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Geedhu Daniel
Department of Biochemistry, Pazhassi Raja College, Pulpally, Wayanad, Kerala, India

Sarun Mani
Senior Technical Specialist, Sedeer Medical Services LLC, Doha, Qatar

Correspondence
Geedhu Daniel
Department of Biochemistry, Pazhassi Raja College, Pulpally, Wayanad, Kerala, India

Quantitative Estimation of Plant Metabolites in Ethanolic Seed Extracts of *Theobroma cacao* (L.) and *Coffee arabica* (L.)

Geedhu Daniel, Sarun Mani

Abstract
In the present study, laboratory evaluations were made to quantitatively assess primary metabolites and secondary metabolites in ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica*. Primary metabolites like total soluble carbohydrates, proteins, total amino acids and secondary metabolites such as flavonoids, total phenols, alkaloids and tannins were estimated using standard procedures. Quantitative analysis is very essential for identifying the compounds present in the medicinal plants. The results obtained from the present study provides evidence that ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica* contains various primary and secondary metabolites and this justifies the use of plant species as traditional medicine for treatment of various diseases. The finding of this study suggests that these seed extracts could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing various diseases. The results are very much encouraging but scientific validation is necessary before being put into practice.

Keywords: Phytochemicals, Primary metabolites, Secondary metabolites, Ethanolic extract, *Theobroma cacao, Coffee arabica.*

1. Introduction
India has a rich, vibrant and diverse cultural history. An important component of this culture and tradition is that of health and healing. India is the largest producer of medicinal herbs and is rightly called the botanical garden of the world. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% of the medicinal needs of the third world countries [1]. Interest in the exploitation of medicinal and aromatic plants as pharmaceuticals, herbal remedies, flavourings, perfumes and cosmetics, and other natural products has greatly increased in the recent years.

Medicinal plants have played an essential role in the development of human culture. Medicinal plants address not only the need for access to medicine as a component of health services but also to the need for increased income for farmers and as a significant contribution to the national economy. Medicinal plants offer alternative remedies with tremendous opportunities. They not only provide access and affordable medicine to poor people; they can also generate income, employment and foreign exchange for developing countries.

Many traditional healing herbs and plant parts have been shown to have medicinal value, especially in the rural areas and that these can be used to prevent, alleviate or cure several human diseases. WHO estimates that more than 80% of the world's population rely either solely or largely on traditional remedies for health care. The medicinal plants have been used by humans from the pre-historical times. Studies have pointed out that many drugs that are used in commerce have come from folk-use and use of plants by indigenous cultures. Medicinal plants are one of the most sensitive commodity areas of research in the world today.

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [2]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [3-5]. Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions.

The phytochemicals are grouped into two main categories [6] namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins,

phenolic compounds etc. [7, 8]. Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticides [9], antibacterial, antifungal [10], anti-constipative [11], spasmolytic, antiplasmodial and antioxidant [12] activities etc. The plants thus find their medicinal value due to respective phytochemical constituents they contains.

Theobroma cacao also called as cacao tree, is a small (4–8 m or 15–26 ft tall) evergreen tree in the family Sterculiaceae (alternatively Malvaceae), native to the deep tropical region of the America. Its seeds are used to make cocoa powder and chocolate. Cocoa, inherently contain high vitamin E which is well-known for its nourishing and moisturizing properties. In addition, cocoa shower has the healing benefit in curing light rheumatism besides refreshing and reenergizing body. Recent studies have shown that dark chocolates are cardio protective. In recent year's cacao and cacao product namely cacao powder, dark chocolate and cocoa chocolate have been shown to suppress the development of atherosclerotic lesion [13] and inhibit the proliferation of human breast cancer cell [14] and exert hypoglycemic properties [15, 16].

