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Effect of quantity of plant extract and maceration period in extraction of phytochemical compounds from six different plants of Cuddalore district, Tamil Nadu, India

Sumathi V and Priyalaxmi Rajesh

Abstract
Plants are considered as a potent and powerful source of drugs that have stood the test of time, where the modern medicine and chemistry development could not replace most of them. But medicinal plants unless thoroughly investigated for its composition, it is dangerous to be used widely. Thus, the main objective of our research work was to analyze the essential quantity (0.5g, 1.0g and 2.0g) and maceration period (6hrs, 12 hrs and 24hrs) required for extraction of phytochemical compounds from 6 different plants (*Albizia saman, Artocarpusm heterophyllus, Melia azedarch, Prunus dulcis*, *Thespesia populnea* and *Vitex negundo*) collected in Cuddallore district. Phytochemical screening of 6 different plants were done for the presence of carbohydrates, alkaloids, proteins, glycosides, reducing sugar, aminoacids, steroids, phytosterol, terpenoids, anthroquinones, saponin, flavanoids, betacyanin, anthocyanin, starch, cardiac glycosides, tannin and phenol using standard methods at different quantities of plant extract (0.5g, 1.0g and 2.0g) and maceration periods (6hrs, 12 hrs and 24hrs). Among them, 1 g extract and 12 hrs of maceration period was found to be essential in extraction of phytochemical compounds.

Keywords: Plant extract, different quantity, maceration period, phytochemical analysis

1. Introduction
The problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries should be emphasized for the need to develop new antibiotics (Prasad *et al*., 2008) [15].

Nature has been a promising source of new therapeutic candidate compounds due to the tremendous chemical diversity found in various species of plants. Plants are considered as a potent and powerful source of drugs that have stood the test of time, where the modern medicine and chemistry development could not replace most of them. Moreover, it has been revealed that more than 50% of all modern drugs contain natural products as the major component of modern pharmaceuticals used for the treatment of human diseases (Rosangkima and Prasad, 2007) [18].

Approximately, 400 herbs have been used widely as therapeutic medicinal substances as narcotics, purgatives and sudorifics, where morphine was first obtained from opium by Serturner in year 1806. Other compounds such as cinchonine, quinine, caffeine and brucine were successfully isolated during the next five years (Yeo *et al*., 2014) [27].

Phytochemicals are naturally occurring bioactive compounds having a defense mechanism to cure and protect from various diseases (Duke, 1995) [7]. They are of two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids whereas secondary constituents contain terpenoids, alkaloids, flavonoids, phenolic compounds and other metabolites which exhibit various important pharmacological activities such as antioxidant, anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities (Prema and Jayanthy) According to world health organization plant derived drugs constitute the mainstay of nearly.

80% of the population for their Primary Health Care (Panda *et al*., 2009; Simbo, 2010; Singh *et al.*, 2012) [12, 22, 23]. The practice of herbal medicine is wide spread in China, India, Japan, Pakistan, Srilanka and Thailand (Deeb *et al*., 2013; Krishnamoorthi *et al.*, 2015; Salisu *et al.*, 2015) [5, 10, 19]. Presently plant based drugs are being used worldwide. Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (Essential and fixed) (Amrit pal., 2005; Suman Kumar *et al*., 2013) [2, 25]. These are either used directly extracted from plants or modified through further synthesis. These specific chemicals belong to plant derived compounds called phytochemicals such as the metabolites of primary and secondary metabolites and Cathrine, 2016) [17].

Many pharmaceutical companies are showing great interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is effective, safer and more reliable than synthetic drugs (Alagesaboopathi, 2012; Seru Ganapthy *et al.*, 2013) [1, 21]. The extensive use of synthetic compounds led to a decline in the use of plants in modern medicine; however, synthetic drugs often cause considerable side effects, and as a result, people are more favouring the use of natural compounds obtained from plants. *Albizia saman* tree is largely reported to possess medicinal properties to treat several ailments. The root decoction is used in hot baths for stomach cancer in Venezuela. Rain Tree is a traditional remedy for colds, diarrhea, headache, intestinal ailments and stomachache. Seeds are chewed for sore throat. The alcoholic extract of the leaves inhibits *Mycobacterium* tubercle growth (Prema and Jayanthy. 2018) [16].

Artocarpus heterophyllus is reported to possess antibacterial, anti-inflammatory, antidiabetic, antioxidant and immunomodulatory properties. The leaves are useful in fever, boils, wounds and skin diseases. The seeds are, diuretic, and constipating. The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent. The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhea. Heated leaves are placed on wounds. Latex is used as an anti-inflammatory agent (Prakash *et al*., 2009) [14].

