPPH radical scavenging activity.

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INTRODUCTION

India is richly endowed with medicinal plants of potential value used by all sections of the society either directly as folk remedies or indirectly as pharmaceutical preparations in the modern medicine. The genus *Fagraea* comprises a diverse group of trees, shrubs, scrambling lianas, and epiphytic stragglers that are found growing at sea level to altitudes of 3000 m, in forest gaps, forest edges, along stream beds in wet tropical forests, and less commonly in mesic forests, savannas and mangrove swamps. The genus is comprised of approximately 70 species, indigenous to Ceylon, India, Southeastern Asia to Southern China, Hainan and Taiwan, northern Australia, New Caledonia and throughout the Pacific Islands, and later introduced into the Hawaiian Islands. [(1)]

Fagraea genera are renowned for hard wood, fragrant and showy flowers. It is an assemblage of approximately 70 species and is distributed from Southeast Asia and Malaysia, to the archipelagos of the Pacific ocean. [(2)] The genus is a fast growing, pioneer and serves as important component in natural ecosystem by providing food and shelter to other forest species. The plant is utilized by Asian, Australian, Malaysian and Polynesian cultures for timber, weapons, crafts, medicine and ornamentals.

Fagraea ceilanica Thunb., is an epiphytic climber which turns into an understorey tree up to 17 m tall when mature. Stipules surround the twigs, leaves are opposite, simple, penni-veined, glabrous and leathery. The flowers measure 60 mm diameter, whitish to orange, and are placed in branched inflorescences. Fruits are 40 mm, rounded, oblong, green-yellowish-whitish berries (Fig. 1). It is distributed in tropical Asia mainly, Sri Lanka, India and South China to New Guinea. The members of loganiaceae are considered to be medicinally potential e.g., *Strychnos, Buddleja, Fagraea* and other genera. Till date, no information is reported on the phytochemical properties and antioxidant activity from the bark of *F. ceilanica* Thunb. Extensive work on the phytochemistry and biological activities has been achieved on the genera such as *Strychnos* and *Buddleja* of the family loganiaceae. The radical scavenging activity of *Fagraea blumei* and *Fagraea racemosa* and ethnobotanical uses of *Fagraea* species are reported. There is no report on the evaluation of phytochemicals and antioxidant activities of *F. ceilanica* Thunb., bark extracts selected for the present investigation.

MATERIALS AND METHODS

The bark samples of *F. ceilanica* Thunb., were collected from the forests of Nelaji, Kodagu district of Western Ghats during November 2012. The specimen of the plant material was identified by the plant taxonomist, Dr. K.K. Sampathkumar, Govt. Science College, Davangere, Karnataka. The samples of *F. zeylanica* bark was cut into small pieces and dried under shade for a day and then in oven at 40°C to remove moisture present in the sample. It was powdered using a blender and the powdered sample was weighed and preserved in ziplock cover.

PHYTOCHEMICAL SCREENING:

Qualitative phytochemical analysis of the crude powder and different solvent extracts were determined using standard procedures .[(3)] The extracts were tested qualitatively for the presence of phytochemical constituents such as tannins, saponins, terpenoids, flavonoids, alkaloids, steroids, cardiac glycosides, phlobatannins, anthraquinones and reducing sugars.

EVALUATION OF ANTIOXIDANT ACTIVITY:

The antioxidant activity from the bark solvent extracts of *F. ceilanica* Thunb., was determined by four assays, estimation of total phenolic content, reducing power assay radical scavenging activity (DPPH) and ferric reducing antioxidant power assay (FRAP) using standard procedures.

Source of chemicals:

1, 1-diphenyl-1-picrylhydrazyl (DPPH) was purchased from M/s Sigma–Aldrich Chemicals Pvt., Ltd., Bangalore, India, Folin–Ciocalteau reagent, ascorbic acid and phytochemical reagents used were of analytical grade.

