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PHYTOCHEMICAL SCREENING OF THE VARIOUS EXTRACTS OF THE PLANT *ELAEOCARPUS TUBERCULATUS* Roxb.

***Rajeswari M.**

PG and Research Department of Botany, Vellalar College for Women, Thindal, Erode, Tamil Nadu, India.

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***Corresponding Author**
Rajeswari M.
PG and Research Department of Botany, Vellalar College for Women, Thindal, Erode, Tamil Nadu, India.

ABSTRACT

In the present study, the phytochemical screening of the powder of leaf, fruit with seed of *Elaeocarpus tuberculatus* Roxb. in different extracts i.e. petroleum ether, methanol, ethanol and water was executed. The result indicated the presence of carbohydrates, proteins and amino acids, alkaloids, flavonoids, tannins, phenols, terpenoids, steriods, triterpenoids, coumarin, saponins, quinine, glycosides, gum, starch and fixed oil. Generally the solvents of both leaf, fruit with seed ethanol, and methanol were more efficient in extracting the phytochemicals than the other two solvents. The quantitative analysis shows utmost phenolic content in methanol extracts of leaf and fruit with seed (42.51 ± 3.23; 35.19 ± 0.43) respectively. The highest flavanoid content was observed in leaf; fruit with seed in methanol and ethanol (49.37 ± 1.24; 19.02 ± 2.18) respectively. The tannin content was maximum in methanolic extract of leaf (48.56 ± 0.78); fruit with seed (26.98 ± 3.17).

KEYWORDS: Elaeocarpus *tuberculatus*, leaf, fruit with seed, phytochemicals, phytoceuticals.

INTRODUCTION

Plants are the backbone and existence of life on this planet and a vivacious reserve for human welfare. The intimate relationship between the human and plants has progressed throughout generations. This approach has been persisted to the contemporary period where people get much of their needs from biological resources (Uraku *et al*., 2015). For millennia, plants are the only pharmaceutical giants we had. For thousands of years mankind is using plant source to alleviate or cure illnesses. The plants epitomize a huge pool of natural resources that can

produce various products and chemicals for the advantage of all life forms. Plants are the greatest astounding chemists. Humans use these plants compounds for health care as drugs and medicines (Pomnha, 2014).

Phytocheuticals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major degenerative diseases (Kelble, 2006). The most important of these bioactive groups of plants are alkaloids, terpenoids, tannins, saponins and phenolic compounds (Edeoga *et al*., 2005). Each class of these functional agents consists of a wide range of chemicals with differing potency. (Tonthubthimthong *et al*., 2001). However, the plants synthesize phytochemicals, which are valuable for our wellbeing as they cannot be produced in the human body (Martinez *et al.*, 2008). Many current studies have showed the urgent need for the development of new, safe and efficacious drugs to help reduce the global burden.

METHODS

Collection of plant materials

The plant parts such as leaf, fruit with seed of *Elaeocarpus tuberculatus* (commonly known as 'Rudraksh') for the proposed study were collected from Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), India and were authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India. The plant materials were dried separately under shade and powdered in a grinder and stored in a closed container for further screening.

Qualitative Phytochemical screening

Phytochemical tests to give general idea regarding the nature of constituents present in crude drug. The phytochemical screening of constituents (carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, phenols, terpenoids, triterpenes, steroids, coumarin, saponins, quinine, anthraquinone, glycosides, gum, starch and fixed oils) present in the powder of leaf and fruit with seed of *E. tuberculatus* in various extracts (petroleum ether, methanol, ethanol and aqueous) were carried out the following standard procedure of Brindha (1981), Harborne (1998), Lala (1993), Trease and Evans (2002), Kokate *et al*. (2004), Edeoga *et al*. (2005), Khandelwal (2008) and Siddiqui *et al*. (2009).

Tests for Carbohydrate

a. **Molisch's test**: To 2-3 ml of extract, two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added

slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

b. **Fehling's test**: An equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagents are mixed along with few drops of extract solution and boiled. A brick red precipitate of cuprous oxide forms if reducing sugars are present.

Tests for protein and amino acid

a. **Biuret test**: To 3 ml test solution, 4% NaOH and few drops of 1% $CuSO_4$ solution were added and observed for violet or pink colour.
b. **Ninhydrin test**: To 3 ml test solution and 3 drops 5% ninhydrin solution were heated in boiling water bath for 10 minutes observed for purple or bluish color.