Cocoa bean contain 10-15% protein with albumin and globulin being the predominant fraction [17]. Used as medicine to relive symptoms associated with sickness of cardiovascular, gastro intestinal diseases etc. [18-21]. Since seventeenth century cacao and chocolate have been described as potential medicine [22]. *Theobroma cacao* have antioxidants and antiradical properties due to the presence of their polyphenolic constituents specially procyanidins and flavan-3-ol [23]. The cacao phenolics is a bioactive compound especially prominent for their metabolic and cardiovascular effect. Other relevant properties include their ability to modulate the immune response [24, 25] and their anti-inflammatory [26, 27] and anti-carcinogenic properties [28, 29]. Consumption of cacao or dark chocolate can also decrease the burden and efficiency of epigenetic carcinogens [30].

Coffea arabica, the scientific name for the coffee tree. It belongs to family, Rubiaceae. They are shrubs or small trees, native to subtropical Africa and southern Asia. Seeds of several species are the source of the popular beverage coffee. Coffee ranks as one of the world's most valuable and widely traded commodity crops and is an important export of a number of countries. Coffee can have a stimulating effect on humans due to its caffeine content. Coffee is a brewed drink prepared from roasted seeds, called coffee beans. Coffee bean is the herbal supplement. Coffee bean is a powerful cardiac and respiratory stimulant that increases heartbeat and blood flow and acts as a bronchodilator to improve breathing.

The pharmaceutical industry incorporates the caffeine in coffee bean into many commercial painkillers, where its stimulating effects help to rush such substances as aspirin and paracetamol into the system and enhance efficacy. Recent reports indicate that coffee bean helps to relieve migraine headache. In homeopathic and alternative medicine, coffee has long been used to relieve tension headaches and reduce hyperactivity.

Coffee bean is considered as central nervous system stimulant. As a brain stimulant, it helps to increase cerebral activity, and one is said to be more alert with a sharper mind. According to the research, caffeine is a cognitive stimulant that helps to reduce levels of the protein called beta-amyloid in the brain, whose accumulation is responsible for Alzheimer's disease but which also occurs in normal ageing. Recent studies have suggested that coffee consumption in middle age could decrease the risk of dementia and Alzheimer's disease by sixty-five percent. Coffee bean has been used to control vomiting and ease nausea. The caffeine in coffee Bean may be of great help in treating diabetes. Coffee may ward off liver damage. The chlorogenic acid and caffeic acid in coffee bean work as powerful antioxidants that absorb free oxygen radicals and prevent destructive, free radical or oxidative damage to tissues or cells.

Keeping this pharmaceutical effects of both the plants, present study aims to screen the metabolites present in ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica* quantitatively.

Materials and Methods

Plant material

Fresh seeds of *Theobroma cacao* and *Coffee arabica* were collected from Wayanad discrict, Kerala. Plant sample was washed and shade dried at room temperature.

Preparation of the extract

The shade dried beans of *T. cacao* and *C. arabica* was powdered and extracted with solvents using soxhlet apparatus. The extract obtained was rotory evaporated and the powder was preserved in an air tight container and stored at 4^0C for further use.

Quantitative determination of primary metabolites

Primary metabolites are of prime importance and essentially required for growth of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs [31, 32].

1. Determination of total soluble Carbohydrates

The total soluble Carbohydrate content was determined according to the method described by Hedge and Hofreider, 1962 [33]. 1 ml of sample was mixed with 4 ml of Anthrone reagent. Incubated in boiling water bath for 8 minutes, after which the absorbance was read at 630 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg / g sample

2. Determination of Proteins

Protein content was determined according to the method of Lowry *et al*., 1951 [34]. 1 ml of sample was mixed with 0.5 ml of 0.1 N NaOH and 5 ml of alkaline coper reagent, incubated the mixture at room temperature for 30 minutes. Added 0.5 ml of Folin-Ciocalteau reagent and incubated again for 10 minutes at room temperature. Absorbance was read at 660 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed mg / g sample.

3. Determination of Total free amino acids

Total free Amino acid (Ninhydrin method) was determined according to the procedure given by Moore and Stein, 1948 [35]. 1 ml of the sample was mixed with 1 ml of Ninhydrin reagent in a test tube. Tubes were kept in boiling water bath for 20 minutes and then added 5 ml of diluent (equal volume of water and n-propanol).Incubated at room temperature for 15 minutes and absorbance were read at 570 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg / g sample.