Estimation of total phenolic content:

The total phenolic content of the plant extracts was estimated by Folin-Ciocalteau (FC) method as per the

procedure of Volluri *et al*. [4] with some modifications. The plant extracts and the standard gallic acid was prepared at one mg/mL concentration. Different concentrations of the plant extracts (20-100 µg/mL) and the standard (5-25 µg/mL) were taken in test tubes and 1.0 mL of FC reagent was added, after 3-5 min 2.0 mL of sodium carbonate solution (20%, w/v) was added and the mixture was allowed to stand for 30-45 min under dark. After the prescribed period of incubation the absorbance was read at 765 nm in a UV-Vis spectrophotometer. The concentration of total phenolics was expressed in terms of µg/gram gallic acid equivalents (GAE).

DPPH radical scavenging activity:

Radical scavenging activity by DPPH method was evaluated according to the procedure of Pannangpetch *et al*. [5]. Different aliquots of standard and plant extracts previously described (1 mg/mL) were taken and the volume was made up to 250 µL using methanol. To this, 1.0 mL of DPPH (4 mg/100 mL) was added and the tubes were kept under dark for 10 min. The incubated mixture was read at 517 nm using UV-Vis spectrophotometer. The radical scavenging percentage was calculated based on the extent of reduction in the color as:

Percentage radical scavenging activity = $A_c - A_s / A_c \times 100$

Where A_c = absorbance of the control

A_s = absorbance of the sample.

Total antioxidant power by ferric reducing antioxidant power assay (frap):

Total antioxidant activity by FRAP assay was determined by using the modified method of Benzie and Strain. [6] The stock solutions of acetate buffer (300 mM), TPTZ (10 mM) in HCl (40 mM) and $FeCl_3.6H_2O$ (20 mM) were prepared. From this stock, a fresh working solution was prepared by adding 25 mL of acetate buffer, 2.5 mL of TPTZ and 2.5 mL of $FeCl_3.6H_2O$. The temperature of the solution was raised to 37°C before use. Different concentration of plant extracts (20-100 µg/mL) as well as the standard Ascorbic acid (5-25 µg/mL) were taken in test tubes and the volume was made up to 3000 µL with freshly prepared FRAP solution and incubated for 30 min under dark condition. The absorbance was measured at 593 nm using a UV-Vis spectrophotometer. Based on the concentration of the plant extract the color changes from light brown to various shades of blue.

Determination of reducing power:

The reducing power of the bark extracts were evaluated according to the procedure of Yen and Chen [7] with some modifications. The concentration of plant extracts and the standard ascorbic acid were prepared as mentioned earlier. Different concentrations of standard (5-25 µg/mL) and plant extracts (20-100 µg/mL) were taken in test tubes and the volume was made up to 500 µL by using phosphate buffer (2.0 M) and potassium ferricyanide (1%), then the tubes were kept at 50°C for 20 min of incubation. After this period, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%) were added. The absorbance was read at 700 nm against a blank sample based on the reduction of yellow color to the various shades of green and blue depending on the concentration of the plant extract.

STATISTICAL ANALYSIS:

All the experiments were done in triplicates. The reported value for each test was calculated as the mean of three measurements by using SPSS ver 16.0.

RESULTS

Preliminary phytochemical analysis in bark extracts of *F. ceilanica* Thunb.

Phytochemical analysis of different solvent extracts of bark *viz*., hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous were conducted. Preliminary phytochemical screening showed the presence of tannins, saponins, flavonoids, terpenoids, steroids, cardiac glycosides, reducing sugars and alkaloids in all the extracts (Fig. 2).

Tannins were detected in the polar solvent extracts such as ethanol, methanol and aqueous extracts. Saponins were present in both the polar as well as non-polar solvents *viz*., hexane, ethanol, methanol and aqueous extracts. Terpenoids were present in all the solvent extracts. Flavonoids were found in hexane, chloroform, ethanol and methanol extracts.

Steroids and cardiac glycosides were detected in the non-polar hexane extract only. Reducing sugars and alkaloids were present in polar solvents such as ethanol and methanol extracts. The phytochemical screening of different solvent extracts of bark is tabulated in table 1.