Tests for alkaloid

a. **Mayer's test**: To 1 ml of extract, add 1ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.
b. **Wagner's test**: To 1 ml of the extract, add 1ml of Wagner's reagent (iodine in potassium iodide solution). Formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for tannin and phenolic compound

a. **Ferric chloride test**: To 2-3 ml of extract, add few drops of ferric chloride solution. Formation of blue-green color precipitate indicates the presence of tannins.
b. A small quantity of the powder was dissolved in 0.5 ml of 20% sulphuric acid solution followed by addition of few drops of aqueous sodium hydroxide solution. It turns blue colour in the presence of phenols.

Tests for flavonoid

a. **Alkaline reagent test**: To the extract solution, add few drops of sodium hydroxide solution; formation of an intense yellow color that turns colorless on addition of few drops of dilute acetic acid indicates the presence of flavonoids.
b. **Ferric chloride test**: To 2-3 ml of extract, add few drops of ferric chloride solution. Blue-green color precipitate indicates the presence of flavonoids.

Test for terpenoid

a. Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicates the presence of terpenoids.

Test for triterpenoid

a. **Libermann-Burchard test**: Extract treated with few drops of acetic anhydride, boiled cooled. Concentrated sulphuric acid is added from the sides of the test tube. Formation of deep red color indicates the presence of triterpenoids.

Test for steroid

a. **Salkowski's test**: The extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterol compounds in the extract.

Test for saponin

a. **Froth formation test**: About 1 ml of alcoholic extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponins.

Test for glycoside

a. **Borntrager's test**: The extract was treated with chloroform and the chloroform layer was separated. To this, dilute ammonia solution was added. Pink colour in the ammonia solution indicates the presence of glycosides.

Tests for anthraquinine

a. 5ml of extract was added with 10 ml of benzene. The mixture was shaken and the appearance of a pink, red or violet colour in the lower phase indicates the presence of free anthraquinine.

b. For combined anthraquinine, 5 ml of extract was boiled with 10 ml of aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of benzene and the organic layer was separated. To half of its own volume, 10% ammonia solution was added. A pink, red or violet colour in the ammonia phase (lower layer) indicates the presence of anthraquinine derivatives in the extract.

Test for quinine

a. To the 2 ml of extract conc. H_2SO_4 was added and shaken well for 5 minutes. Red colour indicates the presence of quinine.

Test for coumarin

a. To 2 ml of extract 10% NaOH was added and shaken well for 5 minutes. Yellow colour indicates the presence of coumarin.

Test for gum

a. A small quantity of extract was slowly added into a test tube containing alcohol with constant stirring. Formation of precipitate indicates the presence of gum.

Test for starch

a. To 2 ml of extract, few drops of I_2KI_2 is added and shaken well for 5 minutes. Red colour indicates the presence of starch.

Test for fixed oil

a. A small quantity of the extract was separately pressed between two filter papers. Appearance of stain in the paper indicates the presence of fixed oil.

Quantitative Analysis of phytoceuticals

Determination of Total Phenolics and Tannin (Siddhuraju and Becker, 2003)

Ten microlitre aliquots of the extracts (10 mg/2 ml) were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteau phenol reagent and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) (Siddhuraju and Manian, 2007). 100 mg of PVPP was weighed into a 100 x 12 mm test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The content was vortexed and kept in the test tube at 4°C for 4 hrs. Then the sample was centrifuged (3000 rpm for 10 minutes at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant

was measured and expressed as the content of non-tannin phenolics on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) - Non-tannin phenolics (%)

Determination of Total Flavonoid Content (Zhishen *et al.*, 1999)

0.5 ml aliquot of appropriately (10 mg / 2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes Absorbance of the mixture was determined at 510 nm versus water blank. The results were expressed as rutin equivalents.

RESULTS AND DISCUSSION

Qualitative phytochemical screening of *Elaeocarpus tuberculatus*

Qualitative phytochemical screening of the leaf and fruit of *Elaeocarpus tuberculatus* of different extracts like petroleum ether, methanol, ethanol and aqueous extracts resulted in the table 1 and 2. The result indicated the presence and absence of carbohydrates, proteins and amino acids, alkaloids, flavonoids, tannins, phenols, terpenoids, steriods, triterpenoids, coumarin, saponins, quinine, glycosides, gum, starch and fixed oil. Anthraquinone is absent in all the leaf extracts. At the same time, gum and fixed oils were absent in petroleum ether and water. Similarly gum is absent in fruit with seed extracts. Generally the solvents of both leaf, fruit with seed ethanol, and methanol were more efficient in extracting the phytochemicals than the other two solvents.

