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A comparative evaluation of maceration, soxhlation and ultra sound assisted extraction for the phytochemical screening of the leaves of *Nephelium lappaceum*. L. (Sapindaceae)

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Abstract

Nephelium lappaceum L. (Sapindaceae) also known as Rambutan is a tropical fruit tree common to Southeast Asia. The Rambutan fruit possesses various medicinal properties such as astringent, stomachic and febrifuge. Traditionally, it was used for the treatment of diarrhoea, dysentery and fever. The antibacterial, antiproliferative as well as the antihyperglycemic effect of the fruits were reported. The objective of the present study was to carry out Successive solvent extraction by Maceration, Soxhlation, and Ultra Sound assisted extraction using different solvents like Petroleum ether, Chloroform, Ethyl Acetate, Methanol and Distilled water as well as the preliminary phytochemical screening and to compare the results.

Keywords: *Nephelium lappaceum* L. maceration, soxhlation, ultra sound assisted extraction, phytochemical screening

Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all independent. The plants are indispensable to man for his life. The three important necessities of life-food, clothing and shelter-and a host of other useful products are supplied to him by the plant kingdom. Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that we possess many effective means of ensuring health care [1].

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal medicinal practitioners. Natural products are the source of synthetic and traditional herbal medicine. The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, flavonoids, gums, tannins, etc. The active principles usually remain concentrated in the storage organs of the plants. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by a selective solvent known as menstruum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. Considering all these facts, the present study was designed to investigate the presence of various phytochemicals present in the leaves of *Nephelium lappaceum* L. using different solvents like Petroleum ether, Chloroform, Ethyl Acetate, Methanol and Distilled water by using different methods of extraction like Maceration, Soxhlation and Ultra Sound assisted extraction. The results so obtained were compared.

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Materials and Methods

Materials

The plant specimens (leaves) for the proposed study were collected from plants located at Vazhayila, Thiruvananthapuram district, Kerala. Eight year old five plants were selected and 60 leaves were collected from each plant. The collected plants were carefully examined and authenticated by Dr. A.G. Pandurangan, Scientist and Head, PS &ES Division, Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram. A Collection number (Collection No. 87269) has been deposited for future reference.

Preparation of Extracts ^[2-5]

Maceration method

Preparation of the different extracts of the leaves of *Nephelium lappaceum* L. has been done by successive maceration with the following solvents.

- a) Petroleum ether (60-80 °C)
- b) Chloroform
- c) Ethyl Acetate
- d) Methanol
- e) Distilled water

a) Petroleum ether extract

The shade dried coarse powder of the leaves of *Nephelium lappaceum* (100g) was taken in 500ml conical flask and was macerated with petroleum ether for 7 days with occasional shaking and filtered. Then the extract was distilled in vacuum in order to remove the solvent completely, dried in a desiccator and calculated the percentage yield.

b) Chloroform extract

The marc left after Petroleum ether extract was dried completely in hot-air oven below 50 °C and then macerated with chloroform for 7 days with occasional shaking and filtered. Then the extract was distilled in vacuum in order to remove the solvent completely, dried in a desiccator and calculated the percentage yield.

c) Ethyl Acetate extract

The marc left after Chloroform extract was dried completely in hot-air oven below 50 °C and then macerated with chloroform for 7 days with occasional shaking and filtered. Then the extract was distilled in vacuum in order to remove the solvent completely and dried in a desiccator and calculated the percentage yield.

d) Methanol extract

The marc left after ethyl acetate extract was dried completely in hot-air oven below 50 °C and then macerated with methanol for 7 days with occasional shaking and filtered. Then the extract was distilled in vacuum in order to remove the solvent completely and dried in a desiccator and calculated the percentage yield.

e) Aqueous extract

The marc left after Methanol extract was dried completely in hot-air oven below 50 °C and then macerated with distilled water for 7 days with occasional shaking and filtered. Then the extract was distilled in vacuum in order to remove the solvent completely and dried in a desiccator and calculated the percentage yield.