Melia azedarach has wider applications. The leaves are leprosy, scrofula, anthelmintic, antilithic, diuretic, deobstruent, resolvent, insecticidal, burns, malaria, gingivitis, piles, pyrexia, chicken pox, smallpox and warts, remove toxins, purify blood and prevent damage caused by free radicals, mosquito coils (Azam *et al*.,2013) [3].

Prunus dulcis almonds are also beneficial to the overall health of the body, being used especially in the treatment of kidney stones, gallstones and constipation. The leaves are used in the treatment of diabetes. The plant contains the antitumour compound taxifolin.

Thespesia populnea are cutaneous infections, skin and liver diseases. Fruit juices are used on rheumatism sprains, scabies, swellings, insect bites and warts. Unripe fruit juice was used to cure piles. Root, fruit and leaf are used in psoriasis, scabies and other cutaneous diseases. Bark was used for the treatment of hemorrhoids and chronic dysentery. Leaf used as an anti-inflammatory. Leaves and bark of this tree are still used to mix with oil for the treatment of fracture wounds and as an anti-inflammatory poultice applied to ulcers and boils, as described in folk medicine (Muthukumar and Sami Veerappa, 2018) [4].

Vitex negundo Linn (Verbenaceae) leaves and the barks are the most important in the field of medicine. Leaves may have both central and peripheral analgesic action and also possesses anti-inflammatory activity by acting through inhibition of prostaglandin biosynthesis. The mature fresh leaves have oral anti-inflammatory, analgesic and antihistamine properties. The decoction of leaves is considered as tonic, vermifuge and is given along with long pepper in catarrhal fever (Devi *et al*., 2017) [6].

To the best of my knowledge, there is no studies to reveal the proper quantity and maceration periods in extraction of phytochemical compounds. But medicinal plants unless thoroughly investigated for its composition, it is dangerous to be used widely (Devi *et al*., 2017) [6].

Thus, the main objective of our research work was to analyze the essential quantity and maceration period required for extraction of phytochemical compounds from 6 different plants (*Albizia saman, Artocarpusm heterophyllus, Melia azedarch, Prunus dulcis*, *Thespesia populnea* and *Vitex negundo*) collected in Cuddallore district.

2. Materials and Methods

2.1. Selection of plants

Healthy and disease free leaves of 6 medicinal plants: *Albizia saman, Artocarpusm heterophyllus, Melia azedarch, Prunus dulcis*, *Thespesia populnea* and *Vitex negundo.*known by the traditional medical practitioners of Cuddallore district, Tamil Nadu, India, were collected.

2.2 Preparation of plant material

The fresh leaves of the collected plants were removed and then washed under running tap water to remove dust. Washed plant materials were air dried, cut into small pieces and pulverized in a mechanical blender and stored in plastic boxes for use. Powdered plant material was used for the preparation of crude extracts.

2.3 Preparation of Crude extract:

The plant powder was taken in a test tube in three different volumes 0.5g, 1g and 2g, respectively and 10 ml of distilled water was added to it such that plant powder soaked in it for different maceration periods 6hrs, 12hrs and 24hrs, respectively and shaken well. The solution then filtered with the help of Whatmann No. 41 filter paper and the filtered extract of the selected plant samples were taken and used for further phytochemical analysis.

2.4 Phytochemical screening

The phytochemical screening of the sample was carried out as described by Jayapriya and Gricilda Shoba, 2015; Bhargavi and Kalpana Kaloori, 2018 [9, 4]. The samples were screened for carbohydrates, alkaloids, flavonoids, phytosterols and steroids, anthocyanin and betacyanin, phenols and tannins, saponin, glycosides, and proteins.

2.4.1 Test for carbohydrates

To 2 ml of plant extract, 1 ml of Molisch reagent and 4 drops of concentrated sulphuric acid were added. Formation

of purple or reddish colour indicates the presence of carbohydrates.

2.4.2 Test for alkaloids
To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then 3 drops of Mayer's reagent were added. Formation of green colour or white precipitate indicates the presence of alkaloids.

2.4.3 Test for Proteins
To 2ml of each plant extract, treated with few drops of concentrated nitric acid formation of yellow colour indicates the presence of xanthoprotein.

2.4.4 Test for glycosides
To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

2.4.5 Test for Reducing Sugar
Mix 1 ml of fehling solution 1 and 1 ml of fehling solution 2. To it add 3 drops of plant extract and keep in waterbath at 60ºC. Presence of green suspension and red precipitate indicates the presence of reducing sugar.

2.4.6 Test for Aminoacids
To 2 ml of plant extract, 4 drops of 0.2% Ninhydrin was added and heated to 100ºC. Formation of blue colour indicates the presence of proteins.

2.4.7 Test for steroids and phytosterols
To 1 ml of plant extract, equal volume of chloroform and 3 drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.