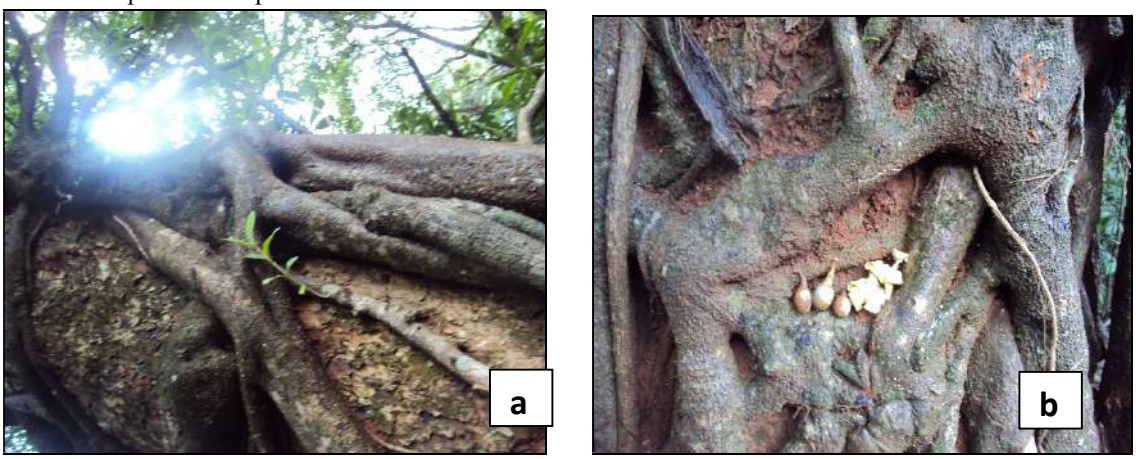

Fig. 1: Habit of *F. ceilanica* Thunb., from Western Ghats
a. Epiphytic habit b. Close-up of the woody stem