Table 1: Qualitative phytochemical screening of the leaf of *Elaeocarpus tuberculatus*.

S. No.	Phytochemical constituents	Reagents used / Chemical tests	Solvent extracts			
			Petroleum Ether	Methanol	Ethanol	Water
1.	Carbohydrates	Fehling's Reagent	+	+	+	+
		Molisch's Reagent	-	+	-	+
2.	Proteins and Amino acids	Biuret Reagent	+	+	+	+
		Ninhydrin	+	+	+	+
3.	Alkaloids	Mayer's Reagent	+	+	+	+
		Wagner's reagent	+	-	+	+
4.	Flavonoids	Extract + $FeCl_3$	+	+	+	+
		Extract + NaoH	-	+	+	+
5.	Tannins	Extract + $FeCl_3$	+	+	+	+
6.	Phenols	Extract + $FeCl_3$	+	+	+	+

7.	Terpenoids	Extract + Chloroform + Conc. H_2SO_4	+	+	+	+
8.	Triterpenoids	Libermann - Burchard's test	+	+	+	+
9.	Steriods	Salkowski Test	+	+	+	-
10.	Coumarin	Extract + 10% NaOH	+	+	+	+
11.	Saponins	Foam Test	+	+	+	-
12.	Quinine	Extract + Conc. H_2SO_4	+	+	+	+
13.	Anthraquinine	Borntrager's Reagent	-	-	-	-
14.	Glycosides	Anthrone + H_2SO_4	+	-	+	+
15.	Gum	Water	-	+	+	-
16.	Starch	I_2KI_2	-	+	+	+
17.	Fixed oil	Spot test	-	+	+	-

"+" = Present; "-" = Absent

Table 2: Qualitative phytochemical screening of the fruit with seed of *Elaeocarpus tuberculatus.*

S. No.	Phytochemical constituents	Reagents used / Chemical tests	Solvent extracts			
			Petroleum Ether	Methanol	Ethanol	Water
1.	Carbohydrates	Fehling's Reagent	+	+	+	+
		Molisch's Reagent	+	+	+	-
2.	Proteins and Amino acids	Biuret Reagent	+	+	+	+
		Ninhydrin	+	+	+	+
3.	Alkaloids	Mayer's Reagent	+	+	+	+
		Wagner's reagent	-	+	+	-
4.	Flavonoids	Extract + $FeCl_3$	+	+	+	+
		Extract + NaoH	-	-	+	-
5.	Tannins	Extract + $FeCl_3$	+	+	+	+
6.	Phenols	Extract + $FeCl_3$	+	+	+	+
7.	Terpenoids	Extract + chloroform + Conc. H_2SO_4	-	+	+	+
8.	Triterpenoids	Libermann - Burchard's test	+	+	-	+
9.	Steriods	Salkowski Test	+	+	+	+
10.	Coumarin	Extract + 10% NaOH	-	-	+	-
11.	Saponins	Foam Test	+	+	+	+
12.	Quinine	Extract + conc. H_2SO_4	-	+	+	+
13.	Anthraquinine	Borntrager's reagent	-	+	+	-
14.	Glycosides	Anthrone + H_2SO_4	+	+	-	-
15.	Gum	Water	-	-	-	-
16.	Starch	I_2KI_2	+	+	+	+
17.	Fixed oil	Spot test	-	+	+	+

"+" = Present; "-" = Absent

Quantitative phytochemical screening

The quantitative estimation of phytoconstituents of plant parts of different extracts of *Elaeocarpus tuberculatus* is depicted in table 3. The quantitative analysis shows utmost

phenolic content in methanol extracts of leaf and fruit with seed (42.51 ± 3.23; 35.19 ± 0.43) and followed by ethanol extracts (33.17 ± 2.43 and 29.32 ± 3.91) respectively. The highest flavonoid content was observed in leaf; fruit with seed in methanol and ethanol (49.37 ± 1.24; 19.02 ± 2.18) followed by methanol and ethanol extracts (46.33 ± 2.14 and 18.21 ± 2.11) respectively. The tannin content also was maximum in methanolic extract of leaf (48.56 ± 0.78) and fruit with seed (26.98 ± 3.17). Noticeable minimum quantity of phenolics, flavanoids and tannin contents were present in water extracts.