2.4.8 Test for terpenoids
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

2.4.9 Test for anthraquinones
To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

2.4.10 Test for saponins
To 2 ml of plant extract, 6 ml of distilled water was added and shaken vigourously for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponin.

2.4.11 Test for flavonoids
To 2 ml of plant extract 1 ml of 2N aqueous NaOH solution was added and observed for the formation of yellow colouration.

2.4.12 Test for anthocyanin and betacyanin
To 2 ml of plant extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100ºC. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

2.4.13 Test for starch
To 2ml of the plant extract, 2 drops of iodine solution was added. Appearance of dark blue black colour indicates the presence of starch.

2.4.14 Test for cardiac glycosides
To 2 ml of plant extract, 1 ml of glacial acetic acid and 5% ferric chloride was added. To these 3 drops of concentrated sulphuric acid was added. Presence of greenish blue colour indicates the presence of glycosides.

2.4.15 Test for tannins
To 1 ml of plant extract, 2 ml of 5 % ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.4.16 Test for phenols
To 1 ml of the extract, 2 ml of distilled water followed by 5 drops of 10% ferric chloride was added. Formation of dark blue or green colour indicates presence of phenols.

3. Results
The present study was conducted with an objective to identify the quantity plant extract and maceration period required for the extraction of phytochemical compounds from the shade dried leaves of 6 plants, *Albizia saman*, *Artocarpusm heterophyllus*, *Melia azedarch*, *Prunus dulcis*, *Thespesia populnea* and *Vitex negundo.* Among the different quantity of extract and different maceration period analysed, 1g of extract in 10ml of distill water with maceration period of 12 hrs was found to best by showing maximum number of phytochemical compound extraction.

Table 1: Phytochemical analysis of *Albizia saman* at different quantities and maceration periods

Chemical Test	0.5g (hrs)			1g (hrs)			2g (hrs)		
	6	12	24	6	12	24	6	12	24
Carbohydrates	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+	-
Glycosides	-	-	-	-	-	-	-	-	-
Reducing sugar	+	+	+	-	+	+	-	+	+
Aminoacids	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-
Phytosterol	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-	-	-	-
Anthroquinones	-	-	-	-	-	-	-	-	-
Saponin	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+	+
Betacyanin	+	+	+	+	+	+	+	+	+
Anthocyanin	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-	-
Phenol	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	+

Table 2: Phytochemical analysis of *Artocarpusm heterophyllus* at different quantities and maceration periods

Chemical Test	0.5ml (hrs.)			1ml (hrs.)			2ml (hrs.)		
	6	12	24	6	12	24	6	12	24
Carbohydrates	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	-	-	-
Glycosides	-	-	-	+	+	+	+	+	+
Reducing sugar	+	+	+	-	-	-	+	+	+
Aminoacids	-	-	-	-	+	+	+	+	-
Steroids	-	-	-	-	+	-	+	+	-
Phytosterol	-	-	-	+	-	-	-	-	-
Terpenoids	-	-	-	+	+	-	+	+	-
Anthroquinones	-	-	-	-	-	-	-	-	-
Saponin	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	-	-	-	-	-	+
Betacyanin	-	+	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	+	-
Phenols	-	-	-	-	-	-	-	-	+
Tannin	-	+	+	+	+	+	+	+	+

Table 3: Phytochemical analysis of *Melia azedarch* at different quantities and maceration periods

Chemical Test	0.5ml (hrs.)			1ml (hrs.)			2ml (hrs.)		
	6	12	24	6	12	24	6	12	24
Carbohydrates	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+
Protein	-	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+
Reducing sugar	+	+	+	+	+	-	+	+	-
Aminoacids	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+
Phytosterol	-	-	-	+	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+	+
Anthroquinones	-	-	-	-	-	-	-	-	-
Saponin	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	-	+	+	-	-	-	-
Betacyanin	+	-	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	+	-	-	+
Cardiac glycosides	-	-	-	-	-	-	-	-	-
Phenols	-	-	-	-	-	-	-	-	-
Tannin	+	+	+	+	+	+	+	+	+

Table 4: Phytochemical analysis of *Prunus dulcis* at different quantities and maceration periods

Chemical Test	0.5ml (hrs.)			1ml (hrs.)			2ml (hrs.)		
	6	12	24	6	12	24	6	12	24
Carbohydrates	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+
Protein	-	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+
Reducing sugar	+	+	+	+	+	+	+	+	+
Aminoacids	+	+	-	-	+	+	-	+	+
Steroids	-	-	-	-	-	-	-	-	-
Phytosterol	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-	-	-	-
Anthroquinones	-	-	+	-	+	+	+	+	+
Saponin	+	+	+	+	+	+	+	+	+
Flavanoids	-	-	-	-	-	-	-	-	-
Betacyanin	-	-	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	-	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+