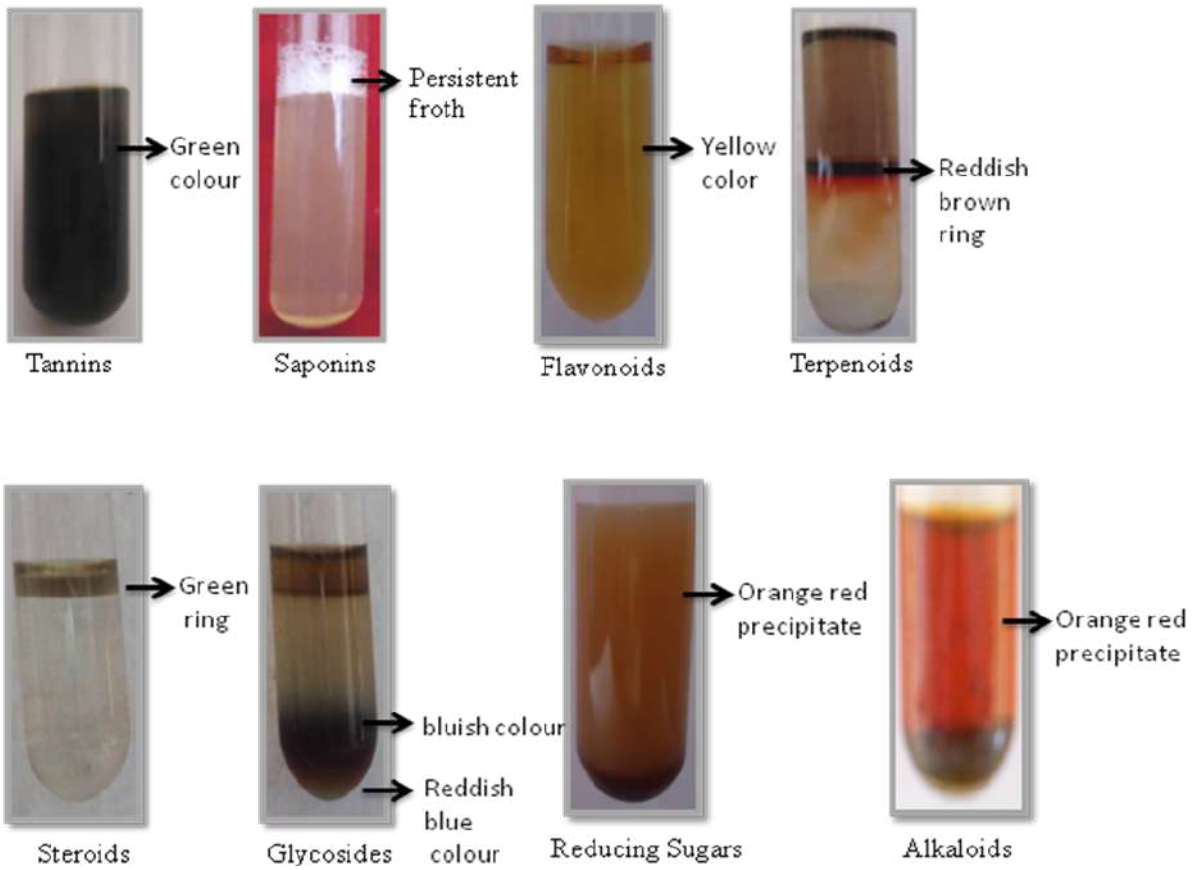

Fig. 2: Phytochemicals tested positive for the stem bark solvent extracts of *F . ceilanica*

Table 1: Phytochemical tests for the stem bark extracts of *F. ceilanica* Thunb.

Phytochemical Tests	Extracts					
	H	C	EA	E	M	Aq
Tannins	-	-	-	+	+	+
Saponins	+	-	-	+	+	+
Flavonoids	+	+	-	+	+	-
Terpenoids	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-
Steroids	+	-	-	-	-	-
Cardiac Glycosides	+	-	-	-	-	-
Reducing sugars	-	-	-	+	+	-
Alkaloids	-	-	-	+	+	-
Anthraquinones	-	-	-	-	-	-

H - Hexane; C - Chloroform; EA - Ethyl acetate; E - Ethanol; M - Methanol and Aq - Aqueous. '+'= presence of phytochemical; '-' = absence of phytochemical in the extracts.

EVALUATION OF ANTIOXIDANT ACTIVITY

In the present investigation the evaluation of antioxidant activity was carried out by the estimation of total phenolic content, DPPH radical scavenging activity, total antioxidant power by FRAP assay and by the determination of reducing power.

Estimation of the total phenolic content:

The total phenolic content of the bark extract revealed the presence of high phenolic content in the methanolic extract (312.5 µg/mL), followed by ethanol (312.5 µg/mL), chloroform (90 µg/mL), ethyl acetate (75 µg/mL), aqueous (60 µg/mL) and hexane extracts (50 µg/mL. The concentration of total phenolics in various solvent extracts is depicted in Fig. 3.

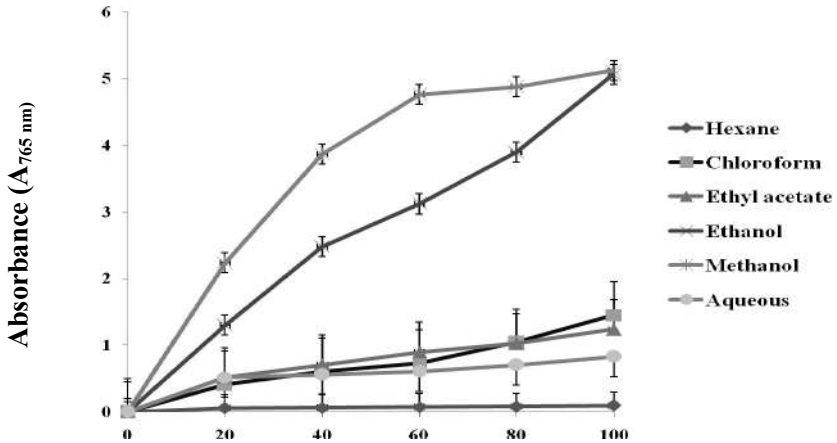

Fig. 3: Total phenolic content in the stem bark solvent extracts of *F. ceilanica* Thunb.

DPPH radical scavenging activity:

The radical scavenging activity of the bark extracts was evaluated using DPPH which showed activity in all the extracts. The activity was high in hexane extract (97%) followed by methanol (96%), ethanol (93%), ethyl acetate (92%), chloroform (91%) and aqueous (81%) extracts respectively. A graph is plotted against concentration versus absorbance based on the absorbance at 517 nm (Fig. 4).