Table 3: Quantification of total phenolics, total flavonoids and tannin content of various solvent extracts of *Elaeocarpus tuberculatus.*

Plant Name	Sample	Extraction Medium	Total Phenolics*	Total Flavonoids**	Tannin***
Elaeocarpus tuberculatus	**Leaf**	Ethanol	33.17 ± 2.43	46.33 ± 2.14	23.22 ± 2.31
		Methanol	42.51 ± 3.23	49.37 ± 1.24	48.56 ± 0.78
		Water	22.13 ± 0.05	32.24 ± 0.08	27.62 ± 0.18
	Fruit with Seed	Ethanol	29.32 ± 3.91	19.02 ± 2.18	23.49 ± 1.06
		Methanol	35.19 ± 0.43	18.21 ± 2.11	26.98 ± 3.17
		Water	26.47 ± 2.33	16.34 ± 0.14	17.02 ±3.28

*mg tannic acid equivalent/g dry weight plant material

**mg rutin equivalent/g dry weight plant material

***mg tannic acid equivalent/g dry weight plant material

Values are mean ± SD (n=3)

For centuries, plants have been utilized by mankind for healing of various diseases. Presently, there was an attention to identify the active constituents or metabolites from medicinal plants. So, isolation methods, purification techniques and structural elucidation by spectroscopic techniques were fostered in order to detect these bioactive plant metabolites. Plants, especially medicinal plants, are still essential sources for the finding of novel pharmacologically active metabolites. The major chemical substances of attention in the earlier reports have been the alkaloids and steroidal sapogenins (saponins) however; other various groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils etc. also have been reported (Farnsworth *et al*., 1966). Similarly, the therapeutic effects of *Moringa oleifera* could be due to the combined actions of various bioactive components located in the plant, including trace metal ions, vitamins, alkaloids, polyphenols and other elements and they collectively act on broad physiological processes including metabolism and immunity (Amaglo *et al*., 2010). In some plant parts the amount of these phytoconstituents was comparatively higher as reported in a study for flower

crude extract of *Bauhinia tomentosa* which showed that amount of alkaloids was (5.6%), amount of flavonoids was (15.8%) and amount of saponins was (2.1%), (Sathya *et al*., 2013). Presence of alkaloids, flavonoids, saponins, sterols and tannins in aqueous, ethanol, ether and chloroform extracts in leaves of Guava *Psidium guajava* was reported by Arya *et al*. (2012). Gupta *et al*. (2013) reported that in pods of *Acacia concina*, alkaloids were presented in aqueous and methanol extracts but absent in chloroform extract.

The preliminary phytochemical screening of selected medicinal plants like *Calotropis procera*, *Lantana camara* and *Mangifera indica* was studied by Khalid *et al*. (2018). The qualitative screening of these medicinal plants showed the presence of carbohydrates, glycosides, flavonoids, phenols in all the three plants. Alkaloids are only seen in *Calotropis procera*; phytosterols present only in *Mangifera indica*. The quantitative analysis expressed that the concentration alkaloid content (10.3 ± 0.11) in *Calotropis procera*, flavonoids in (0.718 ± 0.23), tannins in (1.0 ± 0.05), and phenols in (0.52 ± 0.10). At the same time, concentration of alkaloids (10.2 ± 0.10), flavonoids (0.719 ± 0.19), tannins (2.3 ± 0.09), phenols is 0.48 ± 0.08 and the concentration of alkaloids (10.0 ± 0.8), flavonoids (0.680 ± 0.10), tannins (2.1 ± 0.07), phenols (0.30 ± 0.06) were present in *Lantana camara*, *Mangifera indica* respectively. Kamble (2018) had analyzed the presence of phytochemicals of the plant *Achyranthes aspera* using different solvents such as petroleum ether, chloroform and methanol. In previous studies it was reported that flavonoids and terpenoids were present in aqueous extract of the *Punica granatum* (Pietta 2000) while alkaloids and phlobatannins were found to be absent in it. The above studies are supported the present study which expressed the preliminary phytochemical screening of leaf and fruit with seed extracts showed the presence of phytochemicals such as carbohydrates, proteins and amino acids, alkaloids, flavonoids, tannins, phenols, terpenoids, steriods, triterpenoids, coumarin, saponins, quinine, glycosides, gum, starch and fixed oil.

CONCLUSION

In addition, further research is necessary to identify and purify the active compounds by use of different analytical methods.

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