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Quantitative phytochemical analysis of some edible fruits from Boda and Kolli hills

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Abstract

The aim of this study was to evaluate the phytochemical analysis for fifteen fruits collected from the Boda and Kolli hills. The phytochemicals estimation is screened by using three different solvent such as ethanol, chloroform and aqueous. The wild and edible fruits such as *Coccinia indica*, *Carissa carandas*, *Ficus benghalensis*, *Ficus religiosa*, *Hugnoia mystax*, *Limonia acidissima*, *Morinda pubescens*, *Murraya koenigii*, *Pithecellobium dulce*, *Syzygium cumini*, *Tarenna asiatica*, *Zizyphus mauritiana* and *Zizyphus oenoplia*. The phytochemicals like alkaloids, flavonoids, steroids, phenols and saponins were estimated. The results show the presence of phytochemical constituent was high in ethanol extract. The concentration was varied for the solvent extraction. Hence, these findings confirm that the selected edible fruit have a potential source for the formulation of new therapeutic drug.

Keywords: Boda and Kolli hills, Edible fruits, phytochemical constituent, therapeutic drug.

1. Introduction

Developing countries use traditional based medicines at the primary health care level. The use of traditional medicine is one of the common practices in India due to their wide pharmacological activities. Many currently used drugs are expensive or not readily available and a major reason is continued usage lead a drug resistance^[1,2]. The Kolli and Boda hills are highly enriched with its vegetation including wild edible fruits due to its varied ecogeographical and eco-climatic conditions. Local tribes consume wild fruits as a food supplement. Plant species have long been the practice and principal ingredients of traditional medicine and their uses is the beginning of human civilization^[3,4]. Wild edible plants contribute significant role in nutrition of tribal inhabitants. These foods are consumed by people in fresh or dried forms. During the food shortage, the dependence on these foods increases. Nowadays, a nutritional supplementary transition is replaced of traditional plant-based diets that are rich in fruits and vegetables. These help the tribal people to increase quantity of daily food^[5]. Wild edible foods include fruits, leaves, flowers and seeds from spontaneous trees and shrubs. Of these, the researchers were interested in working on wild edible fruits because of their nutritional value, vitamin and mineral contents and their bioactive compound. Research on wild fruits and other edible parts of plants is also intended to promote the preservation of these species, presently under threat from human activities. In addition to their nutritional value, the preservation of these fruits also has economical advantages, as there is a significant trade in some of these wild edible fruits. These wild fruits are also known to have medicinal properties such as antimicrobial, antioxidant, anticancer etc. some scientific evidence for the health benefits of such wild fruits in addition to their nutritional value would be an added value to the plants producing such fruits. Concerning their medicinal properties, the present study is to find out the phytochemical constituent like alkaloids, flavonoids, phenols, tannins and saponins from the collected fruits such as *Coccinia indica*, *Carissa carandas*, *Ficus benghalensis*, *Ficus religiosa*, *Hugnoia mystax*, *Limonia acidissima*, *Morinda pubescens*, *Murraya koenigii*, *Pithecellobium dulce*, *Syzygium cumini*, *Tarenna asiatica*, *Zizyphus mauritiana* and *Zizyphus oenoplia* from Boda and Kolli hills.

2. Materials and Methods

2.1 Plant collection and identification

Wild edible, mature fruits were collected from the Boda and Kolli hills. The collected fruits specimen was authenticated by Botanical survey of India (BSI), Coimbatore, Tamil Nadu, India and in Department of Botany, National College, Tiruchirappalli.

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2.2 Preparation of Extracts

The fresh fruits were dried in shade for about 3 weeks and ground using a mixer to a coarse powder. 100gm of powdered material was Soxhlet extracted with different solvents, like ethanol, chloroform and aqueous (12 hour each). All the extracts were evaporated in vacuum under reduced pressure and stored in sterile glass bottles at room temperature until screened.

2.3 Quantitative determination of the Phytochemical Screening constituency

The amount of each secondary metabolites from the crude powdered sample were evaluated using standard laboratory procedures based on the methods of Total phenol contents using a spectrophotometric method (Singleton *et al.*, 1999), Flavonoids by the method of Boham and Kocipai-Abyazan (1974), Tannin by the method of Van-Burden and Robinson (1981), Alkaloids by the method of Harborne (1973) and Saponin by the method of Obadoni and Ochuko (2001) [6-10].