Total antioxidant power by FRAP assay:

FRAP activity was estimated in the different solvent extracts of bark which revealed high activity in the methanol extract. The activity increased with an increase in the concentration of the extracts. High activity of methanol extract was followed by the aqueous, ethanol, ethyl acetate, chloroform and hexane extracts (Fig. 5). The absorbance is measured by color change from light brown to varying shades of blue depending upon the concentration of the extracts.

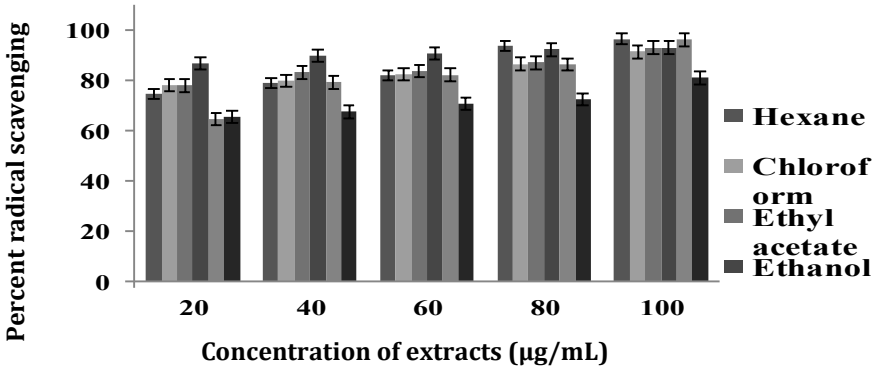

Fig. 4: DPPH radical scavenging potential of stem bark solvent extracts

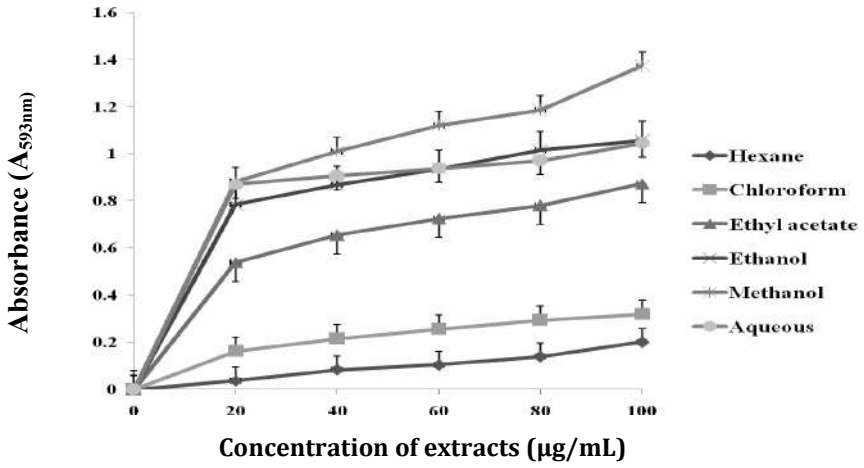

Fig. 5: Total antioxidant power of stem bark extracts by FRAP assay

Determination of reducing power:

The reducing power of the bark extracts revealed the presence of antioxidants in the plant extracts. The reducing power was determined by the reduction in Fe^{3+} to Fe^{2+} ion. The reducing value of the extract is significantly high in methanolic extract than the standard ascorbic acid followed by the aqueous, ethanol, ethyl acetate, chloroform and hexane extracts respectively. The absorbance is measured at 700 nm based on the reduction of yellow color to various shades of green and blue depending on the concentration of the plant extract (Fig. 6).