Quantitative determination of secondary metabolites

Drug discovery from the medicinal plants has played significant role in the treatment of various diseases. Secondary metabolites are important mediators of ecological interactions between plants and their environment.

1. Determination of Total phenols

Total phenol content were estimated in the ethanolic extract by the procedure given by Bray and Thorpe, 1954 [36], Folin-ciocalteu method. To 1 ml of sample added 0.5 ml of Folin-ciocalteu reagent and incubated at room temperature for three minutes. After three minutes 2 ml of 20% Na2CO3 was added, mixed well and incubated the tubes in boiling water bath for 1 minute. Cooled rapidly and read absorbance at 650 nm against reagent blank. The analysis was performed in triplicates and the results were expressed as mg / g sample.

2. Determination of Flavonoids

Flavonoids in ethanolic extracts was estimated by the method proposed by Jia *et al*., 1954 [37]. 1 ml of the was mixed with 0.075 ml of 5% Sodium nitrite solution and incubated at room temperature for 10 minutes. Then added 10% aluminum chloride and incubated at room temperature for 6 minutes. Then added 1 N NaOH and absorbance was read at 510 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg catechin equivalent/ g sample.

3. Determination of Tannins

Estimation of tannins in the extracts was done by the procedure given by standard methods of Bray and Thorpe, 1954 [36]. 1 ml of the sample was mixed with 5 ml of vanillin hydrochloride reagent and incubated at room temperature for 20 minutes. Absorbance was read at 500 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as catechin equivalent / sample.

4. Estimation of Alkaloids

The estimation of alkaloids was done by method of Harborne [38]. Homogenized 10 mg of plant material in a motor and pestle and 20 ml mixture of methanol: ammonia in the ratio 68:2 was added. The ammoniacal layer was decanted and fresh methanolic ammonia mixture was added after 24 hrs. The procedure was repeated thrice and extracts were pooled. The extracts were evaporated using a flash evaporator. The residue was treated with 1 N HCl and kept overnight. The acidic solution was extracted with 20 ml of chloroform thrice; the organic layers were pooled and evaporated to dryness. Basified the acidic layer with concentrated sodium hydroxide to pH 12 and extracted with 20 ml of chloroform thrice and pooled the chloroform layers. Evaporated to dryness over absorbent cotton. The fraction that contains alkaloids was weighed and expressed as mg/100 g.

Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

Results

The results that are obtained are given in tables as follows under each of its respective topics.

Quantitative estimation of Primary metabolites in ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica*

Primary metabolites have a key role in survive of the species, playing an active function in the photosynthesis and respiration. Quantitative analysis of primary metabolites in the ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica* is given in Table 1 and Table 2.

Table 1: Quantitative estimation primary metabolites in ethanolic seed extract of *Theobroma cacao.*

Primary metabolites	Estimated quantity (mg/g)
Total protein	3.55 ± 0.08
Total carbohydrates	2.78 ± 0.09
Amino acids	1.65 ± 0.67

Values are expressed as mean ± SD of three samples.SD: Standard deviation

Table 2: Quantitative estimation primary metabolites in ethanolic seed extract of *Coffee arabica*.

Primary metabolites	Estimated quantity (mg/g)
Total carbohydrates	4.78 ± 0.05
Total protein	2.34 ± 0.02
Amino acids	1.89 ± 0.98

Values are expressed as mean ± SD of three samples. SD: Standard deviation

Quantitative estimation of Secondary metabolites in ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica*

Secondary metabolites are organic molecules that are not involved in the normal growth and development. Quantitative analysis of secondary metabolites in ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica* is given Table 3 and Table 4.