2.3.1 Determination of Total Phenolic contents using Spectrophotometric method

The concentration of phenolics in fruit extracts was determined using Spectro-photometric method. The reaction mixture was prepared by mixing 0.5 ml of ethanol extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 ml ethanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO₃. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extract was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

2.3.2 Determination of flavonoids by the method of Boham and Kocipai-Abyazan (1974)

10 g of the sample was extracted repeatedly with 100 ml of 80% aqueous ethanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.3.3 Determination of Tannin by Van – Burden and Robinson (1981) method

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M FeCl₃ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10 minutes.

2.3.4 Determination of alkaloids using Harborne (1973) method

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol added. The beaker was covered and allowed to stand for 4 hours. It was then filtered

and the extract concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2M) and then filtered. The residue if available, is the alkaloid which is then dried and weighed.

2.3.5 Determination of saponin by Obadoni and Ochuko (2001) method

The samples were ground and 20g of each put into a conical flask followed by the addition of 100ml of 20% aqueous ethanol. They were then heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re – extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n – butanol was added. The combined n–butanol extracts were washed twice with 10 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. Saponin content was calculated as percentage.

3. Results and Discussion

The fifteen edible fruits were collected from Boda and Kolli hills. The fruits such as *Coccinia indica*, *Carissa carandas*, *Ficus benghalensis*, *Ficus religiosa*, *Hugnoia mystax*, *Limonia acidissima*, *Morinda pubescens*, *Murraya koenigii*, *Pithecellobium dulce*, *Syzygium cumini*, *Tarennia asiatica*, *Zizyphus mauritiana* and *Zizyphus oenoplia* are subjected to quantify the phytochemical compound by using standard methods. All the fruit extracts were prepared by using a solvent such as aqueous, ethanol and chloroform. The wild edible fruits are estimated for phytochemical constituent such as alkaloids, flavonoids, phenols, tannins and saponins. The result obtained from the three solvent of all wild fruit extraction showed the presence of phytochemicals from the highest to the least extent. The phytochemical constituents such as alkaloids, flavonoids, phenols, tannins and saponins were estimated. The results were shown in the table -1. The alkaloids compound are range from 9.1 to 32.6 $\mu\text{g}/\text{mg}$ (dry weight of the extract) were shown. The high amount is seen in the edible fruit of *Murraya koenigii* in ethanol extract (32.6 $\mu\text{g}/\text{mg}$ dry weight of the extract) and least amount at 9.1 $\mu\text{g}/\text{mg}$ dry weight of the extract was observed in the aqueous extract of *Carissa carandas*. All other solvent extract has moderate and low concentration of alkaloid compound. Alkaloids have many medicinal properties such as cytotoxicity [11], analgesic [12], antispasmodic and antibacterial [13]. Alkaloids formed as metabolic byproducts and be responsible for the antibacterial activity [14]. The flavonoids concentrations of all the edible fruits were upto 22.1 to 77.3 $\mu\text{g}/\text{mg}$ dry weight of the extract. The higher amounts are seen in the edible fruit of *Syzygium cumini* in ethanol extract (77.3 $\mu\text{g}/\text{mg}$ dry weight of the extract) and least amount is 22.1 $\mu\text{g}/\text{mg}$ dried weight of the extract was observed in the aqueous extract of *Hugnoia mystax*. All other solvent extract has high and moderate concentration of Flavonoids compound according to their solubility in solvent. Flavonoids complex with extra cellular and soluble proteins and with bacterial cell walls. Flavonoids are hydroxylated phenolic substances

known to be synthesized by plants in response to antimicrobial substances against pathogen microorganisms in *in-vitro* studies [15]. They also are effective antioxidant and show strong anticancer activities [16-18].

The phenol concentrations of all the edible fruits were upto 30.2 to 89.55 µg/mg dry weight of the extract. The high amounts are seen in the edible fruit of *Morinda pubescens* in ethanol extract (89.55 µg/mg dry weight of the extract) and least amount is 30.2 µg/mg (dried weight of the extract) was observed in the aqueous extract of *Ficus benghalensis*. All other solvent extract has high and moderate concentration of phenol compound according to their solubility. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [19]. They possess biological properties such as anti-apoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [20]. Phenol has the antioxidant properties [21, 22]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc [23]. The tannin concentrations of all the edible fruits were upto the range of 0.021 to 0.091µg/mg dry weight of the

extract. The high amounts are seen in the edible fruit of *Hugnoia mystax* in ethanol extract (0.091 µg/mg dry weight of the extract) and least amount is 0.021 µg/mg (dry weight of the extract) was observed in the aqueous extract of *Pithecellobium dulce*. All other solvent extract has high and moderate concentration of phenol compound according to their solubility. Tannins bind to proline rich proteins and interfere with the protein synthesis [24]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [25]. Finally the concentrations of saponins were determined all the edible fruit extracts. The saponins are in the range of 0.11-1.35 µg/mg dry weight of the extract. The *Hugnoia mystax* showed highest amounts of saponins (1.35 µg/mg dry weight of the extract) and least concentrations are observed in *Ficus benghalensis* (0.11µg/mg dry weight of the extract). The plant extracts were also revealed to contain saponins, which are known to produce inhibitory effect on inflammation [26]. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [27].