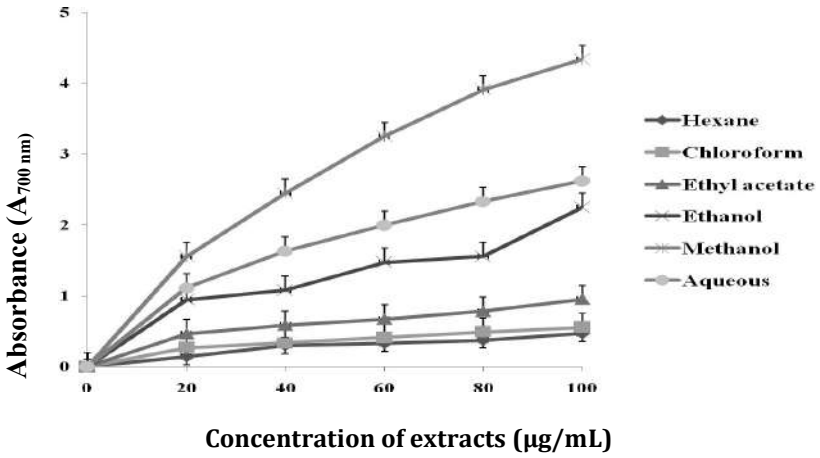

Fig. 6: Reducing power in the stem bark solvent extracts

DISCUSSION

The plants of loganiaceae family are considered medicinally important. The plant serves as an important source of medicine, timber, weapons, crafts and ornamentals. [2]

In the present investigation, the evaluation of phytochemicals and antioxidant activity of *F. ceilanica* Thunb., an epiphytic species was carried out. Phytochemicals are the plant secondary metabolites which are not directly involved in their growth and development of a plant and also serves as a defensive agent against any pathogen. Phytochemical analysis of stem bark revealed the presence of tannins, alkaloids, saponins, flavonoids, reducing sugars, terpenoids, steroids and cardiac glycosides in various solvent extracts.

Tannins are the astringent polyphenolic compounds, used against diarrhoea and as an antidote in poisoning by heavy metals. Their use declined after the discovery of hepatotoxic effects of absorbed tannic acids. Recent studies have reported that tannins have anti-cancer and anti-HIV activities.[8] Alkaloids are the organic compounds containing nitrogen in a negative oxidation state. They are pharmaceutically significant, e.g., morphine as a narcotic analgesic, codeine in the treatment of coughs, colchicine in the treatment of gout, quinine as an anti-malarial, quinidine as an anti-arthritic and atropine as an antisplasmodial agent. [9] Saponins constitute a vast group of glycosides, which occur in many plants. They are characterized by their surfactant properties; they dissolve in water and when shaken, form a foamy solution. They exhibit various pharmacological activities *viz*., anti-inflammatory, expectorant, analgesic and cytotoxic. [10] Flavonoids are a group of polyphenolic compounds that occur naturally in foods of plant origin. They exhibit anti-allergic, anti-inflammatory, antioxidant, anti-mutagenic and anti-carcinogenic activities. [11]

Glycosides are naturally occurring carbohydrates, more in plants, present in flower and fruit pigments known as anthocyanins. They are used as condiments, dyes and heart-stimulating agents as cardiac glycosides. [2] Terpenoids are the largest group of natural compounds with wide range of

biological activities against cancer, malaria, inflammation and a variety of infectious diseases. [12]

Sugars that contain aldehyde groups that are oxidised to carboxylic acids are classified as reducing sugars. They provide energy and serve as the basic building blocks for carbohydrate storage and are excellent scavengers for metal ions. Glucose, fructose and the sugar alcohols (sorbitol and mannitol), have the ability to block the reactive sites of ions such as copper, cobalt and iron to a lesser extent. This characteristic of reducing sugars aids in food preservation by retarding catalytic oxidation reactions. [13] A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other, e.g., the dietary fat cholesterol, the sex hormones estradiol and testosterone and the anti-inflammatory drug dexamethasone. They are used clinically to promote growth and repair of body tissues in diseases. [14]

Phytochemicals have been detected from the stem bark of *Strychnos* and *Buddleja* spp. by many workers. 11-methoxyspennostrychnine, an alkaloid was isolated from *S. rubiginosa* stem bark aqueous extract by Battista *et al*. [15]; Longicaudatine and flavopeirerine from *S. longicaudata* and longicaudatine-1 from *S. ngouniensis* were the most abundant alkaloids isolated by Massiot *et al.* [16]; *S. soubrensis* yielded strychnobrasiline, strychnofendlerine, and isosplendine as investigated by Ohiri *et al*. [17]; the methanolic extract of *B. davidii* yielded six novel lignan compounds named as buddlenols A-F, Penelle *et al*. [18] isolated 5',6'-dehydroguiachrysine from *S. guianensis.* Tchinda *et al*. [19] isolated monoindole alkaloids *viz*., 15 hydroxyvomicine and 12-methoxyicajine from *S. icaja* and *S. nux-vomica* yielded an alkaloid strychnochrysine. [20]