Table 5: Phytochemical analysis of *Thespesia populnea* at different quantities and maceration periods

Chemical Test	0.5ml (hrs.)			1ml (hrs.)			2ml (hrs.)		
	6	12	24	6	12	24	6	12	24
Carbohydrates	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-
Reducing sugar	-	-	-	+	+	+	+	+	+
Aminoacids	+	+	+	+	+	+	+	-	-
Steroids	-	-	-	-	-	-	-	-	-
Phytosterol	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-	-	-	-
Anthroquinones	-	-	-	-	-	-	-	-	-
Saponin	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+	+
Betacyanin	+	+	+	+	+	+	+	+	-
Anthocyanin	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-	-
Phenol	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	+

Table 6: Phytochemical analysis of *Vitex negundo* at different quantities and maceration periods

Chemical Test	0.5ml (hrs.)			1ml (hrs.)			2ml (hrs.)		
	6	12	24	6	12	24	6	12	24
Carbohydrates	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-
Reducing sugar	-	+	+	-	+	+	-	+	+
Aminoacids	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	+	+	-	+	+
Phytosterol	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	+	+	-	+	+
Anthroquinones	-	-	-	-	-	-	-	-	-
Saponin	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	-	-	-	-	-	-
Betacyanin	+	+	+	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-	-
Phenol	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	-

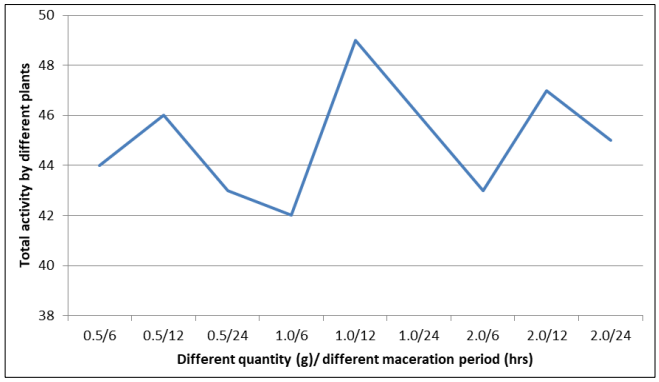

Fig 1: Shows the best quantity and maceration period for extraction of compounds

4. Discussion

Phytochemicals and biological constituents in plants have remarkable contribution towards the drug industry (Srinivasan *et al*., 2007) [24]. The medicinal properties of plants are due to the presence of certain chemical substances which can elicit a definite physiological action in human body. None of these studies revealed the importance of proper maceration periods (Yeo *et al*., 2014) [27]. This became the main objective of the current study.

From the results obtained, a wide spectrum of photochemical compounds were extracted using water as solvent with different quantities of 6 plant extracts (*Albizia saman*, *Artocarpusm heterophyllus*, *Melia azedarch*, *Prunus dulcis*, *Thespesia populnea* and *Vitex negundo*) - 0.5g, 1.0g and 2.0g at different maceration periods – 6 hrs, 12 hrs and 24 hrs. Figure 1 shows that 1.0g and 12 hrs were found to the best quantity and maceration period from analyzing the total 6 plants.

Water is a universal solvent and so was used to extraction of compounds. But, successful determination of phytochemical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Tiwari *et al*., 2011; Phan and Nguyen, 2014) [26, 13]. The variations in different extraction methods that affect quantity and secondary metabolite composition of an extract depends upon type of extraction, time of extraction, quantity of plant extract and soon (Tiwari *et al*., 2011) [26].

The quantity of plant extract was found to be essential for the extraction of compound and the present finding of 1g plant extract was best enough in extraction was supported by several research findings (Janakiraman and Jeyaprakash, 2015; Santhi and Sengottuvel, 2016; Devi *et al*., 2017) [8, 20, 6]. On the maceration time used, 12hrs was found to best in extraction of phytochemical compounds. Similar study on maceration periods in extraction was done (Yeo *et al*., 2014) [27] which shows 6 hrs. to be best in extraction of compounds that show antimicrobial activity from the fruit of *Momordica charanti*. This non-supporting evidence which indicates this might be due to change in location and genetic variation due to cross pollination. The result was supported that the effect of plant phytochemical depends on nature of plant material, its origin, degree of processing, moisture content and particle size (Tiwari *et al*., 2011) [26].

5. Conclusion

The phytochemical screening of six plants collected from cuddallore district with different quantities and maceration periods with water as a solvent revealed their significant influence in their phytochemical activity. Therefore, it is of great important to use an optimum quantity and maceration period in combination with suitable extraction solvents in order to obtain the desirable photochemical activity. However, further study is necessary to quantify, isolate, characterize and evaluate biological activity of the particular compound for drug development.

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