Table 1: Quantitative analysis of secondary metabolites against selected fruits extract

S. No.	Botanical name	Alkaloids			Flavanoids			Phenol			Tannin			Saponin		
		E	A	C	E	A	C	E	A	C	E	A	C	E	A	C
1	<i>Coccinia indica</i>	14.5	11.3	13.5	37.01	33.5	36.5	51.09	45.12	50.34	0.088	0.073	0.084	0.05	0.013	0.06
2	<i>Carissa carandas</i>	10.3	9.1	10.2	45.8	45.2	45.3	53.46	50.3	52.54	0.067	0.053	0.063	0.84	0.83	0.83
3	<i>Carissa spinarum</i>	10.8	10.1	10.4	43.27	41.5	42.3	68.85	63.4	68.72	0.042	0.023	0.036	0.73	0.72	0.78
4	<i>Ficus benghalensis</i>	12.9	12.4	12.3	43.97	41.23	45.9	34.7	30.2	33.3	0.084	0.083	0.084	0.18	0.11	0.16
5	<i>Ficus religiosa</i>	15.7	14.3	14.3	39.9	35.2	36.4	41.80	41.35	41.58	0.056	0.052	0.053	0.29	0.22	0.27
6	<i>Hugnoia mystax</i>	16.8	12.3	15.5	24.5	22.1	24.5	69.79	67.34	69.32	0.091	0.081	0.089	1.35	1.29	1.32
7	<i>Limonia acidissima</i>	13.9	12.7	13.6	48.2	46.3	48.2	58.51	54.43	55.6	0.089	0.083	0.087	0.73	0.74	0.79
8	<i>Morinda pubescens</i>	11.9	11.5	11.9	75.75	65.4	67.5	89.55	81.34	83.06	0.075	0.074	0.075	0.34	0.23	0.37
9	<i>Murraya koenigii</i>	32.6	32.1	32.4	54.86	54.23	54.66	76.94	74.56	76.83	0.079	0.073	0.072	0.54	0.53	0.55
10	<i>Pithecellobium dulce</i>	12.5	10.2	11.7	25.45	23.34	25.16	55.64	55.23	55.47	0.036	0.021	0.032	0.65	0.61	0.69
11	<i>Phyllanthus emblica</i>	14.7	13.7	12.6	41.07	39.4	42.2	62.53	62.4	62.01	0.058	0.052	0.051	0.07	0.06	0.07
12	<i>Syzygium cumini</i>	17.3	15.5	13.9	77.3	75.23	75.3	74.4	72.3	73.1	0.076	0.072	0.074	0.17	0.16	0.17
13	<i>Tarennia asiatica</i>	21.2	21.1	21.3	76.5	74.1	74	82.3	82.5	83.4	0.081	0.085	0.074	0.16	0.19	0.17
14	<i>Zizyphus mauritiana</i>	14.6	16.1	14.6	65.57	56.3	64.8	76.4	74.5	75.7	0.064	0.06	0.063	0.79	0.73	0.73
15	<i>Zizyphus oenoplia</i>	14.8	12.3	13.9	67.65	63.11	67.65	77.7	75.6	76.4	0.062	0.059	0.061	0.63	0.61	0.63

E=Ethanol; A=Aqueous; C= Chloroform

4. Conclusion

The wild edible fruits based bio-active compounds have their effective dosage response with minimal side effects and very low cost than the synthetic compounds. The wild edible fruits such as *Coccinia indica*, *Carissa carandas*, *Ficus benghalensis*, *Ficus religiosa*, *Hugnoia mystax*, *Limonia acidissima*, *Morinda pubescens*, *Murraya koenigii*, *Pithecellobium dulce*, *Syzygium cumini*, *Tarennia asiatica*,

Zizyphus mauritiana and *Zizyphus oenoplia* showed the presences of alkaloids, flavonoids, phenols, tannins and saponins compound. The bioactive compound is responsible for their therapeutic effects. Hence this may further reflect a hope for the development of many more novel drugs. Further research needs to be carried out to identify the active molecules and evaluation of their therapeutic significance in the prevention of diseases.

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