Table 3: Quantitative estimation of Secondary metabolites in ethanolic seed extract of *Theobroma cacao*

Secondary metabolites	Estimated quantity (mg/g)
Total phenols	5.63 ± 0.98
Alkaloids	3.7 ± 0.65
Tannins	2.7 ± 1.10
Flavanoids	3.92 ± 0.44

Values are expressed as mean ± SD of three samples. SD: Standard deviation

Table 4: Quantitative estimation of Secondary metabolites in ethanolic seed extract of *Coffee arabica*

Secondary metabolites	Estimated quantity (mg/g)
Total phenols	5.56 ± 0.06
Tannins	2.7 ± 1.02
Alkaloids	1.76 ± 0.45
Flavonoids	4.78 ± 0.09

Values are expressed as mean ± SD of three samples. SD: Standard deviation

Discussion

Quantitative analysis of primary metabolites in *Theobroma cacao* shows that (Table 1), protein content was found high (3.55 ± 0.08 mg/g) followed by carbohydrate amino acid (2.78 ± 0.09 mg/g) and then amino acid (1.65 ± 0.67 mg/g). Quantitative analysis of primary metabolites in *Coffee arabica* shows that (Table 2), carbohydrate content was found high (4.78 ± 0.05 mg/g) followed by protein content (2.34 ± 0.02 mg/g) and then amino acid (1.89 ± 0.98 mg/g)

Plant sugars can be used as artificial sweetener and they can even help in diabetes by supporting the body in its rebuilding [39]. The presence of higher protein level in the plant parts towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [40].

Secondary metabolites analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. The ethanolic seed extract of *Theobroma cacao* extract (Table 3) showed higher level of phenols (5.63 ± 0.98 mg/g) than the other secondary

metabolites, followed by flavonoids (3.92 ± 0.44 mg/g), alkaloids (3.7 ± 0.65 mg/g) and then tannins (2.7 ± 1.10 mg/g). Ethanolic seed extract of *Coffee arabica* (Table 4) also showed higher levels of phenols (5.56 ± 0.06 mg/g) followed by flavonoids (4.78 ± 0.09 mg/g), tannins (2.7 ± 1.02 mg/g) and then alkaloids (1.76 ± 0.45 mg/g).

Phenol is important in the regulation of plant growth, development and disease resistance. Consumption of diets rich in plant polyphenols offers protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.

Flavonoids have been reported to exert wide range of biological activities. These includes: Anti-inflammatory, antibacterial, antiviral, anti-allergic [41-43], cytotoxic anti-tumour, treatment of neurodegenerative diseases, vasodilatory action 44-46. In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation [43, 45, 47]. These are also reported to inhibit variety of enzymes like hydrolases, hyaluronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase [48].

Alkaloids protect against chronic diseases [49] and earlier recorded that bitter leaf contains an alkaloid that is capable of reducing headaches associated with hypertension. Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase [50].

The Higher amount of Tannin contributes various medicinal properties such as antimicrobial, anti-inflammatory and astringent activity. They have been also reported to have anti-viral [51] antibacterial [52, 53] and anti-parasitic effects.

The phytochemical analysis of the medicinal plants are important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for the treatment of various diseases. Thus, we hope that the important phytochemical properties identified in the present study with ethanolic seed extracts of *Theobroma cacao* and *Coffee arabica* will be helpful in the treatment of various ailments.

Conclusion

Plants and its products are used as medicine from the ancient time. Recently there has been a shift in universal trend from synthetic to herbal medicine [54]. It is estimated by the World Health Organization that approximately 75-80% of the world's population uses plant medicines either partly or entirely as medicine. Interest in plant derived drug increases mainly due to the increasing use, and misuse, of existing synthetic drugs this poses the need for search and development of new drugs to cure diseases. The chemical substances of the medicinal plants which have the capacity of exerting a physiologic action on the human body are the primary features. The bioactive compounds of plants compounds are considered to be most important. The phytochemical research that has been done based on the ethno-pharmacological information forms the effective approach in the discovery of new medicinal agents from higher plants.

The results obtained in the present study indicate *Cacao and Coffee* seed extracts have the potential to act as a source of useful drugs because of presence of various phytochemical components such as various lipids and amino acids. The results are very much encouraging but scientific validation is necessary before being put into practice.

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