Soxhlation method (Hot Continuous Extraction)

Extracts were obtained by continuous hot filtrate extraction method using soxhlet apparatus. Freshly collected leaves were dried in shade and moderately coarsely powdered. 100 gm of leaf powder was passed through the sieve no. 18 and placed in a porous bag or thimble made of strong filter paper, which was placed in a middle chamber of the soxhlet apparatus and extracted using solvents such as petroleum ether (60-80 °C), chloroform, ethyl acetate, methanol, and distilled water as solvents. The filtrate was concentrated in rotary vacuum evaporator, dried in a desiccator and calculated the percentage yield.

Ultra Sound assisted Extraction

Extracts were obtained by ultra sound assisted extraction using Sonicator. Freshly collected leaves were dried in shade for five days and moderately coarsely powdered. 100 gm of leaf powder was passed through the sieve no. 18 and placed in a 500ml Conical flask and placed in the Sonicator for 30 minutes. Then the extract was distilled in vacuum in order to remove the solvent completely, dried in a desiccator and calculated the percentage yield.

Phytochemical Screening

The leaves of *Nephelium lappaceum* L. were subjected to Phytochemical screening to determine the presence of active secondary metabolites using Standard procedures.

Qualitative analysis ^[6]

Detection of alkaloids

About 50mg of solvent-free extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrate was tested carefully with various reagents for the detection of alkaloids as follows:

a) Mayer's Test: A few ml of the filtrates were treated with one or two drops of Mayer's reagent (Potassium Mercuric Iodide).

b) Wagner's Test: A few ml of the filtrates were treated with one or two drops of Wagner's reagent (Iodine in Potassium Iodide).

c) Dragendorff's Test: A few ml of the filtrates were treated with one or two drops of Dragendorff's reagent (solution of Potassium Bismuth Iodide).

d) Hager's Test: A few ml of the filtrates were treated with one or two drops of Hager's reagent (saturated picric acid solution).

Detection of carbohydrates

About 100mg of the extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: 2 ml of the filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand.

b) Benedict's Test: 0.5ml of the filtrates were treated with 0.5ml of Benedict's reagent and heated on a boiling water bath for 2 minutes.

c) Barfoed's Test: 1 ml of the filtrates were treated with 1ml of Barfoed's reagent and heated on a water bath for 2 minutes.

- d) **Fehling's Test:** 1ml of the filtrates were treated with 1ml of each of Fehling's solutions A and B and boiled on a water bath.

Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

- a) **Modified Borntrager's Test (Anthraquinone Glycoside):** 2 ml of the extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.

- b) **Legal's Test (Cardiac Glycoside):** 50mg of the extracts were treated with sodium nitroprusside in pyridine and 10% sodium hydroxide solution.

Detection of Proteins and Aminoacids

- (a) **Biuret test:** To 2ml the extracts, a few drops of Biuret reagent was added.
- (b) **Ninhydrin test:** To 2ml of the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes.
- (c) **Millon's test:** To 2 ml of the extracts, a few drops of Millon's reagent was added.
- (d) **Xanthoproteic Test:** 3 ml of the extracts were treated with 1ml of conc. Nitric acid and Sulphuric acid. Cooled the solution and made alkaline with 10% sodium hydroxide

Detection of Steroids

- a) **Salkowski's Test:** 2 ml of the extracts were treated with 3ml of chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand.
- b) **Libermann Burchard's test:** 2 ml of the extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. 1 or 2 drops of Conc. Sulphuric acid was added.

Detection of fixed oils and fats

- (a) **Spot test:** A small quantity of the extract was pressed between two filter papers.
- (b) **Saponification test:** A few drops of 0.5 N alcoholic

Potassium Hydroxide were added to a small quantity of extract along with a drop of Phenolphthalein. The mixture was heated on a water bath for two hours.

Detection of Phenolic compounds and Tannins

- (a) **Ferric Chloride Test:** 50 mg of the extracts were treated with 3-4 drops of 5% ferric chloride solution.
- (b) **Lead acetate test:** To 50mg the extracts 10% Lead acetate was added.
- (c) **Gelatin Test:** To 50mg of the extract, 2ml of 1% gelatin solution containing 10% sodium chloride was added.

Detection of Triterpenoids

Tin and Thionyl Chloride test: To 50mg of the extracts, Tin and Thionyl Chloride was added.

Detection of saponins

Foam Test: 50mg of extract was diluted to 20 ml with distilled water and shaken in a graduated cylinder for 15 minutes.