Singha *et al.* [21] isolated alkaloids, flavonoids, lignins, glycosides, phenols, saponins, sterols and tannins from stem bark and root extracts of *S. potatorum*; Mallikharjuna *et al*. [22] isolated an alkaloid diaboline and four triterpenes *viz*., isomotiol, sitosterol, stigmasterol and compesterol from seeds, stem bark, root and leaf extracts of *S. potatorum*; Mallikharjuna and Seetharam [23] isolated strychnine, diaboline and triterpenes from the leaves and seeds of *S. potatorum* and Mallikharjuna *et al*. [22] reported the presence of alkaloids, flavonoids, glycosides, phenols, saponins and sterols in seed and leaf extracts of *S. wallichiana*.

Phenolics are ubiquitous secondary metabolites in plants possessing a wide spectrum of biochemical activities such as antioxidant, antimutagenic and anticarcinogenic activities. In the present study, total phenolics by FC method and antioixidant activity by radical scavenging activity (DPPH method), total antioxidant activity (FRAP assay) and the determination of reducing power were evaluated.

In the present investigation, the methanolic (312.5 µg/mL) and ethanolic (312.5 µg/mL) bark extracts showed high phenolic content when compared to other plant extracts like hexane, ethyl acetate, chloroform and aqueous. Investigation by Oyedemi *et al*. [24] revealed the presence of high phenolic content i.e., 48 mg/g in the aqueous bark extract of *S. henningsii.* Phenolics are ubiquitous secondary metabolites in plants, possessing a wide spectrum of biochemical activities. Bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and aging [25].

The radical scavenging activity of any antioxidant is commonly associated with the use of DPPH because it is a reliable method to search the *in vitro* general antioxidant of pure compounds as well as the plant extracts. In the present investigation, all the extracts showed high antioxidant activity i.e., hexane (97%); methanol (96%); ethanol (93%); ethyl acetate (92%); chloroform (91%) and aqueous (84%).

FRAP assay is a simple, automated test measuring the ferric reducing ability of plasma. It is represented as a novel method for assessing the antioxidant power. Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form, by the change of color the concentration of the antioxidant mixture is determined [4].

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that, they are electron donors and can reduce the oxidized intermediates of lipid

peroxidation processes, so they can act as primary and secondary antioxidants. [26]

Total antioxidant FRAP assay was estimated in which methanolic extract showed high antioxidant activity (1.3 µg/mL) than aqueous, ethanol, chloroform, ethyl acetate and hexane extracts when compared to the standard ascorbic acid. Similar work has been done by Adedapo *et al*. [27] on the stem methanolic extract of *B. salinga* and the result revealed that the stem methanolic extract showed low antioxidant activity (1546.98±63.67) compared to the standard vitamin C.

Determination of reducing power in the present investigation revealed the presence of high reducing power in the methanolic extract (4.3 µg/mL) followed by the aqueous, hexane, chloroform, ethyl acetate and ethanol extracts. Similar investigation on the aqueous bark extract of *S. henningsii* was reported by Adedapo *et al*. [27] The antioxidant potentials of the plant extract was estimated from their ability to reduce Fe^{3+} to Fe^{2+}. This was observed from yellow color of the test solution that changed to various shades of green and blue depending on the concentration of the plant extract. The reducing value of the extract was significantly lower than that of BHT, Vitamin C and Vitamin E used as reference compounds.

Since there are no reports on the phytochemistry and biological activity of *F. ceilanica*, the related members *Strychnos* and *Buddleja* species of Loganiaceae have been studied extensively. Therefore, we have analyzed the presence of phytochemicals and antioxidant activity in the bark solvent extracts.

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