Detection of Gums and Mucilage:

- (a) **Precipitation Test:** To 100mg extract in 10ml distilled water 25ml of Absolute alcohol was added with constant stirring.
- (b) **Ruthenium Red Test:** 50mg of the extract was added to swell in water, and a few drops of Ruthenium Red was added.

Detection of flavonoids

Aqueous Sodium Hydroxide: Added Aqueous solution of Sodium Hydroxide to the Sample.

- (a) **Concentrated Sulphuric acid:** Concentrated Sulphuric acid was added to the sample.
- (b) **Shinoda Test:** 50mg of the extract was dissolved in alcohol, a few Magnesium turnings were introduced and a few drops of Hydrochloric acid was added.

Results and Discussion

The Preliminary Phytochemical screening of the leaves of *Nephelium lappaceum* L. revealed that the phytoconstituents extracted by different methods that is Maceration, Soxhlation and Ultra Sonication were different for all the extracts. The yield of each extract is also different for all the three methods. Extraction by Soxhlation shows maximum yield.

Table 1: Colour, nature and percentage yield of extracts of powdered leaves of *Nephelium lappaceum* L.

Sl. No	Extracts	Colour	Nature	Percentage Yield (%w/w)		
				Maceration	Soxhlation	Ultra Sonication
1	Petroleum Ether	Dark Brown	Smooth	1.29	2.41	2.13
2	Chloroform	Greenish brown	Smooth	0.79	1.43	1.07
3	Ethylacetate	Black	Sticky	1.18	2.06	1.64
4	Methanolic	Reddish brown	Sticky	2.22	5.18	4.53
5	Aqueous	Dark brown	Amorphous	0.98	1.38	1.11

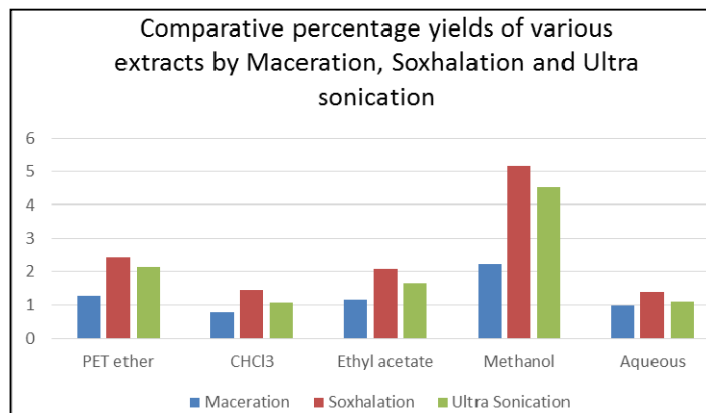


Fig 1: Comparative percentage yields of various extracts by Maceration, Soxhalation and Ultra sonication method

Table 2: Phytochemical Screening of different extracts by Maceration, Soxhalation and Ultra Sound assisted extraction

Extracts	Methods		
	Maceration	Soxhalation	Ultra sound assisted extraction
Petroleum ether (60-80 °C)	Steroids	Tannins	Nil
Chloroform	Cardiac Glycoside	Cardiac Glycoside	Nil
Ethyl acetate	Carbohydrate, Cardiac glycoside	Carbohydrate, Cardiac glycoside	Carbohydrate, Steroids
Methanol	Carbohydrates, Fixed oils and fats, Cardiac glycosides, tannins, Flavanoids, Saponins	Carbohydrates, Fixed oils and fats, Cardiac glycosides, Tannins, Flavanoids, Saponins	Carbohydrates, Steroids, Fixed oils and fats, Cardiac glycosides, Tannins, Flavanoids, Saponins
Aqueous	Carbohydrates, Cardiac Glycosides, Tannins	Carbohydrates, Cardiac Glycosides, Tannins	Carbohydrates

Conclusion

Preliminary Phytochemical screening and comparison of the results obtained by the three different methods of extraction were done. The Preliminary Phytochemical screening of the leaves of *Nephelium lappaceum* L. revealed that the phytoconstituents extracted by different methods that is Maceration, Soxhalation and Ultra Sound assisted extraction were different for all the extracts. The yield of each extract was also different for all the three methods. Extraction by Soxhalation showed maximum yield. The studies have finished a set of qualitative parameters that can serve as important information which can be used to ascertain identity, quality and purity of plant material in future.

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